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Prof. Brian Chung joined the Hong Kong Genome Institute as the Chief Scientific Officer in 2021, and is currently the President of the Asia Pacific Society of Human Genetics. He is a distinguished member of the Canadian College of Medical Geneticists and holds the esteemed title of Founding Fellow of the subspecialty of Genetics & Genomics (Paediatrics) of the Hong Kong Academy of Medicine (HKAM). Leveraging his all-rounded expertise and rich experience in genomic medicine, coupled with key roles in various local and overseas genomics and genetics organisations, Prof. Chung has been aspiring to mainstream genomic medicine in Hong Kong through close collaborations and partnerships with various researchers and clinicians. His notable accolades include the Best Young Investigator Prize of the Hong Kong College of Paediatricians (2017), Sir Patrick Manson Gold Medal (2018), notably the Outstanding Teaching Award, Teaching Excellence Awards, HKU (2019), *etc*.

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Journal of Translational Genetics and Genomics

Editorial

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Personalised genomic medicine is shaping the future of healthcare

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The Human Genome Project, a pivotal moment in genomics, reached a significant milestone in 2003 by completing 92% of the sequencing map of the human genome. As genomic technologies advance, the Telomere-to-Telomere Consortium took on the task of filling in the remaining gaps in the genomic regions in 2022[1]. This endeavour has provided invaluable insights into the field of genomics and its implications for precision health. Precision medicine is an emerging paradigm in precise diagnosis, treatment, and efficient surveillance of diseases, taking into account individual variability in genetics, lifestyle, and environment of each patient[2]. The transformative potential of precision medicine lies in its ability to move away from a one-size-fits-all approach and embrace a patient-centric model[3]. Diverse precision medicine initiatives involving the sequencing of thousands to millions of human genomes have been launched worldwide, aiming to bring genomics into healthcare[2,4,5]. Notable examples include the 100,000 Genomes Project in the United Kingdom, the All of Us Research Program in the United States, and the China Precision Medicine Initiative in China. These collaborative efforts exemplify the global commitment to leveraging advanced technology to generate large-scale genomic data to predict or stratify the health risks of patients. Targeted treatment and personalised care could be provided, unlocking the potential of improving quality of life and helping to bring down healthcare costs. This special issue "Genomics & Precision Health" includes a total of nine publications, leading by showcasing the launch of a population-based genome project in Hong Kong,

namely the Hong Kong Genome Project (HKGP), where their four strategic directions are pertinently mapped with the eight publications in this special issue covering application and review of genomic advancement in rare and common diseases, experience sharing of high-quality sequencing and analysis platforms, and recommendations and strategies in fostering the development, wide adoption of, and access to the benefits of genomics and precision health.

Recognising the immense potential of genomic medicine and the lack of genomic data, many countries and regions have also launched their own genome projects to contribute to the advancement of healthcare in their communities. Hong Kong, as a world-recognised financial centre with a modern city standard and quality of living, also seizes the opportunity to embrace the era of precision medicine for more accurate diagnosis and personalised treatment. The Hong Kong Genome Institute (HKGI), established by the Government of Hong Kong in 2021, implements the city's first large-scale genome sequencing project, HKGP, aiming to perform whole genome sequencing (WGS) for up to 50,000 genomes by 2025. Chu *et al.* presented the potentials and challenges of launching the HKGP as their first paper after the successful implementation of the HKGP[6]. Four main strategic foci pertaining to (1) integrating genomic medicine into clinical care; (2) advancing research in genomic science; (3) nurturing talents in genomic medicine; and (4) enhancing public engagement and genomic literacy were mapped out to work toward achieving HKGI's vision and mission, "to avail genomic medicine to all for better health and well-being". They reviewed the current landscape and specific challenges encountered during the construction of the infrastructure, workflow, and implementation of the pilot phase of HKGP, highlighting the importance of governance, stakeholder engagement, the development of a patient-focused consent protocol, the unique three-tier informed consent process, and the aspiration for developing the genetic counselling profession in Hong Kong. This paper provides insights not only for international counterparts when building similar projects but also into setting a solid foundation for integrating genomics into routine clinical care by starting a new theme "Genomics and Precision Health" in addition to "Undiagnosed Diseases" and "Hereditary Cancers" after the pilot phase with the completion of the first 5,000 genomes. This new theme focuses on driving the incorporation of genomic medicine into mainstream healthcare development in Hong Kong by improving genomic diagnosis, personalised treatment, personalised prediction, and the prevention of disease risks. Collaborative research projects in diverse disease cohorts, beyond the existing undiagnosed diseases and hereditary cancers, that will benefit from WGS in disease diagnosis, treatment, and prevention have been initiated. Sequencing infrastructure and analysis platforms have also been further enhanced with the latest technologies incorporated to facilitate advanced genomic research development and discovery. These pave the way to move toward the four strategic directions to foster the development, wide adoption of, and access to the benefits of genomics and precision health.

(1) Integrating genomic medicine into clinical care

The art of genome sequencing and analysis offers valuable insights into early and precise diagnosis, personalised treatment and management of various diseases, highlighting the expanding influence of genomics across diverse areas of clinical practice. In this special issue, we gathered an application of genomics in prenatal molecular diagnosis of a rare disease, X-linked Bartter Syndrome, as well as reviews on how genomics benefits clinical practice in various common disease cohorts, such as inherited cardiovascular conditions (ICCs), adult myeloid leukaemia (AML), and young-onset diabetes (YOD)[7-10]. Xu *et al.* presented an application of genomics in the prenatal diagnosis of Bartter syndrome (BS), which is important for the early onset of polyhydramnios related to BS[7]. They reported that severe transient X-linked antenatal BS was resulted by the fetus carrying the *MAGED2* hemizygous nonsense variant c.967C>T [p. (Asp323*)] inherited from the pregnant mother. Therefore, a prenatal genetic diagnosis can confirm that

initiating prenatal indomethacin therapy has a beneficial effect on the fetus, emphasising the importance of timely prenatal diagnosis of BS type 5 for guiding appropriate management of polyhydramnios and postnatal symptoms. Loong *et al.* shared the experience from the ICC research program at the National University Heart Centre, Singapore, bringing the notion that genomic medicine has opened the door to precision medicine in cardiology[8]. The clinical frameworks and considerations were presented, providing an overview of the operations of the clinic, including wet and dry lab conditions, work performed by a healthcare professional, and the variety of cases, ranging from cardiomyopathies and arrythmias to aortopathies. Their experience provides insights for international counterparts when implementing similar services in local healthcare centres to address the healthcare burden of ICCs. Leung *et al.* reviewed that the genomic revolution in AML has ushered in a new era of personalised medicine, shifting the treatment paradigm from a generalised approach, which has reached an impasse, to one that targets individual genetic alterations[9]. The integration of genomics, the detection of measurable residual disease, drug sensitivity testing, and single-cell transcriptomics hold tremendous potential for optimising AML management through personalised approach based on genomic and transcriptomic information. Chan *et al.* discussed various aspects of YOD, including the importance of correct diagnosis of maturity-onset diabetes of the young and monogenic diabetes, the use of genomic medicine in diagnosis and classification, and a clinical trial called PRISM that aims to re-define insulin secretion and monogenic diabetes in Chinese patients[10]. This paper emphasises the importance of clinical observations and person-oriented care, maximising the utility of genomic medicine in pursuit of early diagnosis and management of YOD.

(2) Advancing research in genomic science

To maximise the benefits of embedding genomics into routine clinical care, like the aforementioned examples in rare and common diseases, effort in advancing research in genomic science through the establishment and enhancement of standardised high-quality sequencing and analysis platforms with state-of-the-art technologies is extremely important. HKGI has enhanced its genome sequencing capacity and capability to increase patients' accessibility to this advanced technology for precise diagnosis and personalised clinical care. Chu *et al.* published their experience in designing the HKGI laboratory, establishing the genome sequencing workflow, and enhancing the sequencing and analysis platforms[11]. The HKGI drew on recommendations and experience from the Medical Genome Initiative and other sequencing projects to customise hardware and software components of the laboratory to optimise the laboratory design and layout, sequencing workflow, quality assurance and data information management system. A unidirectional workflow that employs a hybrid of manual and automated approaches for proper laboratory practice and operation was established. A list of stringent quality assessments of the samples and sequencing libraries was established. Systems for handling and housing different data types, such as Clinical FrontEnd and LabKey Sample Manager, were tailored and optimised at par with international standards to facilitate and standardise the patient recruitment process, clinical data collection, sample processing, and biobanking. The genome sequencing workflow has been optimised to boost weekly throughput to approximately 350-500 samples, enabling the processing of over 6,600 genomes in the first 24 months since launch. The laboratory has been further enhanced to include cutting-edge techniques, such as long-read sequencing. The performance of long-read GS in detecting variants in complex regions ("dark regions") of the human genome, which are challenging for short-read sequencing, was also illustrated in the example of the polycystic kidney disease 1 gene. Such a precise diagnosis can inform the patient's clinical management and treatment choices, such as the use of Tolvaptan to slow down the progression of kidney failure. The combination of long-read and short-read sequencing offers a more accurate understanding of the genome, enabling the detection of complex genomic rearrangements, large insertions or deletions, and allelic phasing. Broadly visioning the extent of further research advancement in favor of precision medicine, the

laboratory has also established a single-cell sequencing platform to integrate multi-omics information to enhance the characterisation of disease at the cellular and tissue levels and to enable the discovery of biomarkers for therapeutic targets. These hold the promise of transforming healthcare through precise diagnosis, targeted treatment, prognostic prediction, and targeted therapeutic development.

(3) Nurturing talents in genomic medicine

Expediting the advancement of genomic medicine and the promotion of precision health in the healthcare system worldwide cannot be achieved without enlightening work in other disciplinary aspects, like nurturing talents in genomic medicine by facilitating genomic education for medical professionals. Maher *et al*. shared their experience in developing continuing genomics education programs for non-genetics medical specialists to increase their understanding of genomic medicine and its clinical application in Australia, providing insights into the place of online learning and workshops as implementation strategies to translate the use of genomics from research settings to health systems[12]. Additionally, the inclusion of non-genetic specialty peer experts in the co-design and delivery of education is highly recommended to mediate and translate the evidence for the use of genomics in a specialty, to adapt clinical genetics practice as appropriate to the specialty, and to strengthen cross-specialty relationships to practice genomic medicine.

(4) Enhancing public engagement and genomic literacy

The promotion of public engagement and genomic literacy is crucial work in support of genomics and precision health in healthcare service development. Collaboration in research with members of the public and patients is recognised to be indispensable. Hunter *et al*. shared five case studies in a variety of clinical genomic studies in the United Kingdom, highlighting that public and patient involvement has a significant and beneficial influence on research that addresses sensitive and ethically challenging topics in genomic service development[13]. Key recommendations in planning, recruitment and involvement are also identified to further embed good practices across genomic and other health service research. Sharing the same view that public support and engagement in promoting genomics and precision health in the healthcare system is the key to success, especially in the Chinese culture with different socio-cultural views, Chu *et al*. explored the views and concerns of patients and family members participating in the HKGP[14]. A quest for a patient-oriented, transparent, and decommercialised WGS campaign for the population-based genome project is highly important to address the challenge of public distrust as a common obstacle to genomic advancement. Age-specific marketing and publicity strategies are vital for raising public awareness and encouraging public engagement for genomic initiatives. Genomic literacy through tailoring complex genomic topics to diverse audiences ranging from the public and patients of different ages to highly educated professionals is a priority to facilitate the integration of genomic medicine into mainstream healthcare. Insights into the long-term promotion of public engagement and education can serve as a guiding beacon for international counterparts in navigating the genomic medicine era.

In conclusion, the publications gathered in this special issue "Genomics & Precision Health" aim to showcase collaborative efforts in multidisciplinary aspects to promote genomics and precision health. Implementation of a population-based genome project with advanced sequencing and analysis technology can bring the era of precision medicine into clinical practice. Equipping healthcare professionals and the general public for a better understanding of genomics and precision health leads the journey to unlock the full potential of genomics for personalised and patient-centred care. With strategies to integrate genomic medicine into clinical care, advance research in genomic science, nurture talents in genomic medicine, and

enhance public engagement and genomic literacy, the goal of bringing genomic and precision medicine into our healthcare with substantial clinical and economic benefits is not far to reach.

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Potentials and challenges of launching the pilot phase of Hong Kong Genome Project

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Abstract

Genomic medicine and precision medicine initiatives have taken centre stage in scientific, clinical, as well as health economics and utility research on the global scene for the past decade. It is clear the important role genomic advancement has played in enhancing diagnostic rate, streamlining personalised treatment, and improving efficacy of the overall clinical management of undiagnosed, rare, and common diseases for humankind. The Hong Kong Genome Institute (HKGI) was established in May 2020 within the Food and Health Bureau, Hong Kong Special Administrative Region, to integrate genomic medicine into mainstream healthcare. The main goals of setting up HKGI are to (1) improve the diagnostic rate and future care for individuals affected by undiagnosed diseases and hereditary cancers using whole genome sequencing; (2) advance research in genomic science; (3) nurture talents in genomic medicine; and (4) enhance public genomic literacy and overall engagement through the launching of the Hong Kong Genome Project (HKGP). In this paper, we review the current landscape and specific challenges encountered during the construction of the infrastructure and implementation of the pilot phase of HKGP. Through reviewing what has been achieved and established to date, and the potentials and prospects that have emerged in the process, this paper will provide insights into planning the main phase of HKGP, and considerations for our international counterparts when building similar projects.

Keywords: Hong Kong Genome Project, genomic medicine, whole-genome sequencing, informed consent, genetic counselling

INTRODUCTION

The rapid advancement in genomic medicine has offered huge potential and opportunities in accurate diagnosis, personalised treatment, and efficacious surveillance and prevention of both undiagnosed, rare, and common diseases. Since the completion of the renowned Human Genome Project in 2003, progresses in next-generation sequencing and bioinformatic technologies have accelerated the execution of large-scale human genome projects.

A growing number of countries have invested immense resources to utilise the human genome in view of the importance of integrating genomic medicine into future medical and health development[1]. Precision medicine initiatives involving sequencing of thousands to millions of human genomes are being launched worldwide, and experiences are shared via publications and exchanges in international conferences. The largest projects in terms of scale are the 1+ Million Genomes Initiative, which is a collaboration among 24 European Union countries; and the All of Us Research Program of the United States (US), both aim at recruiting and analysing genomes of one million participants[1].

Many of these genomic initiatives strategically focus on rare disease patient cohorts [Table 1]. Affected individuals are often difficult to diagnose and have very long diagnostic journeys, and thus they are more likely to benefit from comprehensive and unbiased genome sequencing. Studies did not only show the diagnostic and clinical utility of genome sequencing in rare disease cohorts in ending the diagnostic odyssey at a personal level, but there is also the extended impact on the social and health economics aspects[2-4].

These initiatives have facilitated the reform of healthcare in numerous areas. For example, the 100,000 Genomes Project led by Genomics England has proven to be a well-recognised success, based not only on their gene discoveries and scientific publications, but also by their impact on the nation's healthcare ecosystem[5]. The Project has enabled and driven the necessary changes and reform in the National Health Service (NHS) by offering whole-genome sequencing as routine.

Challenges and barriers of genetic services development: global and local perspectives

Although countries such as the United Kingdom, US, and China strive to be global leaders by launching ambitious population-based genome sequencing projects, experts in the field who are heavily involved pointed out that many overlooked the importance of understanding and managing the "public appetite" while designing the projects[6]. In an era in which the public highly values data security and personal privacy, public trust remains a universal challenge for implementing any genome initiatives and campaigns.

Albeit the difficulties and uncertainties in managing public trust and confidence, the design of these national whole-genome sequencing projects shares one humane mission - the elimination of the major barriers to accessing genomic services. Cost and limitations of an accessible service infrastructure for targeted patients have remained significant hurdles to genomics and genetics services worldwide.

Prior studies pinpointed patients' difficulties in getting proper and timely referrals to genetic testing services (for rare, undiagnosed, or hereditary cancer syndromes) as a universal obstacle. Pragmatic difficulties in many institutions lie in the lack of well-established multi-disciplinary genetic clinics and clearly delineated triage system for managing referrals and arranging timely genetic consultation[7-9].

Table 1. Examples of large-scale genomic projects with target size larger than 20,000 genomes include patients with rare and undiagnosed diseases

Country	Project/institute name	Target size	Years active	Status	Genomic sequencing modality
Australia	Australian Genomics Health Alliance[i]	> 25,000	2016-ongoing	Active	Depending on the flagship projects
Australia	Genomics Health Futures Mission[ii]	200,000	2018-ongoing (targeted completion in 2028)	Active	Depending on the projects
China	China Precision Medicine Initiative[iii]	100,000,000	2015-ongoing (targeted completion in 2030)	Active	WGS
Denmark	Danish National Genome Center[iv]	60,000	2021-ongoing (targeted completion in 2024)	Active	WGS
France	France Genomic Medicine 2025[v]	235,000 per annum	2015-ongoing (targeted completion in 2025)	Active	WGS/WES/RNA seq
Hong Kong	Hong Kong Genome Project[vi]	50,000	2021-ongoing (targeted completion in 2026)	Active	WGS
Japan	GEnome Medical alliance Japan[vii]	Nationwide	2018-ongoing	Active	WGS
Saudi Arabia	Saudi Human Genome Program[viii]	100,000	2018-ongoing (targeted completion in 2030)	Active	WGS/WES/panel
Thailand	Genomics Thailand Initiative[ix]	50,000	2019-ongoing (targeted completion in 2024)	Active	WGS/WES/Microarray
Turkey	Turkish Genome Project[x]	100,000	2017-ongoing (targeted completion in 2023)	Active	WGS
United Kingdom	100,000 Genomes Project[xi]	100,000	2013-2018	Completed	WGS
United Kingdom	Our Future Health[xii]	5,000,000	2020-ongoing	Active	Depending on the projects
United States	NHGRI Centers for Mendelian Genomics[xiii]	Nationwide	2011-ongoing	Active	Depending on the projects
United States	NIH Undiagnosed Diseases Program[xiv]	Nationwide	2008-ongoing	Active	WES/Microarray

NHGRI: National Human Genome Research Institute; NIH: National Institutes of Health; RNA Seq: RNA sequencing; WES: whole-exome sequencing; WGS: whole-genome sequencing. [i]https://www.australiangenomics.org.au/our-history/ and https://www.sciencedirect.com/science/article/pii/S0002929719302289; [ii]https://www.health.gov.au/initiatives-and-programs/genomics-health-futures-mission; [iii]https://www.sciencedirect.com/science/article/pii/S0002929718304221 and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7657949/; [iv] https://eng.ngc.dk/Media/637614364621421665/Danish%20Strategy%20for%20personalised%20medicine%202021%202022.pdf; [v] https://solidarites-sante.gouv.fr/IMG/pdf/genomic_medicine_france_2025.pdf; [vi] https://www.fhb.gov.hk/download/press_and_publications/otherinfo/200300_genomic/SCGM_report_en.pdf; [vii] https://www.amed.go.jp/en/aboutus/collaboration/ga4gh_gem_japan.html and https://www.ga4gh.org/news/gem-japan-releases-largest-ever-open-access-japanese-variant-frequency-panel/; [viii]https://shgp.kacst.edu.sa/index.en.html; [ix]https://www.nature.com/articles/d42473-020-00211-y and https://genomicsthailand.com/Genomic/Activities; [x]https://www.bbmri-eric.eu/news-events/turkish-genome-project-launched/ and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5606892/; [xi]https://www.nejm.org/doi/full/10.1056/NEJMoa2035790 and https://www.genomicsengland.co.uk/the-100000-genomes-project-by-numbers/; [xii]https://ourfuturehealth.org.uk/research-programme/ and https://www.gov.uk/government/publications/genome-uk-2021-to-2022-implementation-plan/genome-uk-2021-to-2022-implementation-plan; [xiii]https://www.nih.gov/news-events/news-releases/nih-genome-sequencing-program-targets-genomic-bases-common-rare-disease; [xiv] https://www.genome.gov/Current-NHGRI-Clinical-Studies/Undiagnosed-Diseases-Program-UDN

Besides the lack of access to well-trained clinical laboratory and healthcare professionals, the Asian socio-cultural beliefs of families and genetics are also major impediments in the Asian region such as Singapore[10], Malaysia[11] and Hong Kong[12]. Perceived difficulties and inadequateness do not only originate from patients. Studies also showed that frontline clinicians felt unequipped to make accurate referrals[13-15]. A recent local study on the attitudes and clinical practices of primary care physicians (PCPs) showed that 68% (104/151) of the surveyed PCPs in both public and private service organisations did not know the referral pathway for patients with suspected or confirmed genetic disorders[16].

While genetic testing is a critical part of diagnostic journeys of patients with rare disease and cancer, cost remains a critical hurdle in genetic services in Hong Kong. As the public service only sponsors and covers essential healthcare for all, a limited number of genetic tests are covered[12,17]. Private financing schemes, including employer-based and privately purchased insurance as well as household out-of-pocket payment, represent common forms of healthcare financing in Hong Kong. As health insurance is not mandatory, 49.2% of the people in Hong Kong are not covered by any form of medical insurance and many would have to bear the cost of genetic and genomic tests out-of-pocket[18]. In a local study investigating the cost-effectiveness of using chromosomal microarray as the primary test for prenatal diagnosis, only 41.8% (300/717) of pregnant women were willing to pay fully out-of-pocket to undergo the test. In scenarios where the test was subsidised at an increasing portion, more women were willing to undergo and benefit from the diagnostic test[19]. Another local study on Chinese females at-risk of Hereditary Breast and Ovarian Cancer Syndromes showed that sponsored genetic counselling and testing were crucial for them as the majority would not have opted for self-financed testing[20].

Some Asian countries have also started national genome projects to advance genomic medicine [Table 1]. A fundamental change and reform of the current genomic landscape is undoubtedly an essential first step in expediting the advancement in precision medicine. Without that, it is difficult to set the stage to facilitate the breakthrough in improving the diagnostic rate and clinical management of rare and undiagnosed diseases.

The characteristics of Asian robust city - Hong Kong

Chinese represents the largest population in the world, and Hong Kong has become a Special Administrative Region of the People's Republic of China since 1997. As a world recognised financial centre, with a modern city standard and quality of living, Hong Kong retains its own economic, legal, social, healthcare, and welfare infrastructures.

According to the latest population projections announced by the Census and Statistics Department (C&SD)[21], Hong Kong's population is projected to increase from 7.51 million in 2019 to 8.10 million in 2039. With reference to the 2016 by-census[22], over 90% of the Hong Kong population is ethnic Chinese and other ethnic groups constitute the remaining 8% of the population. With a relatively homogenous population, Hong Kong is therefore often being seen as a strategic location to conduct health-related studies on Southern Chinese populations.

Current landscape of genetics and genomics in Hong Kong

Hong Kong has a well-established dual-track healthcare system comprised of 43 (and growing) public hospitals managed by the Hospital Authority. Majority of the population (around 85%) uses public healthcare, while the remaining population has convenient access to self-financed medical services. The present paper systematically explores the history and background of the genomic and genetic services in the context of the existing health service landscape, which serves as the backbone of the strategy and implementation of the first large-scale genome project - The Hong Kong Genome Project (HKGP).

Hong Kong is not building genomics and genetics work from ground zero. Clinicians at the Department of Health (the Government's health adviser and agency which executes health policies and statutory functions), along with the Hospital Authority, medical schools of universities, and private hospitals, have been providing continuous clinical genetics service and research support to the public in Hong Kong for decades.

For historical reasons, Clinical Genetic Services of the Department of Health provides the majority of public diagnostic and counselling services to families with inherited genetic disorders. These services include diagnosis of genetic disorders, genetic screening, genetic counselling, and genetic testing in relation to disease management. Post-test clinical management and periodic screening have usually been taken up by Hospital Authority via its public hospitals' infrastructure. Local academic researchers and scientists, usually with a job-induced passion, opportunities, and research/charitable fundings, have attained internationally recognised achievements in genomic research. As mentioned above, the majority of the patients access genetic services provided by the Department of Health or Hospital Authority in Hong Kong. Since only essential investigations are covered, advanced genomic tests such as genome sequencing are either sent to overseas clinical laboratories with patient bearing the full cost or done in a research setting[12].

Despite the hard work and contributions by various parties, the medical-academic-scientific fields in Hong Kong have identified enduring issues with the above set-up and workflow. The genomic services provided by the two medical schools are often research-based and lack the capacities to be used as routine clinical services. The Government acknowledged in the policy address of 2017 that there was an urgent need to explore a more standardised and better co-ordinated clinical pathway, invest in the training and nurturing of related professionals, and improve the overall management of genetic and genomic services in Hong Kong. A Steering Committee was thus formed to review the landscape of genetics and genomics in Hong Kong and map out the strategies for developing genomic medicine.

Steering committee recommendations

After reviewing the experiences of other national sequencing projects and deliberating the local environment, the Steering Committee published its report and proposed the eight recommendations to promote local development and integration of genomics into our healthcare system[12].

1. Launching the HKGP

2. Enhancing clinical services in genetics and genomics

3. Nurturing talents in genomic medicine

4. Enhancing public engagement in genomic medicine

5. Enhancing the laboratory network with effective referral mechanism and centralisation of advanced genetic and genomic tests

6. Facilitating the establishment of a biobank network for genomic research

7. Enhancing the regulation on use of genetic data for insurance and employment purposes

8. Promoting the proper use of genetic and genomic tests

THE LAUNCH OF HONG KONG GENOME PROJECT: PILOT PHASE

Based on the recommendations from the Steering Committee, the Government set up the Hong Kong Genome Institute (HKGI), a company wholly owned by the Government, to implement the HKGP in partnership with the Food and Health Bureau, Department of Health, Hospital Authority, and local

universities. The Government reserved HKD$1.2 billion (USD$150 M) for the Project, the first population-scale whole-genome sequencing project in Hong Kong with the aim to sequence up to 50,000 genomes by 2025.

Leveraging the experience from other sequencing projects, the HKGP is expected to serve as a catalyst to advance the development of genomic medicine in Hong Kong. In addition to the clinical benefits, it would also strengthen the local talent pool, establish infrastructure and protocols, and drive new policy measures in the field of genomic medicine. The data generated from HKGP would form one of the largest health-related local databases and create new research opportunities for studying various diseases.

Since its incorporation in May 2020, the HKGI has been fully engaged in recruiting professionals in the field and setting up various hardware and software necessary for implementing the HKGP. It would pioneer the development of a clinical genomic platform for the population of Hong Kong, with continuing contributions from all members of HKGI under the governance of six committees, overseen by the Board of Directors [Figure 1].

The implementation of HKGP is being conducted in two phases, namely the pilot phase and the main phase. Under the pilot phase of the Project, approximately 2000 cases (~5000 genomes, as majority of the cases will be subjected to trio analysis) will be sequenced. The recruitment eligibility criteria include patients with undiagnosed diseases, hereditary cancers (genetic predisposition to cancer), and their family members. During the short-term and long-term strategic planning process, the current landscape of genomics and genetic services in Hong Kong was carefully reviewed to identify key issues and challenges facing HKGI in the future. Lessons learnt during the pilot phase would guide the directions of the Project's main phase as it grows and expands its coverage into other disease areas and research cohorts. The main phase will sequence approximately 18,000 cases (~45,000 genomes).

Participants enrolled in the HKGP receive WGS analysis in addition to the usual clinical care. A trio-based approach would be adopted in majority of the cases, where feasible, to assist genomic data interpretation. Phenotypic data is recorded using Human Phenotype Ontology (HPO) terms to standardise clinical data input. A whole blood sample is collected from all participants for DNA extraction and subsequent WGS analysis in the HKGI laboratory and sequencing centre at a public-funded university. For participants with suspected hereditary cancers, an additional tumour sample, preferably fresh frozen sample, could be obtained to compare against the germline genome for the detection of somatic variants.

Engagement with frontline healthcare and medical-academic systems

The recruitment structure of HKGP is built on the establishment of partnerships with existing healthcare systems. For the pilot phase, HKGI has established and fully funded the operations of three HKGP Partnering Centres at the university-affiliated teaching hospitals within the public healthcare system to ensure equitable access to the Project. The Partnering Centres play important roles in providing clinical support, such as recruiting participants, providing genetic counselling, collecting samples, handling enquiries, delivering genome sequencing results, and liaising with clinical teams of the hospitals [Figure 2]. The Partnering Centres' participation in HKGP not only introduces whole-genome sequencing (WGS) in the public healthcare system, but also offers a unique opportunity to provide experiential learning and training for the workforce in the Partnering Centres, pathing the way for clinical implementation of genomic medicine.

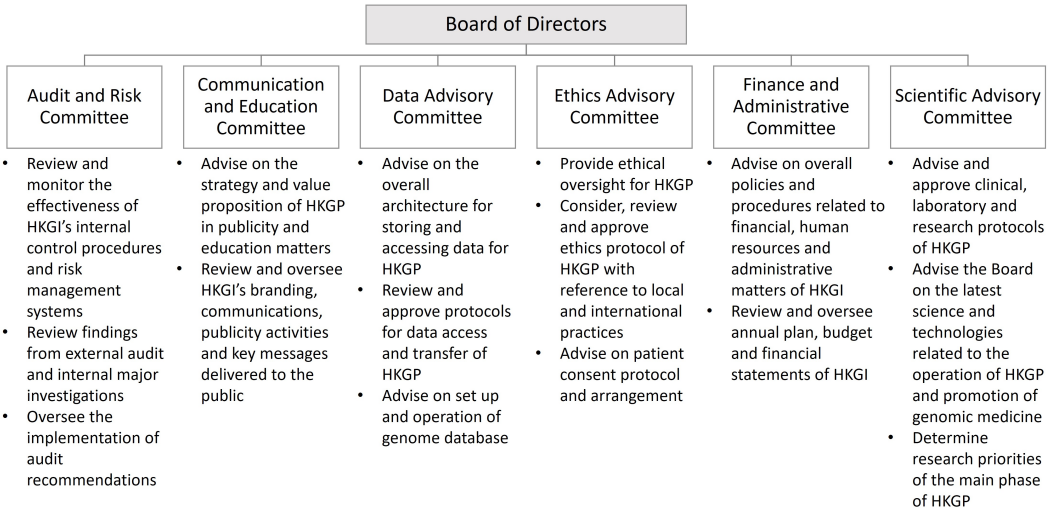

Figure 1. Governance of the Hong Kong Genome Institute. HKGI is overseen by the Board of Directors and six Advisory Committees for effective implementation of HKGP. HKGI: Hong Kong Genome Institute; HKGP: Hong Kong Genome Project.

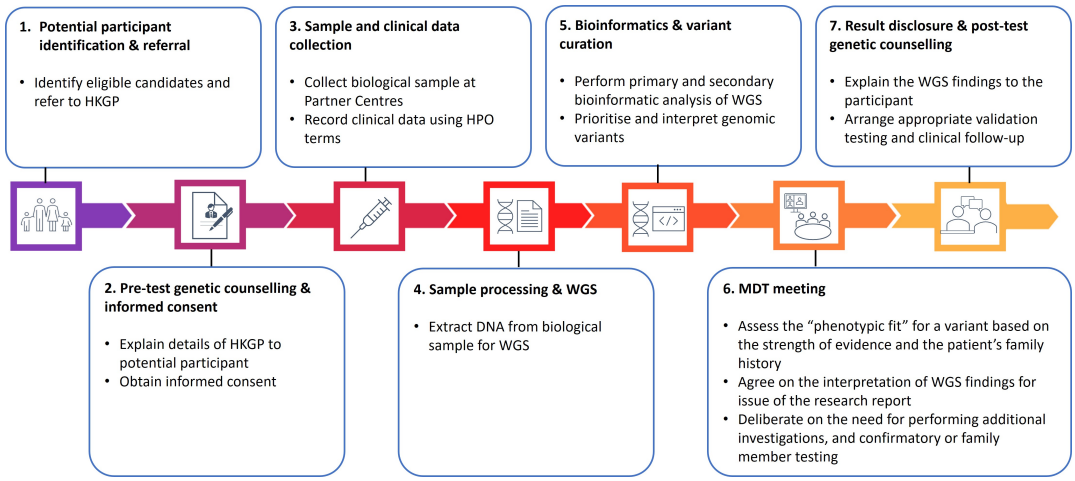

Figure 2. Operational workflow of Hong Kong Genome Project. HKGI: Hong Kong Genome Institute; HKGP: Hong Kong Genome Project; HPO: Human Phenotype Ontology; MDT: multi-disciplinary team; WGS: whole-genome sequencing.

Genome project for the people: public trust and confidence

As mentioned above, public trust and understanding of genomics unquestionably remain a global challenge to the success of all genomics campaigns. With the aim to facilitate public awareness and understanding, the HKGP spearheads the development of guidelines and standardised protocols on informed consent and the collection, storage and sharing of genomic data in a secure, ethical, and responsible manner.

HKGI respects and values the public opinions and perception of the HKGP and carried out three focus groups (undiagnosed disease patient groups, hereditary cancer patients and family members, and clinicians) to understand the people's general opinions on genomic testing and studies. A recurring theme mentioned in the focus groups was the emphasis on making the process as "transparent" and "fully informed" as

possible. The findings were highly valued and shaped the current structure of the informed consent and patient recruitment process, publicity, and public engagement strategies of the Project.

For genomic medicine to be widely adopted, there is also a need for public engagement in the areas of science and medicine to improve genomic literacy and foster public support. The Project provides a good opportunity to enhance the public's understanding of the benefits and limitations of genomic medicine and genome sequencing on healthcare decisions, as well as relevant ethical and privacy standards. To complement and promote the launch of HKGP, a dedicated project website with user-friendly information, videos, and publications on genomic medicine are developed to draw public's interest in genomics and enhance genomic literacy (Available from: https://hkgp.org/en/). With the governance of its Communications and Education Committee [Figure 1], HKGI will continue to do pulse checks on all public engagement and awareness strategies adopted by the Project.

Other than project marketing and promotion strategies to manage public opinions, high-level of transparency, clear, and continuous communication between healthcare professionals and patients is a crucial pillar of building public trust and credibility[23]. One major priority in the implementation of HKGP is the design of a patient-focused and ethical consent protocol. A truly thorough "informed consent" practice for complicated WGS testing must be done in a thorough, colloquial, non-directional, and humane manner.

The thorough process in developing a patient-focused consent protocol

In the era of genomic sequencing, the models of consent have stretched the traditional approaches as a vast amount of genetic information can be obtained from a single test. The expanded scope of testing puts pressure on the width and depth of pre-test discussion on several emerging areas, including but not limited to genomic data handling and privacy, uncertain results, unexpected or secondary findings and the associated psychosocial and familial impact. There is still a lack of consensus in the genetic community about the standard constitution of informed consent in genomic settings owing to its complexity.

During the design process of HKGP consent protocol, consent protocols of other large-scale genomic projects such as the 100,000 Genomes Project, the latest consent policy and consent clauses for genomic research from the Global Alliance for Genomics and Health (GA4GH) were referenced, with the understanding that these frameworks have considered the opinions of the professionals and public[24-27]. To address the specific project details of HKGP, the Ethics Advisory Committee (EAC) [Figure 1] of HKGI advises on ethical issues in relation to the design and implementation, including the endorsement of the consent protocol of HKGP. The formation of EAC comprises external experts and professionals from diverse backgrounds to offer crucial perspectives on relevant ethical decisions, including bioethicists, lawyers, patient group representatives, and clinical geneticists. Opinions from the participating local bodies, including the Equal Opportunities Commission, Privacy Commission for Personal Data, and legal advisers, are gathered in addition to comments from the EAC to ensure concerns from other stakeholders are well-addressed before the submission to the Institutional Review Boards (IRB).

One of the challenges of achieving informed consent for genomic sequencing is often related to the subjective and poorly defined line of adequate information and comprehension[28]. With an aim to meet the specific needs of individuals and facilitate the consent process, the consent materials of HKGP adopted a colloquial and well-organised presentation according to carefully designed sequences and levels of detail. The consent form seeks broad consent for using participants' donated samples, collecting clinical information for genetic analysis, and sharing de-identified data for approved research studies. It also

provides options for participants to receive secondary findings or to provide contact information for participation in future research outside the scope of HKGP. For participants who consented to receive secondary findings, HKGP will return pathogenic/likely pathogenic variants in a well-defined list of genes that are in line with the international practice. Thirteen genes are currently included in the analysis and the reporting approach is age-dependent [Table 2]. While the gene list and approach will be reviewed periodically, participants are informed of the scope and practice during the consent process and have access to the gene list through our website.

Apart from the consent form, two versions of information booklets and 11 videos are produced to provide detailed and step-by-step information for potential participants [Figure 3]. Since the potential participants are from a wide age range, including adults, teenagers, and children, the entire content is presented in age-appropriate and colloquial dialects and enriched with coloured infographics and animated cartoons to facilitate easy understanding of informed consent.

As emphasised, public trust and confidence in genomic testing are vital of the success of any large-scale genomic project. Background of the Project, recruitment information and consent materials can be easily accessed via HKGP website. This also helps set the inaugural move in enhancing public literacy and promoting awareness of genomic medicine in Hong Kong.

Three-tier informed consent process

HKGP recruits participants of all ages and different versions of consent forms are formulated to customise their needs. Like any research involving child participants, their parent or legal guardian is involved in the consent process and will provide written consent on the child's behalf as a proxy. While obtaining assent is a universal aim in both clinical and research consent, there is no single practice internationally. The concept of assent and its application varied in different countries, ranging from the most paternalistic approach of adhering strictly to legal provisions to the most liberal approach of respecting child/young participants' wishes as much as possible.

A unique three-tier model of informed consent was designed and implemented in the HKGP after thorough discussions at EAC [Table 3]. The model is adopted to delineate the consent and assent rationale to balance the paternalistic and liberal approaches in the international arena. In Hong Kong, the legal age is 18 years old; therefore, adult participants aged 18 or above would sign the consent form themselves. Participants aged 16 and 17 would co-sign their consent form with parents/legal guardians. This approach aims at recognising and maximising the autonomy and best interests of adolescents who have sufficient capacity to understand the Project entirely. If there is any difference in opinions between the participants and their parents/legal guardians, further discussion would be encouraged, and the participants would not be recruited. For participants aged below 16, their consent forms would be signed by parents/legal guardians. Verbal assent would be taken wherever possible, in accordance with the child participant's level of understanding. When child participants reach legal age, a formal re-consent process will be conducted.

The entire consent, pre-test counselling, and withdrawal process (if applicable) of HKGP are based on this three-tier model, with an emphasis on open, pro-active, and respectful engagement of all participants about their involvement in the Project in an age-appropriate, humane, and ethical manner.

Needs, challenges, and aspirations for developing the genetic counselling profession in Hong Kong

The previous section depicted the importance of informed consent and pre-test genetic counselling as the first step of patient engagement of HKGP. The process is performed by genetic counsellors specifically hired

Table 2. List of secondary findings reported by the Hong Kong Genome Project

Conditions	Genes	Adults	Children
Lynch syndrome	*MLH1*	√	x
	MSH2	√	x
	MSH6	√	x
Familial adenomatous polyposis	*APC*	√	√
MYH-associated polyposis	*MUTYH*	√	x
Hereditary breast and ovarian cancer syndrome	*BRCA1*	√	x
	BRCA2	√	x
Von Hippel-Lindau syndrome	*VHL*	√	√
Multiple endocrine neoplasia	*MEN1*	√	√
	RET	√	√
Familial hypercholesterolaemia	*LDLR*	√	√
	APOB	√	√
	PCSK9	√	√

Table 3. The three-tier model of informed consent of Hong Kong Genome Project

Category	Age (years)	Consent	Assent
Adult participants	≥ 18	Signed by participant	Not applicable
Child participants	16 to 17	Signed by parent/legal guardian and co-sign by child participant	Materials aiding assent process
	< 16	Signed by parent/legal guardian	Materials aiding assent process or verbal assent (whenever possible)

and trained to execute this Project.

Genetic counselling is a relatively novel and unorganised profession in Hong Kong. Our existing genetic counselling related services have been supported by a group of dedicated and on-the-job trained frontline medical personnel (with or without an overseas board-certified qualification). As the demand for genomic and genetic services has expanded rapidly in the past decade, there is an urgent need to standardise the genetic counselling practice and facilitate training in Hong Kong.

One of the prevailing challenges is the lack of accredited programmes and Board to train and register new genetic counsellors or those who are practising in the field. This further exacerbates the insufficient pool of professional genetic counsellors to meet the rapid growth in service demands in Hong Kong. There is also a pressing need to delineate the scope of practice, code of ethics, and quality assurance of the industry. Without the start of a blueprint or a governing body, it is difficult to plan and implement continuous training and development of genetic counselling practice.

HKGP acts as a catalyst and sets the stage in providing a platform to nurture a group of genetic counsellors by providing funding and resources for Partnering Centres to hire designated genetic counsellors. Through enabling the role of genetic counsellors in the operational workflow, awareness and knowledge amongst other healthcare professionals concerning the scope of practice of genetic counsellors can be enhanced.

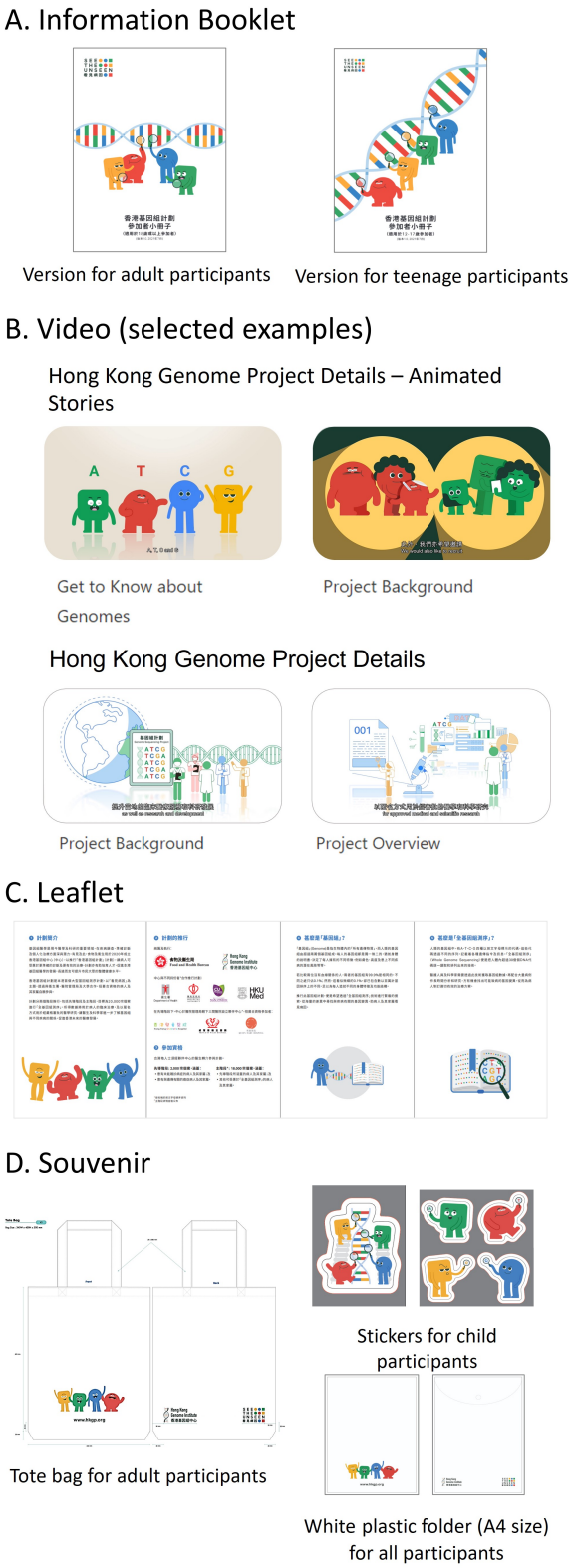

Figure 3. Informational and promotional materials for Hong Kong Genome Project. Cartoon characters that represent the four nucleotide bases in human genome are included in all materials as mascots of the Project. (A) Information Booklet (available in Traditional Chinese and English). Versions with different levels of detail and styles of language are prepared for adult and teenage

participants aged 12-17, respectively. (B) Examples of video on HKGP webpage (bilingual subtitles in Traditional Chinese and English). (C) Leaflet (available in Traditional Chinese and English). (D) Souvenir.

Learning opportunities for genomic professionals

Other than paving the way for the genetic counsellors, HKGP also serves as an important platform to provide continuous enrichment to clinicians, laboratory scientists, bioinformaticians, genomic variant curators, nurses and trainees. The Project offers experiential learning opportunities to different specialities in a collaborative environment.

One of the challenges of working with various professionals from a multi-disciplinary background is the lack of a common language; it is understandable that specialities may use different vocabularies to provide descriptions of the same observed phenotype. Hence, HPO has become the standard for phenotype exchange in the age of genomic medicine, describing human phenotypes systematically and enabling computational inference in genotype-phenotype analyses[29]. It has been used extensively to support diagnostic interpretation of genomic variations in rare diseases and adopted by many large-scale genome projects such as the 100,000 Genomes Project and the Undiagnosed Disease Network[30]. In alignment with international standards, the HKGP also adopts the use of HPO terms to record clinical features, facilitating effective communication amongst professionals of diverse backgrounds.

Apart from using HPO as the "common language" amongst professionals and the computational analysis system, HKGP takes on a multi-disciplinary approach in achieving the project outcomes. Post-analysis multi-disciplinary team (MDT) meeting is one of the core components of the HKGP workflow, allowing inputs from relevant specialists to achieve consensus on the genetic diagnosis and patient's management plan. For example, genomic variant curators will work closely with the referring clinicians to determine the cases that would require further in-depth discussion in an MDT meeting, where the referring clinician will present phenotypes of the participant and the genome variant curators will present the variant finding and interpretation.

Further to providing means of communication for a diverse group of professionals, we hope HKGP serves as an active, experiential learning platform to enable members and trainees to appreciate and familiarise the integration of genomic medicine into practices.

CONCLUSION

HKGI takes on the pioneering role in developing genomic medicine in Hong Kong. Through the launch and implementation of HKGP, it aims to overcome the major hurdles of cost and lack of access to WGS in the existing services. It provides free-of-charge WGS for individuals and families suffering from the clinical odyssey of rare diseases, hereditary cancers, and other undiagnosed diseases. Since HKGP is the first large-scale genome project in Hong Kong, it lays the groundwork for developing the infrastructure and workflow to prepare for the integration of genomic medicine into routine clinical care in the foreseeable future. It also equips the existing workforce, trains the next-generation of genomic experts, and prepares the general public for the age of precision medicine.

With the tight timeline to recruit and analyse an ambitious number of genomes, this is only the beginning of a challenging and farsighted mission. To measure the success of the programme, a panel of international scientists will evaluate the structure, process and outcome of the HKGP independently. We look forward to sharing the evaluation outcome and other progress of the Project as separate publications and case reports. With the perseverance, unyielding efforts, and dedication of all the stakeholders, we aim to live up to our

vision "To avail genomic medicine to all for better health and well-being".

DECLARATIONS

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Authors' contributions

Contributed intellectually and practically to this piece: Chu ATW, Fung JLF, Tong AHY, Chow SM, Chan KYK, Yeung KS, Lo HM, Hong Kong Genome Project, Chung BHY

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Opinion

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Public and patient involvement in research to support genome services development in the UK

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Abstract

Public and patient involvement (PPI) - the collaboration in research with members of the public and patients with relevant experience - is becoming well established in health service research in the UK. It is supported by funders and academic institutions. Published principles and guidelines for researchers, developed through consultation and consensus building, are available. Meanwhile, as genome sequencing is adopted into routine health care, translational genomics research and research to evaluate new genomic services are growing. Given the ethical and social implications of offering genome sequencing within a national health service, it is important that researchers give full consideration to planning and implementing meaningful PPI. Here we present five case studies of PPI in a variety of clinical genomic studies, including commentary on positive impacts and suggestions for improvements. We call for funders and academic institutions to continue and increase their efforts to enable and promote PPI across genomic and other health service research.

Keywords: PPI, patient involvement, genome sequencing, health service research

INTRODUCTION

"Public and patient involvement" or PPI, is becoming an established feature of health research in the UK. For example, it is mentioned in 12 of the 33 articles published in 2022 by the National Institute for Health Research (NIHR)[1]. It is the practice of working in partnership with people who have life experience relevant to the research - enabling them to inform, influence and actively collaborate in research - and is distinct from recruiting study participants and from "engagement", which focuses on disseminating findings outside academic journals and conferences[2]. Efforts have been made to produce frameworks that capture the variety of strategies developed for effective PPI, activities that PPI participants can undertake, and principles to optimise outcomes[3-6]. Discussion in the literature is expanding into standardised reporting of PPI and the finer points of representation on PPI advisory boards by inviting individuals with and without some level of research experience[7,8].

Two key motivators behind the growth in PPI activity are the ethical and democratic imperative of giving the ultimate beneficiaries of research an opportunity to influence its direction, and the related argument that it can improve research and service development outcomes and relevance. These arguments apply along the pipeline from basic to clinical and translational research, although the benefits arising from PPI for laboratory research may be less tangible[9]. Translating new knowledge into clinical services, however, requires an understanding of a wide range of potential impacts on the users of the services and their families, including those that researchers might not anticipate. It is perhaps easier to comprehend the impact that involving PPI contributors can have on research that aims to design and evaluate new clinical services.

The growing body of literature about measuring the impacts of PPI includes a focus on clinical trials[10] and on those involved in research themselves[11], although the value of quantitative metrics and whether measuring impact per se is the most useful way forward are still debated issues[12,13].

The growing evidence around the practice and outcomes of PPI has gone hand in hand with the development of resources by UK-based organisations which aim to support researchers in establishing meaningful PPI. These take the form of national standards, sets of principles, guidelines and toolkits[14-17] developed through consultation and consensus building exercises, plus mechanisms to link interested participants and researchers with each other[18,19]. Standards and principles focus variously on deciding who to involve and how to involve and support PPI members, good communication and documenting PPI processes and impact. Major UK health research funding organisations now encourage or even require PPI planning to form part of applications for funds[20-24].

In the arena of genomics, the UK continues to be a significant actor in the development of the technology and its application in health services. Large-scale projects such as the UK Biobank, the NIHR BioResource and the 100,000 Genomes Project have generated vast numbers of highly cited publications, and the latter led directly to the establishment of the new Genomic Medicine Service (GMS) in the NHS[25-30]. The GMS aims to promote equity of provision and includes new testing services, such as rapid turnaround exome/genome sequencing for fetal anomalies and for seriously ill children who are in hospital without a diagnosis[31,32]. Still at the research stage but embedded within the NHS is the Newborn Genomes Programme which will evaluate the application of genome sequencing for newborns, its impact on the NHS and the risks and benefits for individuals and families[33].

APPLIED GENOMICS AND MEANINGFUL PPI

The culture of a positive approach to PPI among health researchers, and the mature ethical, legal and social research community in the UK, have facilitated the adoption of PPI within the evaluation of proposed and live pilot genomics services. In many ways, the core principles of good PPI apply as with any other health research - but there are potential conflicts between social and scientific perspectives of genomics, and particular issues for patients such as the benefits and risks of knowing certain types of genomic information, such as variants of unknown significance and secondary findings, which means a particularly attentive approach to PPI is warranted[34,35]. PPI strategies that include a focus on underserved populations are also an important part of helping to address the issue of a lack of diversity among the participants in genomics research to date[36,37].

This article describes recent and current PPI case studies [Tables 1-5] from research focused on delivery of genomics in the NHS, and what lessons can be learned. The case studies are presented following Donabedian's three components approach, originally designed for evaluating the quality of care[51]. The components are: structure (attributes of the case study), process (the systems and processes adopted to deliver the desired outcome) and outcome (impact on the project, researchers, and PPI participants themselves).

PPI in the evaluation of new genomics services [Tables 1-3]

PPI in new applications of genomics [Tables 4 and 5]

CONCLUSION

The five case studies presented illustrate that PPI can bring significant and beneficial influence to research that addresses sensitive and ethically-challenging topics in genomics service development. The case studies point to PPI advisors directly impacting study protocols, budgets and materials, refining recruitment approaches for parents who may be bereaved or traumatized and improving diversity, and giving invaluable contextual information to support the interpretation of findings. Integrating PPI contributors in this way has provided invaluable insight to the research teams, which should also benefit their future research work.

Some limits to the benefits of PPI can be directly linked to the existence of barriers or the lack of enablers, which can reduce the effectiveness of PPI activities undertaken by researchers[52]. The case studies explore a range of both organisational enablers (such as resourcing and training) and those at a personal level (such as a collaborative approach that involves PPI contributors at every stage, with clarity around roles and expectations). Further, greater awareness of the imbalances of power inherent in the way PPI partnerships are established could lead to better quality outcomes. For example, we recognise in our case studies the tendency for researchers to set meeting agendas and make decisions about how PPI contributors will engage, and that a more flexible collaborative approach, such as inviting PPI contributors to lead PPI groups, can be very valuable. Our case studies also clearly illustrate challenges in achieving equality and diversity in recruitment and involvement -careful planning and resourcing, and early consultation with patient or community groups, are important to address this. Finally, long-term partnerships with individuals over the course of several studies might lead to a narrowing of the viewpoints being offered - conversely, PPI teams will always be too small to be representative of the study population, and their input must be sought and valued in that context.

Table 1. Case study 1 - reflections on PPI structure, process and outcome

PPI in "evaluation of the NHS Genomic Medicine Service for paediatric rare diseases"
Structure
Research aim: • To understand how genome sequencing for paediatric rare diseases is being delivered in the new Genomic Medicine Service (GMS)[38], with a focus on barriers and enablers to successful delivery. PPI team: • Representatives from Genetic Alliance UK and Unique, and three parent representatives recruited through SWAN UK (Syndromes without a name)[39-41], who sit on the study advisory board
Process
Recruitment: • By advertisement distributed through the patient groups' social media channels explaining the role, who the researchers were looking for (parents with experience of an undiagnosed child, and experience of genetic or genomic testing), and what the expectations of the PPI group would be. The lead researcher spoke to potential participants by phone, and both were recruited. • Diversity: the researchers stated in recruitment advertisements that they were keen to include representation from different ethnic groups, and from fathers. One mother with South Asian heritage applied and joined, but no fathers applied. It was important to recruit individuals with lived experience to complement the patient organisation representatives who may not be representative of the wider community. Supporting involvement: • Expectations of the PPI team set out clearly by the PI in the terms of reference: support development of the study protocol, information sheets and ethics application; assist in developing topic guides and questionnaires to ensure the topics covered are important and relevant to patients and families; develop strategies to troubleshoot any problems, e.g., with recruitment; assist in data analysis, in particular interpreting how the findings may be of relevance to patients and families, and to support translating the findings into recommendations for practice for clinicians and policy makers; and develop plain language summaries and support other effective methods of communicating findings to a wider audience. • The PPI team was integrated into the main advisory group which meets twice each year, but the initial meeting was limited to parent members of the PPI team to build rapport, discuss the study and air questions and concerns. Parent participants were generally contacted by phone after advisory group meetings to discuss the feedback that they did not feel able to share during the meeting. • Meetings have been virtual, and ad hoc phone or email contact is made between meetings to ensure relationships are maintained. • Plans are in place to deliver online training for those wanting to upskill (to be determined by the PPI team but could include data analysis, writing and presenting)
Outcome
Positive impacts on PPI contributors and on the study: • The feedback from parent participants during post-meeting phone calls is that they feel able to ask questions and participate in the discussions about the study. • The PPI team has reviewed and commented on study documents and provided input into which measures to include in a survey for parents of children having WGS. As a result of their feedback, the survey includes a measure of parental health and family functioning which was seen as an important outcome of testing. • A number of the PPI team were co-authors on the published research protocol[38] and they will be invited to co-author further academic publications from the project. There will also be the opportunity to co-present some of the research findings at conferences. Limitations of PPI in this study: • Two parent participants had to drop out due to other commitments. One has been replaced, but it would have been preferable to recruit more participants at the start to allow for this possibility. • It would have been beneficial to build training and support into the grant application to allow one of the parents to act as a lay co-researcher on the team ("an expert by experience"), to access the parent interview transcripts and provide a counter-perspective to analysis by the social scientist

The authors of the case studies have identified improvements that could have been made in their approaches. There is scope for funders and academic institutions to take steps to further embed good practices across genomic and other health service research. Concrete actions are important in themselves, such as communicating clearly about what funders and academic institutions expect researchers to do, signposting to existing PPI resources, and providing financial and practical support - but these steps are also necessary if we are to create and sustain an academic culture where effective PPI is a given.

Table 6 provides the key recommendations identified by the authors for fellow investigators in health service research, arising directly from the case studies presented.

Table 2. Case study 2 - reflections on PPI structure, process and outcome

PPI in "evaluation of rapid genomic sequencing for critically ill children (rGS Study)"
Structure
Research aim: • Rapid genomic testing can offer faster diagnoses and much earlier decisions about care for babies and children when they are critically ill and a monogenic condition is suspected[42]. This mixed-methods study is looking at the delivery of this test in the NHS from the perspective of parents and professionals to facilitate optimal care and support for children and their parents[39]. PPI team: • There are four arms to the PPI Team. ○ 1. Two of the co-applicants are from patient organisations (Genetic Alliance UK and Alstrom Syndrome UK)[39,43] and sit in the research team, bringing experience with a range of research projects and in setting up PPI advisory groups, and a broad perspective on rare disease. The Genetic Alliance UK representative leads on the PPI elements of the evaluation. ○ 2. There is a social science researcher based at a patient organisation (Genetic Alliance UK). ○ 3. A PPI Advisory Group with patient organisation representatives and individual parents. The individual parents offer their lived experience of having a child who was cared for in intensive care and/or offered exome or genome sequencing. ○ 4. A representative of a patient organisation, who is not part of the PPI Advisory Group, sits on the main study steering group alongside clinicians and researchers to bring a patient voice to those meetings
Process
Recruitment for the PPI Advisory Group: • Parent members (of children with a developmental disorder, or a suspected/diagnosed rare condition) were recruited through advertisements and completed a short application form to allow for selection based on diversity as well as experience in genome sequencing and/or neonatal or paediatric intensive care. A father was recruited after additional calls were made through support groups for fathers, and a mother with South Asian heritage was recruited by invitation. Five parents in total were recruited. • Relevant patient organisations were approached and invited to suggest a representative who could join the PPI advisory group. Supporting involvement: • PPI members of the core research team have contributed to funding applications, study design and development from the outset. • Because of the sensitive nature of the topic area, and to help members feel comfortable sharing their experiences, a separate PPI advisory group was set up rather than only including PPI members within the wider study advisory group (including researchers and clinicians). • The PPI Advisory Group meets on an ad hoc basis when feedback is needed. Members are paid for their time. • One of the social science researchers is based at Genetic Alliance UK to further strengthen the links between PPI input and research processes
Outcome
Positive impacts on PPI contributors and on the study: • PPI input into study materials and recruitment planning is particularly important for this research due to (1) the sensitive nature of the planned interviews with parents whose child has been very unwell or may have died; and (2) the need to consider diversity in the study when exploring parent experiences and equity of access to testing. • The PPI advisory group has given detailed feedback on the wording and images for an online parents' survey and the participant information sheets. The diverse experiences of the group have been especially helpful in alerting the researchers to wording that can impact, for example, bereaved parents, those not biologically related to their child and same-sex couples. • The group has helped find meaning in the survey results and given advice on topics to include in the parent interviews. They will be invited to give feedback on themes and quotes from the interview analyses to inform interpretation. • In the future, it is hoped that this group will help with the preparation of publications and the development of recommendations for practice that are focused on parent and patient priorities and needs. • Having PPI co-applicants ensured PPI input from the initial design of the study, and informed budget decisions such as funding for translation of study materials and options for other formats such as audio and video. They are part of the research team, which means that there is iterative feedback between the PPI and research teams throughout the study. Limitations of PPI in this study: • Within the PPI advisory group, it was not possible to find representatives of all parent experiences that are relevant to the study. For example, very few fathers put themselves forward to be involved. • The researchers tend to direct the PPI input, setting the meeting agenda, setting questions and drafting materials for comment. It may be helpful to be less prescriptive and allow the PPI input to be more iterative and open

Table 3. Case study 3 - reflections on PPI structure, process and outcome

PPI in "optimising exome prenatal sequencing services (EXPRESS study)"
Structure
Research aim: • EXPRESS is a mixed-methods research project studying the roll-out of prenatal exome sequencing as part of the NHS Genomic Medicine Service[44]. Prenatal exome sequencing is offered when ultrasound scans show a baby is not developing as expected and doctors suspect a monogenic condition. Expectant parents who are offered the test will have been faced with uncertain scan findings and will then be asked to make decisions about further testing and the future management of their pregnancy. They may be offered the option to terminate their pregnancy. PPI Team: • There are three arms to the PPI Team. 1. Two funding co-applicants and core members of the research team are from the patient organisations (Alstrom Syndrome UK and Antenatal Results and Choices (ARC))[43,45]. The Director of ARC leads the PPI elements of the research. 2. There is a social science researcher based at a patient organisation (ARC).

3. A PPI Advisory Group was established with representatives of parent organistations and an individual member with relevant experience

Process

Recruitment:
• ARC provided guidance on appropriate recruitment of parents to the study PPI: the group's members include representatives from a number of parent and patient support organisations, and a researcher who has personal experience in prenatal testing and bereavement.
Supporting involvement:
• The group has been asked to focus on the qualitative arm that is investigating parents' views and experiences of prenatal exome sequencing, and the ethics workstream that aims to explore associated ethical issues.
• The group meets quarterly and members are paid for their time.
• One of the researchers is embedded at ARC and received training to work on the charity's helpline to gain an in-depth understanding of what parents face when making decisions around testing, diagnosis and termination of pregnancy

Outcomes

Positive impacts on PPI contributors and on the study:
• Including patient group representatives as co-applicants ensured that they helped inform the overall study design.
• The PPI group gave feedback on interview topics and questions, recruitment methods, the design of multiple formats of patient information, and the creation of a newsletter about the research for patients to make sure they are clear and inclusive.
• The PPI group gave feedback on themes arising during interview analysis to inform interpretation and are co-authors on the published research protocol[44].
• Embedding the researcher at ARC allowed them to fully focus on parents' experiences and provide an active link between the research team and the patient organisation, which in turn helps the wider research team to maintain a focus on parent priorities.
Limitations of PPI in this study:
• English-speaking white middle-class participants are overrepresented at the time of writing. PPI involvement in designing a specific budget and plan for targeted methods of recruitment, e.g., using community groups, may have helped us reach potential participants from underrepresented demographics

Table 4. Case study 4 - reflections on PPI structure, process and outcome

PPI in "genomics england newborn genomes programme"
Structure
Research aim: • Genomics England's Newborn Genomes Programme will launch in 2023 and is a co-designed research study, i.e., it develops the PPI approach such that patients and public partners actively influence decision-making in the project design and operation. The programme will explore the benefits, challenges, and practicalities of sequencing newborns' genomes[33]. An in-depth and early phase of consultation with stakeholders as part of the research design has been carried out to focus on how to "choose conditions", i.e., determining which rare genetic conditions, out of the many potential options, should be looked for as part of the study. A six-month process was designed to establish a set of underpinning principles. Note: This case study illustrates an approach to PPI that is "modular" - in addition to integrating PPI advisors for the lifetime of a project (drawing on the long-standing Genomics England Participant Panel - a key advisory group for Genomics England consisting of patients, family members and carers who have had genome sequencing)[46], the scale of this work requires additional consultation with distinct (and relatively large) groups of people at discrete stages of the study as part of the co-design. As there are over 6000 known rare conditions with varying levels of impact on health and quality of life, it was important to capture as wide a range of views as possible. It was also critical to include the perspectives of those who do not have experience in rare conditions, as most babies who take part in the study will not receive a positive result
Process
• First, a working group was established comprising healthcare professionals, scientists, ethics and policy researchers, and representatives from patient groups and the public. A member of Genomic England's standing Participant Panel was included. • Principles were proposed by the group, then tested at online workshops with members of the public, people with experience in rare conditions, and healthcare professionals. Each principle was debated in order to capture participants' concerns and interests. • A series of explanatory materials, including presentations and videos, was generated to support workshop participants, and a member of the programme team was available to answer questions at each workshop. • Deliberations were led by expert facilitators from a public participation charity[47]. The Participant Panel at Genomics England was also consulted about the principles in an additional session
Outcome
Benefits of PPI in this study: • Four final principles emerged from the workshops, which will underpin the design of the programme. They relate to validity of the test, severity of the condition, benefits of intervention and equity of access to interventions (e.g., through the UK's NHS)[33]. • Carrying out these workshops early in the programme means that participants' diverse views are integral to the design phase of the work. Limitations of PPI in this study: • PPI endeavours correspond to a moment in time with a wide variety of participants who might all have different views. When making decisions such as which conditions will be looked for in this research study, it is difficult to achieve consensus, and inevitably it is impossible to incorporate some individuals' views. For this reason, decisions that incorporated PPI input should be revisited in light of changing practices. This revisiting is something that the Newborn Genomes Programme is committed to throughout the duration of its study

Table 5. Case study 5 - reflections on PPI structure, process and outcome

PPI in "The secondary cardiac findings evaluation (SCARFE) study"
Structure Research aim: • The SCARFE study was developed to understand the benefits and risks of informing people about a secondary genomic finding, specifically in an inherited heart condition[48]. Secondary findings are genomic changes that are not related to a patient's known health condition but might indicate a risk of a separate serious condition[49]. It is not yet clear whether looking for secondary findings is beneficial. For example, informing a patient about a secondary finding might enable healthcare actions that would detect a health condition early, allowing medical intervention - but being told about a secondary finding might cause people long-term anxiety, and if the risk of disease development is very low, people might undergo tests and treatment they do not need. Inherited heart conditions are a group of disorders that can occasionally lead to sudden cardiac death; if people are genetically at risk because they carry a variant associated with an inherited heart condition, screening tests such as echocardiogram and ECG can identify people whose hearts are affected, and measures can be taken to manage their risk
Process Recruitment: As a new area of study with the potential to inform people about a serious health risk, it was important to involve patients and the public from the outset. PPI members were recruited from an existing Genomics PPI group based in Oxford, including people who had a rare disease or cancer, and their relatives and carers. Supporting involvement: • The research team presented the study aims to the PPI participants, and invited questions and discussion during an informal round-table; all PPI participants were encouraged to voice opinions
Outcome Benefits of PPI to this study: • A key question was how best to contact participants eligible for the study from the NIHR BioResource Rare Disease Study[27] (who had agreed to be contacted about future studies). Potential participants would need enough information to make a decision about taking part in SCARFE without causing alarm or breaching their right not to know unexpected genomic information. Through discussion with the PPI group, the research team was prompted to explore the use of a pre-invitation opt-out letter that would be sent to all BioResource participants. • The resulting letter was based on other stakeholders' deliberation[50] and advised BioResource participants that if they did not wish to be contacted about studies which might tell them about their risk of other health conditions, they could decline the approach [Supplementary Material]. The PPI group was invited to review the document, and the opt-out process was included in the SCARFE protocol submitted for research ethics committee approval

Table 6. Key recommendations for PPI in health service research

Planning
• Researchers should keep in mind that PPI in health research is an active collaboration and two-way process for mutual benefit. PPI teams should find the experience both enjoyable and rewarding, and their impact/influence should be made clear to the whole study team, including the PPI contributors. • Plan for early training opportunities to ensure PPI contributors feel comfortable with technical aspects of the research, and for the research team to learn about lived experiences. • Perform evaluation from the start of the project: what approaches could be used to evaluate the benefit to the PPI contributors, the research team and the study itself? Document and share learnings. • It is helpful both for PPI participants and researchers to set out clear criteria for membership of the PPI group or for PPI members of a study advisory group, along with a clear outline of what is being asked of them and how the group will operate - everyone should be assured that meetings are interactive with an emphasis on the ability to ask questions and on listening and respecting all views. • To facilitate effective planning for PPI, include people with PPI expertise as co-applicants on the initial funding application and include a budget for PPI activities. To improve diversity and representation from underserved groups, a budget is also needed to support targeted methods of recruitment to advisory groups, e.g., through community groups. • Where possible, embed researchers in patient/parent support organisations to gain in-depth experience of the research context. This is particularly valuable when doing research on sensitive topics, for example, prenatal testing or seriously ill children
Recruitment • Consider involving both individuals with lived experience (not necessarily patients) and representatives of patient groups. The latter can bring a broad perspective that complements individuals' experiences, and access to networks of affected individuals. If patient group representatives have experience in academic research, they may be able to advise on what PPI can bring to a study, what is involved in setting up a PPI advisory group within the processes and constraints of research, and what worked and what did not in previous projects. In addition, they may be well placed to support PPI members of advisory groups new to research. • Researchers and patient organisations can build close relationships over time - this, plus the "small world" nature of rare conditions, means that the same people are often invited to take part in PPI for multiple studies. The case studies presented illustrate this. Researchers should always consider which are the most relevant organisations that could contribute (and which staff), and whether "new" organisations could be approached in order to optimise the independent perspective that PPI can bring. Think about the size of the PPI advisory group: there is no one "patient voice", so the group should be large enough to represent a suitable diversity of opinion and experience but small enough to allow full and open discussions. Plan meetings according to the needs of the PPI participants: many find remote meetings convenient, but face-to-face meetings can build rapport. Consider informal settings and what practical support might be needed for face-to-face meetings
Involvement • Involve PPI participants as far as possible in all stages of the study, from planning the grant application to interpreting findings (not just at the manuscript review stage) to helping develop recommendations for practice. Early consultation with PPI participants will facilitate consideration of the ethical and practical issues that studies can raise, and will improve protocol development.

• Keep in touch with PPI participants, both between meetings (e.g., by email) to ensure their continued interest, and immediately after meetings (offer phone calls to discuss issues that might have been too difficult to bring up in a group, and signposting to independent support organisations).

• Provide opportunities for the PPI team to co-author papers or co-present research at meetings. Not only does this ensure they are acknowledged for their role in the study and have some ownership of the results, but it can also support parents/the public to develop new skills.

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Review

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Genomics in practice - a review of inherited cardiac conditions

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Abstract

Interest in inherited cardiovascular conditions (ICCs) is fueled by resources devoted to its diagnosis, management, and research. The rapid advancement of DNA genomic sequencing deepens our understanding of ICCs. The ICC genomic landscape empowers the development of diagnostic guidelines and the discovery of potential therapeutic targets and promises novel therapeutics, especially in precision cardiology. Therefore, it is essential for healthcare institutes and systems to develop contextual frameworks based on current guidelines to provide holistic care for patients with ICCs. The clinical frameworks and considerations described in this review provide an overview of the operations of an ICC clinic, including wet and dry lab conditions, work performed by a healthcare professional, and the variety of cases, ranging from cardiomyopathies to arrythmias to aortopathies. Insights from our experience in an ICC clinic in Singapore add to the discussion of the challenges and benefits for patients and clinicians who serve them.

Keywords: Clinical genomics, genetic testing, inherited cardiac condition, cardiomyopathy, arrhythmias, sudden cardiac death

INTRODUCTION

Interest in inherited cardiovascular conditions (ICC) has risen as resources are devoted to its study[1]. Cardiovascular diseases are the most common cause of death worldwide, with numbers increasing yearly[2]. A significant proportion of these diseases are ICCs, notable for their high degree of heritable features. ICCs are broadly classified as cardiomyopathies, channelopathies, vasculopathies, heritable lipid conditions, and neuromuscular disorders involving the cardiovascular system.

The rapid advancement of DNA sequencing, from Watson and Crick's description of the three-dimensional structure of DNA in 1953, to Sanger and Gilbert's development of Sanger sequencing in 1977, to the most recent advanced third-generation long-read sequencing offered by Oxford Nanopore Technologies and Pacific Biosciences expands our understanding of ICCs[3]. An elucidation of the ICC genomic landscape enables the development of diagnostic guidelines, the discovery of potential therapeutic targets, and the creation of novel therapeutics. These have opened the field to precision and translational medicine in cardiology. One recent example is the US Food and Drug Administration's approval of the first-ever drug for symptomatic obstructive hypertrophic cardiomyopathy (HCM) that works by modulating cardiac myosin[4,5].

Against this background, it is essential for healthcare institutes and systems to develop contextual frameworks making use of updated guidelines to provide holistic care for patients with ICCs[6,7]. For example, we must tackle the characterization of variants of uncertain significance (VUS), report incidental findings, navigate the myriad ethical considerations, and address the socioeconomic implications for the successful implementation of ICC genetics.

In this review, we offer insights from our experience as an ICC clinic in Singapore and introduce three case examples from our clinic.

Importance of setting up an ICC program

Current studies suggest that the prevalence of HCM, dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (ACM), and channelopathies are approximately 1/500, 1/250, 1/5000, and 1/2000 to 1/5000, respectively[8-12]. These are derived predominantly from studies in Western populations and extrapolated to estimate prevalence worldwide[8,11]. It is challenging to define the epidemiology due to the heterogeneity of clinical presentations and outcomes. Many ICC symptoms are shared with other common non-ICC cardiac conditions. Epidemiological estimates are likely to be conservative. One such example is HCM, where the phenotype of left ventricular hypertrophy (LVH), although sometimes accompanying unique echocardiographic features of the systolic anterior motion of the mitral valve for HCM, can be confounded by more common conditions such as hypertension or physiological LVH in athletes[13]. An additional issue is age-related penetrance or incomplete disease expression, which also hinders the diagnoses, leading to conservative estimates.

The high proportion of sudden death among the young linked to ICC[14,15] and the high prevalence of other ICCs suggests a significant burden to healthcare worldwide. To address this burden, accurate diagnosis is imperative, followed by early lifestyle and medical modifications and cascade gene testing. For example, following the diagnosis of long QT syndrome (LQTS), lifestyle changes, β-blocker therapy, left cardiac sympathetic denervation, and device therapy can be implemented early to reduce the risk of severe morbidities or sudden cardiac death (SCD)[16]. We have worked toward a systematic workflow to identify and manage ICC patients in our tertiary referral center. We follow the recommendations for a comprehensive ICC program highlighted in published genetic testing guidelines[6,7].

Overview of cases in the ICC clinic

The National University Heart Centre, Singapore (NUHCS) ICC research program was initiated in the early 2010s for patients with HCM, DCM (including arrhythmogenic right ventricular cardiomyopathy [ARVC, or now called ACM]), inherited arrhythmias and inherited aortopathies. Over time, these have been expanded to include systemic conditions with cardiac involvement, including familial hyperlipidemia and Anderson-Fabry disease. In this section, we offer a breakdown of the conditions, with relevant genetic and clinical details pertinent to the respective conditions. We only reviewed conditions that were frequently seen in our adult ICC program. For the section on inherited aortopathies, we included Marfan syndrome (MFS) because that is the most common patient we see routinely. Other aortopathies, such as Ehlers-Danlos syndrome and Loeys-Dietz syndrome, are rarely seen in our ICC program as of the writing of this manuscript; our pediatric colleagues manage them.

Inherited cardiomyopathy

Inherited cardiomyopathies form the most significant proportion of cases among our patients, with cases being classified as HCM, DCM, ARVC, and left ventricular non-compaction (LVNC).

HCM

HCM is characterized by LVH unexplained by secondary causes such as hypertension, aortic stenosis, or physiological enlargement, usually with preserved or increased ejection fraction. It is estimated to be present in 1 of 500 individuals[17]. The phenotypic presentation of HCM is heterogenous in progression and demographics, especially age, leading to conservative epidemiological statistics[18]. While most HCM cases follow a relatively benign course, HCM remains a significant cause of SCD, especially among younger patients[14,19].

Pathogenic variants in genes encoding sarcomeric proteins, including *MYBPC3, MYH7, TNNT2, TNNI3, TPM1, ACTC1, MYL3*, and *MYL2* contribute most (up to 50%) of disease-causing variants, and these genes are commonly on HCM-specific gene panels[20,21]. Disease-causing variants contribute to varying degrees to the phenotypic presentation. For example, disease-causing *TNNT2* variants are associated with poor prognosis and a high risk of SCD, while disease-causing *MYBPC3* variants are associated with a delayed onset of disease and more favorable outcomes[22,23]. Risk stratification based on the mutated genes has a limited impact on patient management[18].

Substantial attention has been paid to genotype-negative HCM cases (i.e., cases lacking disease-causing variants in known HCM genes), the absence of prior family history for HCM, and the possibility of *de novo* HCM (cases where the variant is present in the proband but not the parents), where the disease is potentially non-familial or non-Mendelian in nature[24]. This phenomenon impacts cascade testing. In some rare instances, genotype-negative HCM cases may be explained by storage disorders (phenocopies) such as *LAMP2*- or *PRKAG2*-associated multisystem glycogen-storage disease, where the clinical presentation may not differ substantially from HCM[25].

Research is underway to establish guidelines for genotype-negative HCM cases and provide management strategies and therapeutics for the various HCM subsets. Management includes symptomatic treatment and prevention of SCD, with strategies aimed at addressing complications of the disease, such as LV obstruction or heart failure, and prophylactic measures, such as implantable cardioverter defibrillator (ICD) implantation in high-risk individuals[26,27]. It is encouraging that the first cardiac myosin inhibitor (Mavacamten) was recently approved by the United States Food and Drug Administration to treat obstructive HCM[5]. This approval opens the doors for future drugs targeting the underlying pathophysiology of HCM and (broadly) ICC in the move toward precision cardiology.

DCM

DCM is characterized by left ventricular or biventricular dilation and impaired contraction unexplained by abnormal loading conditions (e.g., hypertension and valvular heart disease) or ischemic heart disease[28]. DCM is a common cause of heart failure and a common indication for cardiac transplantation[9]. There is an estimated prevalence of 40 cases per 100,000 individuals, with differences between demographic groups such as race and age of presentation[9,29]. Notably, DCM also accounts for 60% of cardiomyopathies in childhood[30,31]. The etiology of DCM varies, with contributions commonly from genetic/familial causes and non-genetic causes. The latter includes familial DCM resulting from sarcomeric gene variants, neuromuscular disorders (e.g., Duchenne's muscular dystrophy), inherited mitochondrial disorders, drugs (e.g., antineoplastic drugs), infections (e.g., infectious myocarditis) and others[9,32].

Familial DCM is associated with more than 50 genes, many of which encode for sarcomeric proteins and the well-known *LMNA* gene[33,34]. Up to 25% of familial DCM bear causal pathogenic variants in the *TTN* gene, whereas *LMNA* and *MYH7* contribute 6% and 4%, respectively[32]. Genetic testing is indicated to diagnose DCM, and the diagnostic yield for genotype-positive DCM can be as high as 25%[34,35]. Interestingly, studies reported an overlap of disease-causing variants in a significant proportion of DCM patients, with 38% of 639 DCM patients having compound or combined variants with HCM and channelopathy-causing variants[36]. This finding highlights the need for precise phenotyping and additional research to understand DCM's molecular interactions and genotype-phenotype correlations.

Genetic testing also plays some role in outcomes prediction, with some studies demonstrating a higher risk of ventricular arrhythmias and SCD in those harboring specific variants in *LMNA*, PLN, RBM20, and FLNC[37-42]. Another observational study reported that genotype-positive DCM patients had worse outcomes with increased rates of major adverse cardiovascular events and end-stage heart failure than genotype-negative patients[37]. Among genotype-positive DCM patients, the clinical course of DCM differs depending on the implicated gene[37]. Additional research is needed for validation and replication.

DCM management focuses on symptomatic treatment and preventing sudden death, including managing heart failure, its complications, and ICD implantation[27,28]. Currently, targeted therapeutics are being studied, with several novel therapeutic approaches, including new myosin activators[43] showing some promise. In addition to the standard therapies in the guidelines for DCM care, more are needed to advance DCM management[44].

ACM

ACM is a group of familial cell-to-cell junction cardiomyopathies underpinned by cardiac desmosome abnormalities, resulting in myocyte detachment and alteration of intracellular signal transduction. Pathological features include myocyte loss, and fibrofatty replacement of myocardium[45]. ACM predisposes patients to sustained ventricular arrhythmias, progressive ventricular dysfunction, and a high risk of SCD, especially among the young[12]. The literature estimates that the incidence of ACM is anywhere between 1/1000 and 1/5000 individuals. Guidelines suggest that the diagnosis of ACM requires a high degree of clinical suspicion with investigations such as imaging (transthoracic echocardiography or cardiac magnetic resonance imaging [cMRI]), tissue characterization (endomyocardial biopsy), repolarization and depolarization abnormalities (electrocardiography [ECG]), arrhythmia (Holter ECG monitoring), and family history[46].

Numerous pathogenic variants have been reported for ACM, with approximately half of ACM patients showing a pathogenic variant in one or more desmosome genes. Pathogenic *PKP2* variants are most

common, followed by pathogenic variants in *DSP, DSG2, DSC2*, and *JUP*[47,48]. Smaller subsets of ACM patients carry disease-causing variants in *SCN5A, LMNA*, and *TTN*, which are also implicated in other ICCs (i.e., DCM and Brugada syndrome [BrS]).

Genotype-negative ACM cases are similar regarding disease progression compared to genotype-positive ACM. However, different causal genes are associated with different, sometimes worse outcomes among genotype-positive ACM cases[49]. For example, pathogenic variants in *PLN* and *DSP* are more likely to be associated with heart failure[50]. In addition, a disease-causing desmosomal variant among patients with ACM often implies a worse arrhythmic course and a higher risk of SCD[38]. ACM management includes cascade testing for relatives and treatment for heart failure and arrhythmia[48].

LVNC

LVNC is a disorder of endomyocardial morphogenesis characterized by numerous and excessively prominent ventricular trabeculations and deep intertrabecular recesses; it was first described in 1984[51,52]. The diagnosis requires cMRI or echocardiography[53,54]. Its pathogenesis is not yet fully understood, explaining the lack of clarity of its genetic underpinnings. Nevertheless, because over 44% of pediatric LVNC cases are familial, it is hypothesized that gene variants disrupt the physiological compaction of the developing embryonic myocardium, resulting in LVNC[55,56].

A genomic study of LVNC implicated some genes coding for proteins of the sarcomere, Z-disk, and nuclear-envelope structures, including *ACTC1, MYH7, MYBPC3, TNNT2, TPM1, TTN, LDB3, LMNA, RBM20*, and *DTNA*[56-59]. Genotype-positive LVNC patients have poor outcomes, and closer follow-up is recommended for these patients[60,61].

There have been difficulties in establishing a consensus for the management of LVNC because of the lack of large clinical trials. Understandably, the standard of care for DCM has been extended to LVNC patients with reduced ejection fraction, with an increased focus on anticoagulation (to reduce the risk of thromboembolic stroke) and the primary prevention of SCD[62,63]. While the current management of LVNC is prophylactic and symptomatic, more is needed to improve the guidelines.

Transthyretin amyloid cardiomyopathy

Transthyretin is a ubiquitous transporter protein produced by the liver. It is a tetramer but can potentially dissociate into monomers and misfold into insoluble amyloid proteins. Transthyretin amyloid cardiomyopathy (ATTR-CM) is a progressive, life-threatening disease caused by an end-organ accumulation of these misfolded transthyretin amyloid proteins, with the nerves and heart being exceptionally susceptible[64]. The disease results in a slowly progressive peripheral sensorimotor or autonomic neuropathy with progressive cardiomyopathy[65]. ATTR-CM may be associated with aging ("wild-type", wtATTR-CM, i.e., the absence of any pathogenic variants in the *TTR* gene) or the *TTR* variant, resulting in an inherent instability of the transthyretin protein (variant ATTR-CM). More than 110 *TTR* gene variants have been classified as pathogenic for genotype-positive ATTR-CM[66].

While not a new disease entity, ATTR-CM has received more attention in the medical community in the past decade because of the field's rapid development. There are non-invasive diagnostics using nuclear scintigraphy that supplant the previous invasive endomyocardial biopsy[65]. More importantly, disease-modifying treatment is now available in the TTR tetramer stabilizer Tafamidis, significantly improving outcomes in patients with ATTR-CM[67].

Several registries focused on studying ATTR-CM provided many insights into this condition[68,69]. It is now widely understood that the underlying *TTR* variant significantly affects the clinical presentation of patients with TTR amyloidosis[66]. Variants such as NM_000371.4:c.88T>C (p.Cys30Arg) and NM_000371.4:c.349G>T (p.Ala117Ser) are associated with neurological features. Others, such as NM_000371.4:c.424G>A (p.Val142Ile), are associated with cardiac features. For the commonly encountered NM_000371.4:c.148G>A (p.Val50Met) variant, early-onset disease is associated with prominent neurological dysfunction with favorable outcomes following isolated liver transplantation, while late-onset NM_000371.4:c.148G>A (p.Val50Met) is associated with mixed neurological and cardiac dysfunction[66].

With such distinct genotype-phenotype correlations for vATTR disease presentation, accurate genotyping will enable more effective clinical management. The genotype also affects treatment decisions, especially regarding selection for liver transplantation, which is reserved mainly for early-onset NM_000371.4:c.148G>A (p.Val50Met), given the favorable outcomes of this genotype following liver transplantation[68].

Inherited channelopathies

Inherited channelopathies comprise the next most significant disease caseload in the ICC clinic. These may be spontaneously manifest or only revealed on provocation testing and systematic workup. The latter include sudden cardiac arrest (SCA) survivors with normal coronary arteries and structurally normal hearts, for whom identification of an underlying channelopathy has therapeutic and familial implications. This section covers the common inherited channelopathies: LQTS, BrS, and catecholaminergic polymorphic ventricular tachycardia (CPVT).

BrS

BrS is characterized by a coved-type ST segment elevation in the right precordial leads of an ECG and increased risk of SCD in the absence of structural abnormalities; it was first recognized in 1992 by the Brugada brothers[70]. The characteristic ECG patterns can present spontaneously or are unmasked upon provocative by sodium channel blockers such as ajmaline, procainamide, or flecainide[71]. BrS can be potentially life-threatening, with patients presenting with syncope, seizures, and nocturnal agonal breathing due to polymorphic ventricular tachycardia or ventricular fibrillation (VF). SCD may result from sustained polymorphic ventricular tachycardia or VF[71,72]. Because BrS is associated with a familial carriage, researchers have studied its genetic basis. The first genetic evidence for this condition reported in 1998 pointed to the *SCN5A* gene[73].

Various sodium, calcium, and potassium channel genes have been implicated in BrS pathophysiology. These include *SCN4A, SCN10A, KCNH2, CACNA1C*, and *CACNA2D1*[74]. However, only the *SCN5A* gene is a "definite" gene, according to an expert panel from the ClinGen consortium[75]. BrS is a monogenic Mendelian disease with an autosomal dominant inheritance pattern[74,76,77]. However, affected families frequently show incomplete penetrance, with up to 60% presenting with no family history[78]. *SCN5A* pathogenic variants contributed to increased severity, allowing for genetic-based risk stratification[79]. Importantly, a genome-wide association study suggested that BrS was associated with common variants, contributing to another hypothesis that BrS is a complex polygenic disease[80].

Nevertheless, genetic testing offers genetic confirmation and risk stratification[74,81]. Indeed, clinical guidelines recommend that high-risk patients receive an ICD to prevent SCD[82]. However, this decision requires careful consideration and counseling, owing to many long-term psychological sequelae aggravated by inappropriate shocks from the ICD[83]. Despite recent advances in clinical diagnoses and genetic testing,

risk stratification and management remain challenging in clinical practice, not least because of the lack of clarity between the monogenic or polygenic disease causality, the frequently incomplete penetrance, and the lack of other applicable non-genetic-based stratification criteria[74,84].

LQTS

LQTS is a life-threatening inherited arrhythmia characterized by a prolonged QT interval and the onset of syncope or cardiac arrest, sometimes precipitated by emotional or physical stress[85]. LQTS can be lethal, with untreated symptomatic patients having a high mortality rate of 21% a year following syncope[86]. Mortality can be reduced to ~1% with proper and timely intervention involving a formal diagnosis, early detection, and management[16] (3, 4). Genetic testing is indicated when the clinical suspicion of LQTS is high, and the "Schwartz score" is greater than or equal to 3.5 or QTc>499 msec across multiple ECGs[87].

With advances in clinical genomics, robust phenotype-genotype correlations have been established for LQTS, making genomic analysis for this syndrome relatively more straightforward[85]. LQTS is divided into 16 different subtypes, with each subtype corresponding to a specific gene involvement[88]. By far, the first three subtypes (LQT1, LQT2, and LQT3) contribute most, corresponding to the genes *KCNQ1, KCNH2*, and *SCN5A*, respectively[89]. Among these, there is some evidence for genetic risk stratification. For example, a variant in *KCNQ1*, A341V, results in 80% of individuals being symptomatic, with more than 30% experiencing SCA or SCD[90]. By contrast, pathogenic variants associated with a lower frequency of SCA or death are those with LQT2 (*KCNH2*) and LQT3 (*SCN5A*) disease-causing variants, with a comparatively lower risk for life-threatening arrhythmias during exercise[85].

After the diagnosis of LQTS is established, the cornerstone for management for symptomatic patients lies with beta-blocker therapy and lifestyle modification[85]. Different or adjunctive therapeutic options can also be considered for various genetic subtypes, including sodium channel blockers for LQT3 or Andersen-Tawil syndrome (LQT7) patients[91] or calcium channel blockers for Timothy syndrome (LQT8). Other essential aspects are treating patients using left cardiac sympathetic denervation and ICD for cardiac arrest survivors[16]. While the genotype-phenotype relationship of LQTS has been extensively elucidated, understanding its complex genetic architecture might yield new management approaches.

CPVT

CPVT is a rare arrhythmogenic disorder characterized by adrenergic-induced bidirectional and polymorphic VT. The polymorphic VT tends to be reproduced during exercise, intense periods of emotions, or isoproterenol infusion and can cause syncope and SCD at a young age in the absence of structural cardiac conditions[92]. The mortality of untreated CPVT is estimated at > 31% when the patient is 30 years old, and the eight-year cardiac event rate of patients not under beta-blocker therapy is 58%[93]. CPVT is a significant cause of sudden death in the young[94]. Patients with CPVT are often missed or misdiagnosed due to the lack of obvious clinical signs or structural cardiac abnormalities, highlighting the critical role of pre-emptive genetic testing[95].

Genotype-phenotype correlations are well established for CPVT, with most carrying pathogenic *RYR2* (autosomal dominant) or *CASQ2* (autosomal recessive) variants. Other disease-causing variants in *TRDN* and *TECRL* have been less often implicated[92,95]. Hayashi *et al.* showed that cardiac and lethal event rates were similar between 50 probands and 51 affected family members[96]. This finding provides strong evidence for the importance of cascade testing in newly-diagnosed CPVT probands because affected relatives are at similarly high risk of adverse cardiac events[96].

Inherited aortopathies

MFS

MFS is an autosomal dominant multisystem connective tissue disorder associated with pathogenic variants in *FBN1*[97]. It is characterized by aortic root aneurysm, aortic dissection, ectopia lentis (ocular lens dislocation), and skeletal abnormalities usually involving disproportionate long bone overgrowth[98,99]. The clinical diagnosis of MFS is made using the revised Ghent Nosology, which can diagnose or exclude Marfan syndrome in 86% of cases[97,98]. With a positive diagnosis of MFS, early initiation of long-term beta-blocker therapy is essential, with angiotensin-converting enzyme receptor blockers[100]. Quinolone should be avoided because it increases the risk of aortic dissection, especially in individuals with MFS[101]. In addition to medical therapy, surgical repair is considered for significant aortic root dilation, mitral valve regurgitation, or other vascular abnormalities[100].

Up to 90% of MFS is caused by pathogenic variants in the *FBN1* gene[102]. FBN1 is a large structural macromolecule found in the extracellular matrix, essential to the integrity and function of connective tissues, especially in arteries, the perichondrium, and in components of the eye. While identifying *FBN1* variants is not essential for the diagnosis, it is helpful to differentiate MFS from other inheritable syndromes with similar clinical presentations[99,103]. For example, Loeys-Dietz syndrome and familial thoracic aortic aneurysms and dissections have a similar clinical presentation of skeletal abnormalities and are genetically associated with pathogenic variants in receptor genes *TGFBR1* and *TGFBR2* which have also been seen in MFS[104]. *FBN1* variants can result in conditions other than MFS, including Weill-Marchesani syndrome, familial thoracic aortic aneurysms/dissections, acromicric dysplasia, and geleophysic dysplasia, making the clinical evaluation of the patient critical[105]. Special care must be taken when investigating individuals presenting with *form fruste* MFS, with atypical symptoms, or an incomplete presentation of typical MFS symptoms[106]. Genetic testing is warranted in individuals suspected to have MFS, especially when family history is unknown or ambiguous.

CLINICAL FRAMEWORK FOR PATIENTS WITH ICCS

The NUHCS ICC program was launched in 2012. This project was initiated as a research program and developed into a clinical workflow for diagnosing and managing individuals with ICC. Figure 1 summarizes the workflow for ICC evaluation.

Patients were identified and invited into the study if they presented symptomatically at a healthcare institute, if they were found to have phenotypes suggestive of ICC during general screening, or if they were identified as part of a cascade testing workflow. These individuals were referred to the NUHCS ICC clinic for further phenotyping and genotyping.

The phenotypic assessment was performed by clinicians using clinical examination, relevant blood tests, electrocardiography (ECG), and cardiac imaging. Cardiac imaging included echocardiography, cMRI, or computed tomography (CT) with contrast, depending on the assessed case. Following the investigations, the clinicians offered the initial explanation of the ICC to the patient and referring cardiologist/physician, with the strength of diagnosis graded according to international guidelines. Careful and accurate phenotyping is essential for solid genotype-phenotype assessments, which are also required for the genetic variant curation process.

Exome sequencing (WES) is performed for cases in the ICC clinic using next-generation sequencing, with a FASTQ file generated from the sequencing procedure. Variant calling from the FASTQ file is performed using the GATK pipeline (a bioinformatics tool that aligns the sequencing reads), presenting the overall

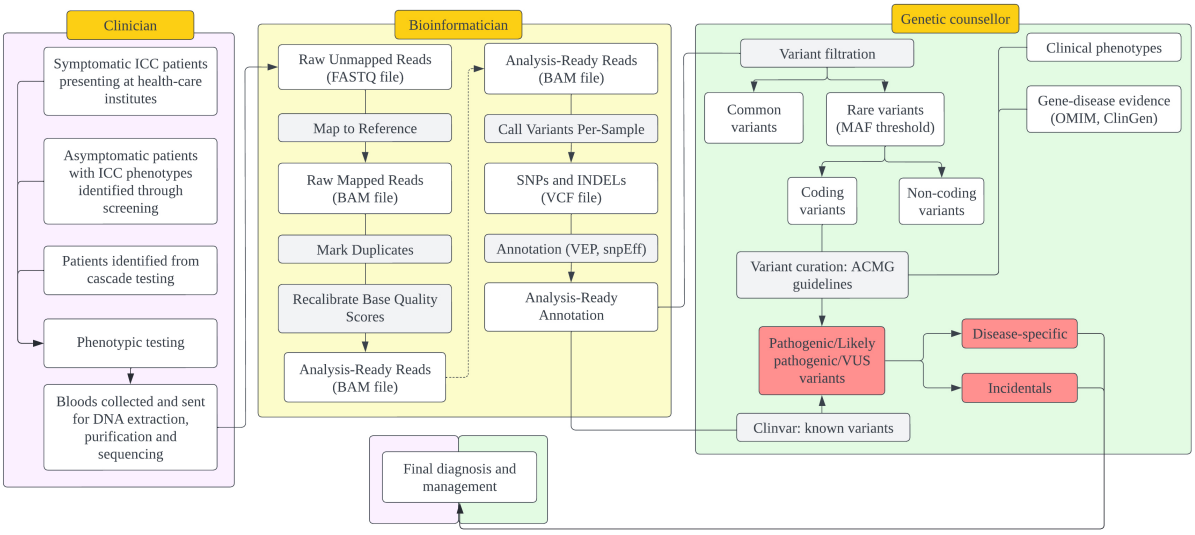

Figure 1. An overview of the process of variant-calling and gene variant curation. DNA: Deoxyribonucleic acid; SNP: single nucleotide pleomorphism; BAM: binary alignment map; INDELs: insertions and deletions; VCF: variant call format; VEP: variant effect predictor; MAF: minimum allele frequency; ACMG: American college of medical geneticists; OMIM: online mendelian inheritance in man.

results in a variant-called format (VCF)[107]. The VCF file contains genomic variants, categorized broadly into single nucleotide polymorphisms, insertions, and deletions. The VCF file is annotated using Variant Effect Predictor software and SnpEff[108,109]. Annotated VCF files contain details such as the variant type, *in silico* predictions, allele frequencies, and database (e.g., ClinVar) annotations, among others. Annotated files were then used for variant curation.

Variant curation

Variant curation is carried out by a genetic counselor trained to assess the pathogenicity of variants based on the American College of Medical Geneticists (ACMG) guidelines and, after that, counsel ICC probands and their families. As the analysis of ICC focuses on rare to ultra-rare variants with large effect sizes, the first step involves filtering variants based on allele frequency (AF) thresholds to compile for these rare coding variants. The AF thresholds for the different ICC are determined based on a model involving factors such as penetrance and population control allele frequencies[110]. AF information is compiled from international and population-specific databases of AFs with a Singapore population AF database - the SG10K_pilot database[111]. This procedure is performed so that only variants with an AF lower than maximal credible AF to suspect pathogenicity are included in the subsequent analysis because a significant pathogenicity criterion is the rarity of the variant in healthy population databases[112]. As soon as the AF threshold is implemented, the number of variants for analysis is substantially reduced.

Specific gene panels are built around published literature to associate with the patient's presenting symptoms or suggested diagnosis. Gene panels are obtained from resources such as ClinGen and PanelApp, where experts in each field have curated disease-specific gene panels[75,113]. Genes with moderate to definite evidence of pathogenicity are included in our assessment panel, including other genes identified through our own experience [Table 1][114]. Established patterns of inheritance are also derived from international databases, consolidating available research to arrive at an expert consensus.

The filtered variants are next curated based on other published ACMG guidelines[112]. Factors consider population data, allelic data, *in silico* analysis, functional data, segregation data (if available), and *de novo*

Table 1. ICC genes are routinely used in our assessment panel consisting of genes identified through our own experience and those in Illumina Trusight panel[114]

No. of genes	Genes
176	ACTA2, ACTC1, APOB, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, KCNH2, KCNQ1, LDLR, LMNA, MYBPC3, MYH11, MYH7, MYL2, MYL3, PCSK9, PKP2, PRKAG2, RYR1, RYR2, SCN5A, SMAD3, SMAD4, TGFBR1, TGFBR2, TMEM43, TNNI3, TNNT2, TPM1, ABCC9, ABCG5, ABCG8, ACTA1, ACTN2, AKAP9, ALMS1, ANK2, ANKRD1, APOA4, APOA5, APOC2, APOE, BAG3, BRAF, CACNA1C, CACNA2D1, CACNB2, CALM1, CALM2, CALM3, CALR3, CASQ2, CAV3, CAVIN4, CBL, CBS, CETP, COL5A1, COL5A2, COX15, CREB3L3, CRELD1, CRYAB, CSRP3, CTF1, DES, DMD, DNAJC19, DOLK, DPP6, DTNA, EFEMP2, ELN, EMD, EYA4, FBN2, FHL1, FHL2, FKRP, FKTN, FXN, GAA, GATAD1, GCKR, GJA5, GPD1L, GPIHBP1, HADHA, HCN4, HFE, HRAS, HSPB8, ILK, JAG1, JPH2, JUP, KCNA5, KCND3, KCNE1, KCNE2, KCNE3, KCNJ2, KCNJ5, KCNJ8, KLF10, KRAS, LAMA2, LAMA4, LAMP2, LDB3, LDLRAP1, LMF1, LPL, LTBP2, MAP2K1, MAP2K2, MIB1, MYH6, MYLK, MYLK2, MYO6, MYOZ2, MYPN, NEXN, NKX2-5, NODAL, NOTCH1, NPPA, NRAS, PDLIM3, PLN, PRDM16, PRKAR1A, PTPN11, RAF1, RANGRF, RBM20, SALL4, SCN1B, SCN2B, SCN3B, SCN4B, SCO2, SDHA, SELENON, SGCB, SGCD, SGCG, SHOC2, SLC25A4, SLC2A10, SNTA1, SOS1, SREBF2, TAZ, TBX20, TBX3, TBX5, TCAP, TGFB2, TGFB3, TMPO, TNNC1, TRDN, TRIM63, TRPM4, TTN, TTR, TXNRD2, VCL, ZBTB17, ZHX3, ZIC3

data (if available), among others. *In silico* tools used to categorize and predict implications of the variant include Polyphen2[115], SIFT[116], LRT[117], MutationTaster[118], Fathmm[119], phyloP[120], CADD[121], and DANN[122]. These provide functional predictions for the variants, such as whether the amino acid substitution affects protein structure and function. Functional data include previous *in vivo* or *in vitro* studies published on specific variants and genes. To achieve high confidence, the studies must model the natural disease state as closely as possible. Family history of similar phenotypes is considered in segregation analysis, which also affects the variant classification based on the condition's inheritance pattern. Of note, the segregation of a particular variant with a phenotype in a family is evidence for the linkage of the variant to the disorder but not any indication of the level of pathogenicity of the variant. Conversely, the absence of segregation provides evidence of the benign nature of the variant unless incomplete penetrance is considered. *De novo* variants are considered strong support for pathogenicity if the parents of the patients are sufficiently evaluated clinically and genetically.

All variants are cross-referenced with other curations recorded in the literature, including disease-causing variants flagged in ClinVar[123]. The variants are then analyzed in the context of the patient's clinical history to identify (1) the disease-specific variant(s); and (2) incidental findings. To accomplish this, variants are classified according to ACMG classifications: benign, likely benign, VUS, likely pathogenic, and pathogenic.

Genome, exome, and panel sequencing

The choice of genome sequencing (WGS), exome sequencing (WES), or panel sequencing in assessing ICC considers factors such as the evidence surrounding gene-disease implications, cost-effectiveness, and depth of coverage. With recent technological advances, the cost of WGS and WES has decreased significantly without sacrificing the depth of sequencing coverage[124]. WES/WGS allows for cost-efficient diagnosis for ICC, especially for those with more than one gene implicated. WES/WGS allows for subsequent re-analysis, when additional genes can be interrogated given new research evidence or progression in the patient's clinical condition, compared to the limited nature of gene panels[125].

Additionally, WGS/WES assesses incidental genomic findings, such as for other non-ICC conditions. Comparing WGS and WES, WGS allows for assessing the exonic and intronic genome, as opposed to WES, which covers only exonic regions. Given ever-decreasing costs, WGS may become the preferred mode of sequencing[126]. Panel sequencing may be preferred when investigating ICC with a few genes implicated, such as CPVT or LQTS, as it is targeted and results are easily interpretable. However, it lacks the benefits of WGS/WES[127].

Cascade testing

Once a disease-causing variant is identified in a proband, steps are taken for their family members to undergo similar genetic testing and phenotypic evaluation, with appropriate consent, because the patient's immediate family members are at increased risk of harboring the same disease-causing variant[6]. From a macro perspective, cascade testing can save a healthcare system, owing to early detection and management of high-risk individuals. This sequence significantly decreases morbidity and mortality and reduces the resources required to manage these patients[128]. One signature example is familial hypercholesterolemia, where cascade testing is a proven, cost-effective means for disease early detection[129].

Cascade testing begins with the immediate family members, followed by the second-, then third-degree relatives, until all at-risk family members are identified and tested to the extent possible. The clinician and geneticist work with the proband to this end. This procedure establishes a well-annotated pedigree tree and improves the confidence of ICC diagnosis[112]. While cascade testing is critical upon discovering a disease-causing variant, there remain significant barriers and challenges to its practice. These challenges include ensuring the confidentiality of the index case (proband), the non-acceptance of such testing among family members, cultural and ethical considerations, insurance coverage, and resource considerations in these families[130]. Appropriate measures must be taken to explain the purpose and obtain informed consent before approaching family members for genetic testing[131].

Incidental secondary genetic findings

While analyzing genomic results, disease-causing variants for unrelated medical conditions may be identified incidentally. These are more likely to occur with WES/WGS sequencing. The variants may not relate to the original phenotype with which the patient presented. Relevant consent and genetic counseling must be clearly and expressly carried out before genetic testing in anticipation of these outcomes. In various cases, these incidental findings would belong under predictive diagnostic testing. Care must be applied to ensure adherence to country-specific regulations for genetic testing[132].

The ACMG lays out clear guidelines for returning incidental medically-actionable disease-causing variants in 73 genes[133]. These guidelines focus on highly penetrant genetic disorders using established management guidelines to prevent or significantly reduce mortality and morbidity. Examples of the ACMG medically-actionable incidental findings gene list include *BRACA1* and *BRACA2* for hereditary breast cancer, *ATP7B* for Wilson's disease, and *NF2* for neurofibromatosis type II[133]. Following the discovery of incidental pathogenic variants in the genes highlighted by the ACMG committee, the multidisciplinary team initiated phenotypic testing, cascade testing, and guideline-based interventions, depending on appropriate consent. Other pathogenic variants on genes not included in the ACMG list are under constant evaluation, and additional studies are required to identify the relevant incidental findings to be reported.

Patient management

After confirming a diagnosis of ICC, the long-term management of the patient and any affected family member takes the form of a multidisciplinary approach. This management begins with cardiologists and genetic counselors directing the patients' evidence-based management, social workers assisting with long-term socioeconomic issues, and many allied health professionals. Genotype-positive and phenotype-negative family (pre-clinical) members are under increased surveillance to monitor phenotype progression[134].

Phenotype-positive, genotype-negative individuals are managed based on their clinical presentation and the latest guidelines. For specific ICCs, prognostic implications are offered. For example, better long-term outcomes are reported in genotype-negative DCM patients[37]. These are also used for decision-making, such

as determining the length of time before a subsequent follow-up visit. Notably, VUS in this group of patients is subject to later reinterpretation, when the variant may become reclassified as pathogenic/likely pathogenic or benign/likely benign when new evidence appears. New research may also find that other genes are implicated in the disease condition, prompting a re-evaluation of the patient's genotype status[6]. The latter highlights our preference for WES/WGS over panel sequencing[124].

ESSENTIAL CONSIDERATIONS FOR AN ICC CLINIC

Resource considerations in the establishment of a cardiovascular genetics program

Significant resources are required to establish a cardiovascular genetics program. In addition to the recruitment and training of a multidisciplinary team to fill the roles of cardiologists, genetic counselors, bioinformaticians, and nurse coordinators, among others, there are pertinent requirements that must be addressed for the long-term success of an ICC program. Some examples include sufficient computational power to analyze genomic information, ample database space to store genomic datasets, and a clinical and scientific framework that begins from patient recruitment and continues to cascade testing and case management. An alternative option is to outsource the process of DNA sequencing, variant-calling, annotation, and curation to an external commercial entity.

Training and education are critical for an ICC program. These include multidisciplinary meetings to ensure that team members are up-to-date with the latest guidelines and cases, journal clubs to facilitate knowledge expansion, and educational materials. Many cardiologists receive variable and often insufficient training in ICC and genomic medicine. There is also a shortage of genetic specialists, especially in less-developed jurisdictions, complicating the training in this area (8). On a positive note, the increased interest in ICCs among cardiovascular clinicians might lead to an increase in clinicians familiar with ICC diagnosis and management[135,136].

Informed consent

Bioethical considerations are essential in the practice of genomic medicine. Valid consent for genetic testing must be obtained from the patient/parent or guardian for underaged patients. They must be adequately informed regarding the details, risks, benefits, and alternatives. Consent must be voluntary and obtained by someone competent, such as a physician with relevant clinical genomics knowledge or a trained genetic counselor[131,137]. This may present challenges in less-developed countries with limited access to resources such as healthcare and education. The basis of genomics may confuse the layperson due to its highly scientific nature[138]. In addition to traditional beliefs and cost considerations, such knowledge gaps are barriers to collecting informed consent and patient's willingness to undergo such tests[131]. The healthcare professional must be able to communicate the various implications of undergoing a genetic test, including its indications, risks, benefits, and alternatives, to ensure patient autonomy, reflecting the informed consent procedure[138]. Efforts such as providing comprehensive educational materials explaining the process help bridge the knowledge gap for patients and their families. In addition, a healthcare provider must be aware of hurdles involving the genetic testing of minors[131,138]. The provider should seek the informed consent of the minor patient's parents (or guardian) and engage the patient in decision-making at a developmentally appropriate level, keeping with ethics guidance[131,139].

Provision of genomic services in Singapore

To ensure a responsible and comprehensive provision of clinical genetic testing, the Singapore Ministry of Health (MOH) has released a code of practice on the standards for the provision of clinical genetic and clinical laboratory genetic services. This code outlines the requirements for healthcare providers before offering genetic testing services, considering factors such as the competency levels of practitioners, types of genetic services, and clinical indications of such tests. It provides guidelines to ensure that a fully-trained

Table 2. Summary of the MOH - LIA moratorium protections on genetic testing and insurance. Reproduced from the MOH website: https://www.moh.gov.sg/resources-statistics/moratorium-on-genetic-testing-and-insurance, accessed 21st October 2022

1. Insurers are not allowed to:

a. Ask the applicant to take a genetic test (whether diagnostic or predictive) as part of their insurance application
b. Ask the applicant to disclose and use the result of any predictive genetic test for assessing/deciding the outcome of their insurance application if the test was taken for biomedical research
c. Ask applicant or medical providers to disclose, and use the result of any predictive genetic test for assessing/deciding the outcome of their insurance application if the insurance or test is any one of these:
i. Health insurance, including Integrated Shield plans
ii. General insurance
iii. Group insurance
iv. Any other insurance not covered by the Moratorium
v. Direct-to-consumer genetic testing
vi. Testing is done on another person (e.g., a blood relative)
vii. Testing is taken after the insurance coverage had started (unless the applicant agreed to take the test before the coverage started).

2. Insurers are allowed to:

a. Ask the applicant to disclose, and use the result of a predictive genetic test for assessing/deciding the outcome of his/her insurance application if all of the following conditions are met:
i. The insurance applied for is one of the following: life, total permanent disability, long-term care, critical illness, and disability income insurance;
ii. The sum assured/pay-out of insurance applied for exceeds the financial limits specified in the moratorium;
iii. The applicant has taken a predictive genetic test from the list of approved predictive genetic tests specified in the moratorium for medical conditions such as Huntington's disease and breast cancer
b. Ask the applicant to disclose, and use the result of any diagnostic genetic test done for clinical care for assessing/deciding the outcome of his/her insurance application
c. Use the result of any predictive genetic test (whether provided by the applicant or another person, voluntarily or accidentally, or otherwise) if the result is favorable to the applicant.

provider orders genetic tests under appropriate clinical indications. These are essential because genomic testing elements such as consent taking, variant curation, and genetic counseling are complex processes and carry many implications for the patients and their families[140]. The MOH Code of Practice safeguards the interests of the healthcare provider and the patient.

Regulation of the access to genomic data

Access to genomic data and patient health data should be governed to ensure that the information is not abused. Such abuse could come in the form of unauthorized sharing of genomic information, using samples collected for purposes other than for the consented reason, or the unauthorized commercial selling of information, among others[141]. Secure databases, information security training, and regulatory bodies ensure that all data collected during these tests are managed responsibly and with sufficient accountability[142]. These factors ensure the privacy of the data and enhance public trust in the medical institutes providing genomic services. These steps go far toward assuring public receptibility to genomic testing.

Insurance (moratorium)

Genetic information can be used for many purposes, including disease prediction, disease management, or life-changing decisions. This information could also be used for insurance and employment. A key issue from the public perspective is the insurability of patients undergoing genomic testing, whether for research or medical indications. It is in the interest of insurance agencies to make use of genomic test results to assess the probability of individuals developing both rare and complex genetic diseases. This might impact the individual's ability to buy insurance and procure insurance claims due to genetic discrimination by insurance companies. To protect their citizens, many countries have introduced legislation and agreements to minimize the risk of discrimination[143].

In Singapore, the MOH and the Life Insurance Association (LIA) have developed the "Moratorium on Genetic Testing and Insurance" to support the development of precision medicine. The LIA is a not-for-profit trade organization representing life insurance products and life reinsurance providers based in

Singapore. The moratorium aims to deter individuals from undergoing clinical genetic tests for any medical indications or participating in precision medicine research due to concerns about insurability. Under this moratorium, insurance companies in Singapore are not allowed to use predictive genetic test results to assess or decide the outcome of insurance applications under various circumstances[144]. A summary of the moratorium is provided in Table 2.

Case studies of ICC patients from our clinic

In this section, we discuss three case examples of ICC. We present a case of cardiomyopathy, LQTS, and Marfan syndrome. These exemplify the real-life application of the workflow described in the above sections.

Case 1: A patient with DCM

The proband was a 24-year-old female at the time of presentation at the ICC clinic after a referral by her primary cardiologist given a family history of SCD. She has three brothers, one of whom passed away from SCD at age 22 shortly before the visit. Thus, she had a strong family history of DCM, with her father and deceased brother having the condition, both diagnosed through echocardiography measurements. Her family history was highly suggestive of ICC, so she was referred to the ICC clinic. [proband's father's echo (Supplementary Material 1)]

At the time of presentation, the proband was asymptomatic. Her transthoracic echocardiogram revealed a mildly dilated left ventricle with an ejection fraction of 52% on transthoracic echocardiography [proband's echo (Supplementary Material 2), Figure 2A]. Due to the familial history and clinical suspicions of DCM, the proband underwent WES.

A rare variant was found on the *TNNC1* gene upon genetic testing and variant curation. This gene *TNNC1* is commonly implicated in DCM and HCM in an autosomal dominant inheritance pattern. The variant, in this case, was: *NM_003280.3*:c.452A>T (p.Asp151Val)[145]. This variant was classed as likely pathogenic by our variant curation [ACMG guidelines: PM1, PM2, PP1, PP3 (Moderate)][112]. The base position is strongly conserved, and the base change is not present in gnomAD or local databases (SG10K_pilot database) with adequate sequence coverage[111]. Pathogenic variants in the *TNNC1* gene have been linked to DCM and HCM according to various databases such as OMIM, and it was labeled as "definite" according to curation by the ClinGen consortium[75]. The *TNNC1* domain has 34 missense/in-frame variants (five pathogenic and 29 VUS), and the pathogenicity of the missense variant = 14.7%. In addition, clear segregation was established according to cascade testing results of the patient's first-degree family members. *In silico* prediction tools, SIFT and DAMN, also offered pathogenic predictions on the variant. The variant was further confirmed through gold-standard Sanger sequencing. Eventually, cascade testing was initiated to examine other family members of the proband. The proband's father, who bore the same variant in *TNNC1*, showed clinical features of DCM: dilated ventricles with a low ejection fraction of 35% [Figure 2A] and multiple hospital admissions for acute decompensated heart failure. We did not initiate testing on her deceased brother. Cascade testing for the patient's two other brothers and mother revealed the absence of the *TNNC1* variant [Figure 2B]. Clinically, they did not present with signs or symptoms of DCM or other significant cardiovascular conditions. Serial transthoracic echocardiograms of the proband's two living brothers were normal. We took this information to represent genotype-phenotype segregation, strengthening the status of the variant as the disease-causing "pathogenic" variant.

The proband's subsequent transthoracic echocardiogram three years after her initial presentation showed a worsening left ventricular ejection fraction of 37%. She was started on guideline-directed heart failure medications and remained asymptomatic in her follow-up visits with her primary cardiologist.

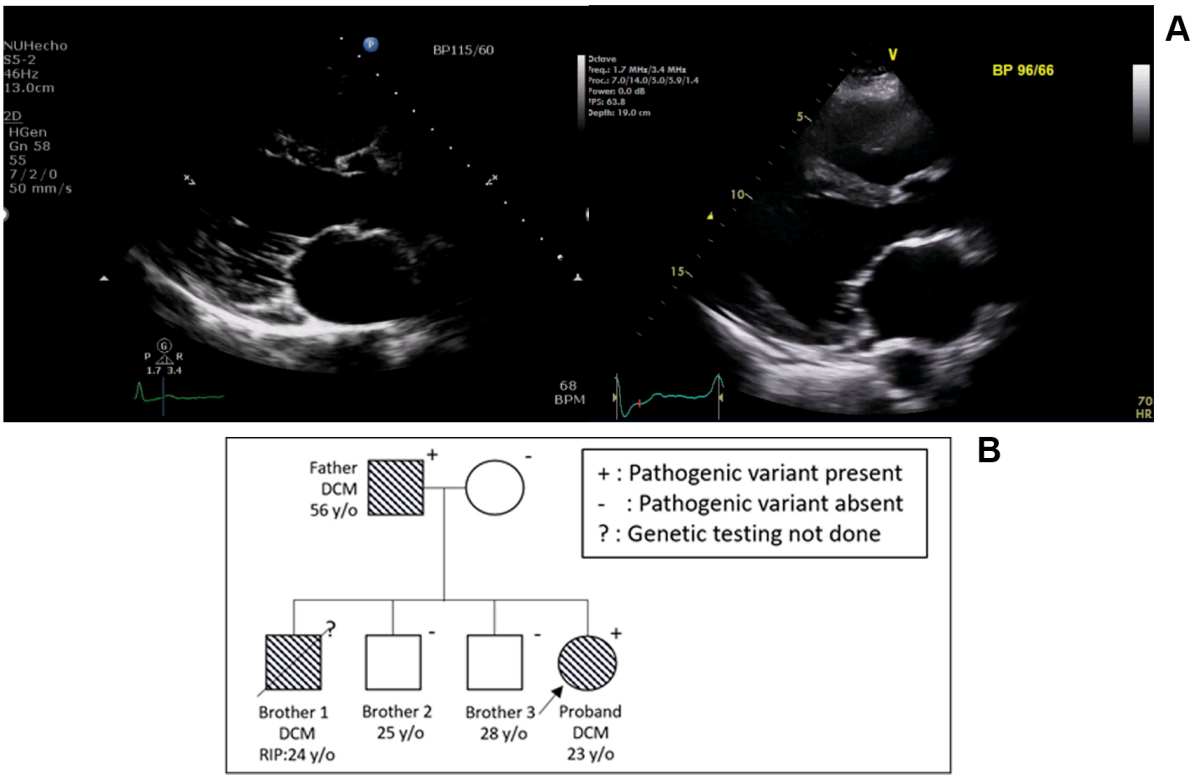

Figure 2. Echocardiographic images and a pedigree tree relevant to this proband. (A) Parasternal long axis view of a transthoracic echocardiogram. The left image belongs to the proband, and the right image belongs to the proband's father. (B) Pedigree chart of the immediate family members. Shaded figures indicate a phenotype suggestive of ICC.

This case brings us through the entire framework of the ICC clinic, focusing on the importance of cascade testing and providing an example of how a pathogenic variant may result in heterogeneous phenotypic presentation.

Case 2: A patient with suspected LQTS

The proband was a 26-year-old female referred to the ICC clinic by her primary cardiologist given multiple episodes of arrhythmias. There was a history of two episodes of out-of-hospital VF collapse two years earlier, diagnosed as an LQTS type 2, given long QT patterns noted on the ECG [Figure 3]. Emotional outbursts seemingly triggered the two episodes. The patient was eventually fitted with a subcutaneous ICD as prophylaxis for future occurrences of VF. The patient's DNA sample was sent for WES.

Upon genetic testing and variant curation, no pathogenic variants were found on the genes *KCNQ1*, *KCNH2*, or *SCN5A* commonly implicated in LQTS. However, a likely pathogenic variant (ACMG criteria: PM1, PM2, PP2, PP3, PP5) was noted: *NM_001035.3:c.14695G>A (p.Asp4899Asn)*. Based available from: https://www.ncbi.nlm.nih.gov/clinvar/RCV000182849.2/ 32681117 on LQTS testing guidelines, this patient was counseled on lifestyle modifications and started on anti-arrhythmic medications[16]. However, the patient presents with genotype-negative LQTS, with only one ECG showing a prolonged QT segment (other ECGs showed normal QT segments, QTc < 480 ms) and a phenotype of exertional collapse/VF; we wondered if CPVT was a differential diagnosis[146]. Pathogenic variants in *RYR2* are strongly associated with CPVT, in an autosomal dominant inheritance pattern, and are less frequently associated with LQTS[146,147]. Other studies reported cases where CPVT was misdiagnosed as LQTS, with one study reporting that nearly

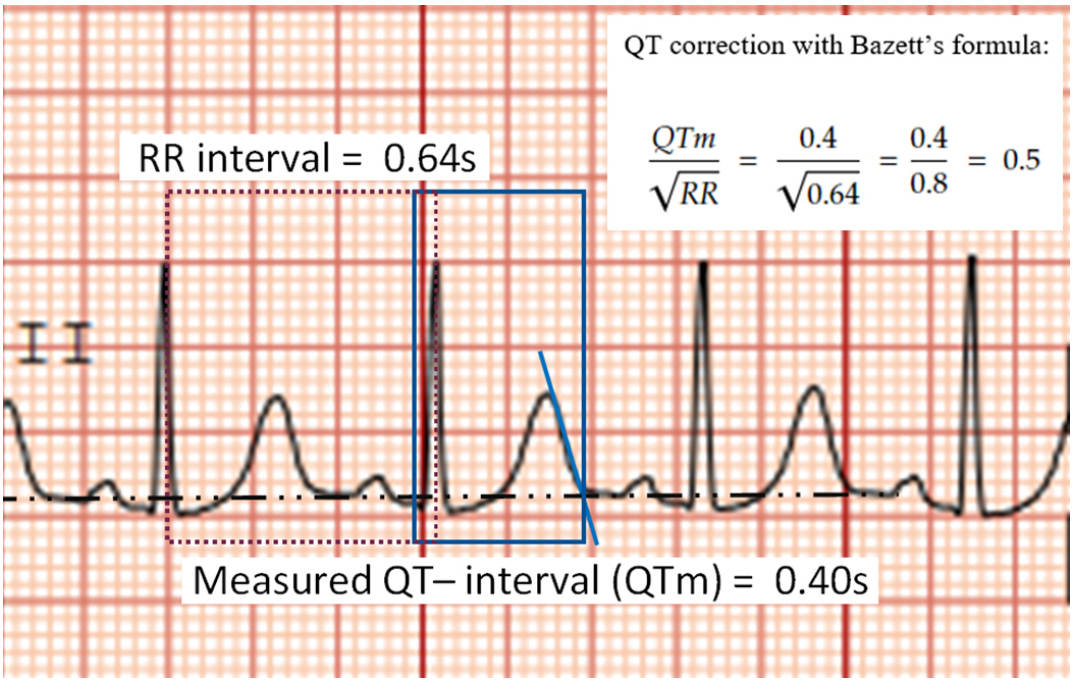

Figure 3. Lead II ECG of the proband. The calculated QTc value for this patient is 0.50 s based on this ECG (normal = 0.36-0.46 s in females).

30% of CPVT are misdiagnosed as concealed LQTS[148-150]. The patient's primary cardiologist was advised that a stress ECG would be required to elucidate her precise diagnosis. The proband has three older sisters and one younger sister, who are asymptomatic and have no known cardiovascular conditions. Cascade testing has been initiated for the pathogenic *RYR2* variant.

This case illustrates the value of genetic testing in diagnosing inherited arrhythmias, such as BrS, CPVT, and LQTS. The test offered a means to differentiate between two conditions (LQTS and CPVT), providing the chance for a more confident diagnosis and evidence-based management for the patient.

Case 3: A patient with Marfan syndrome

This proband was a 57-year-old female patient at the time of presentation to the ICC clinic. She was referred because of her significant family history of MFS. The proband's sister was diagnosed with MFS earlier that year, and her mother was deceased due to complications likely arising from MFS. The patient was sent for a two-dimensional echocardiogram, which revealed a dilated left ventricle with multiple regional wall motion abnormalities and moderate-to-severe aortic regurgitation. CT aortography was performed to assess her aortic root, given the high possibility that the patient has MFS. The CT aortogram showed no dissection. The aortic root z-score was calculated > 3. A diagnosis of MFS was made based on the 2010 Revised Ghent Nosology, with a positive z-score and a strong family history[98]. Genetic testing was initiated to identify the genetic basis of the patient's condition.

Upon variant curation, a heterozygous pathogenic variant was identified on the *FBN1* gene: NM_000138.5:c.4567C>T (p. Arg1523Ter). This variant has been previously annotated as pathogenic in multiple ClinVar submissions and in publications on MFS[151-154]. Variant curation also confirmed the absence of pathogenic variants in other genes, *FBN2*, *TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*, *TGFB3*, *COL3A1*, or *COL3A2*. A diagnosis of MFS was made with high confidence based on the pathogenic *FBN1* variant and clinical features.

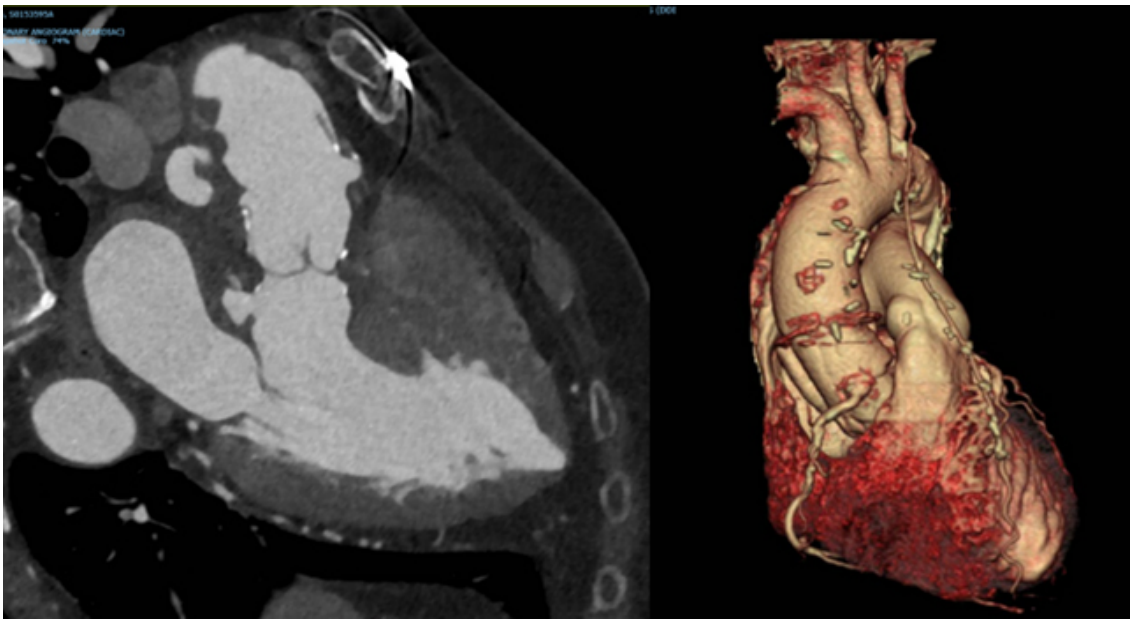

Figure 4. Radiographic images taken of the proband's heart. Images were taken five years after the patient underwent a modified Bentall procedure. (left) MRI image of the patient's heart post-modified Bentall procedure. (right) Three-dimensional reconstruction of the heart, using cardiac CT scans.

Given the diagnosis of MFS and a dilated aortic root, a modified Bentall procedure was carried out to definitively correct the aortic root dilation, and beta-blocker therapy was initiated. Cascade testing was irrelevant because there were no known extended family members, and both parents of the patient were deceased at the time of diagnosis. Five years after the bioprosthetic conduit insertion, a subaortic false aneurysm was detected on cardiac MRI during follow-up, and the patient underwent a definitive patch repair of the aneurysm [Figure 4]. Since her diagnosis and the initiation of appropriate management plans, the patient has been managing well (ten years since diagnosis).

This case displayed the relevance of a genetic test even when clinical features and family history were clear.

CONCLUSION

The clinical framework and considerations described in this review offer clinicians and researchers an overview of what goes on in an ICC clinic and the variety of cases seen, ranging from cardiomyopathies and arrhythmias to aortopathies. While significant issues must be overcome before such a service can be implemented in healthcare centers, it is nevertheless necessary, owing to the evident burdens and needs in the community. We discussed the challenges and benefits of this service for patients and clinicians.

DECLARATIONS

Authors' contributions

Contributed to the conception and design of the study: Loong SSE, Gan LH, Klinzing DC, Tomar S, Foo RSY

Contributed to the writing of the manuscript as well as administrative, technical, and material support: Loong SSE, Gan LH, Lim YC, Ng MMQ, Wang Y, Koo SH, Chew NWS, Yeo C, Tan VH, Leong KMW, Wong RCC, Lin W, Kuntjoro I, Klinzing DC, Tomar S, Foo RSY

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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Marketing and publicity strategies for launching the pilot phase of the Hong Kong Genome Project

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Abstract

Aim: Public trust and confidence determine the acceptance of any population-based genome project. The Hong Kong Genome Institute (HKGI) was established in May 2020 by the Food and Health Bureau (Currently the Health Bureau) to spearhead the integration of genomic medicine into mainstream healthcare. One of HKGI's goals is to enhance public genomic literacy and engagement by launching the Hong Kong Genome Project (HKGP).

Methods: Three focus groups (undiagnosed and rare disease patients and their families, hereditary cancer patients and their families, and clinical geneticists and other medical subspecialists) involving 20 patients, family members, and healthcare professionals were completed in mid-2021 by an independent party. The aim was to harness insights into stakeholders' views, concerns, and aspirations on issues related to genomic studies and the HKGP: (1) the decision to undergo genetic testing; (2) concerns; (3) campaign format; and (4) other strategic suggestions for the Pilot Phase. These issues are complex and multifactorial and have not been documented in Chinese populations. The qualitative approach facilitates such exploration.

Results: Four themes emerged from the thematic analysis: (1) decisional considerations of undertaking genetic testing: perceived benefits and motivators; (2) concerns and worries: personal, familial, and societal concerns; (3) a quest for a patient-oriented, transparent, and decommercialized whole-genome sequencing campaign; and (4) communicating genomics efficaciously: the importance of informational support and literacy enhancement.

Conclusion: Our results shaped the strategies for publicizing the Pilot Phase of HKGP and laid a patient-oriented foundation for HKGP's Main Phase.

Keywords: Hong Kong Genome Project, genomic medicine, whole-genome sequencing, public awareness, genomic literacy

INTRODUCTION

Public distrust as a common obstacle to genomics advancement

With the advancement of genomic technologies and discoveries, scientists and researchers can perform accurate variant interpretation to solve clinical mysteries and inform medical diagnoses and treatment in actionable and precise ways. These interpretations can only be realized if DNA and supplementary medical data are provided for substantial numbers of patients and the public for research and clinical initiatives. The support and trust of patients and the public in collecting and using genomic data are central to the successful implementation and sustainability of any large-scale genomic research project[1]. Despite the contributions of genomic testing to precision medicine, public distrust remains a significant obstacle for health policymakers globally[2-4].

With reference to the collaborative efforts in global genome project initiatives, the substantial volume of data from China contributes to understanding human genomes and genomic diversity. The future of precision genomic medicine in the Chinese population relies on the ability of researchers and clinicians to access substantial quantities of genomic and health data. The Hong Kong Genome Institute (HKGI) was established in May 2020 by the Food and Health Bureau (currently the Health Bureau) to spearhead the integration of genomic medicine into mainstream healthcare. As mentioned in our previous publication, the Government of the Hong Kong Special Administrative Region established the Steering Committee on Genomic Medicine in 2017 to formulate strategies for the development of genomic medicine in Hong Kong; one of its objectives is to enhance public engagement in genomic medicine by launching the Hong Kong Genome Project (HKGP)[5].

The HKGP is the first large-scale genome sequencing project in Hong Kong that aims to (i) utilize whole-genome sequencing (WGS) to identify disease-causing mutation(s) in patients with undiagnosed disorders and cancers with a possible hereditary component; and (ii) enhance the application of genomic medicine to benefit patients and their families with precise diagnosis and personalized medicine. The goal is to facilitate public awareness and understanding, the foundation of building public trust in genomic medicine. HKGP spearheads the first "See the Unseen" campaign involving the efforts of genomic-science professionals in HKGI and other medical fields in Hong Kong.

HKGP - a participant-oriented project for the Hong Kong population

Since its incorporation in May 2020, the HKGI has been recruiting talent in the field, establishing the necessary hardware and software for implementing the HKGP. It is governed by six committees, overseen by the Board of Directors. Among the six committees, the Communications and Education Committee (CEC) is critical for (1) harnessing advice on the strategy and value proposition of HKGP in publicity and education matters; and (2) reviewing and overseeing HKGI's branding, communications, publicity activities, and critical messages delivered to the public.

A strategic focus of the three-year Strategic Plan (2022-2025) of HKGI spelled out the specific strategies for improving awareness and knowledge of genomic medicine in the general population[6]. The primary directive is to engage potential patients, targeted stakeholders, and the public to enhance their

understanding of genomic medicine by developing authoritative and user-friendly information and publications. It also sought to formulate strategic engagement plans for targeted stakeholders, including academic sectors, patient groups, and professional bodies, to promote awareness of genomic medicine and its benefits.

Since its formulation in 2021, the Clinical Operations unit of the Scientific Team and the Corporate Communications Unit of HKGI have been working tirelessly with the CEC to ensure that the Pilot Phase of HKGP synchronizes with the will of the potential participants. The way this large-scale genomic project is presented to the public is critical as the preliminary step in enhancing public genomic awareness. One important preparatory task was to review the focus group results with the HKGI team to map out the marketing and launching strategies for the first public HKGP campaign, "See the Unseen". These issues are complex and multifactorial and have not been documented in Chinese populations. Therefore, this qualitative study was designed to collate insights by exploring the themes integrating participants' views, concerns, and aspirations on issues related to genomic studies and the HKGP.

METHODS

To harness unbiased information directly from stakeholders, an independent agency specializing in social service marketing research was engaged to conduct three focus group sessions involving (1) undiagnosed and rare disease patients and their family members; (2) hereditary cancer patients and their family members; and (3) clinical geneticists and other medical subspecialists. Patients and family members of undiagnosed and rare diseases and hereditary cancers, and healthcare professionals experienced in working with these patients were eligible to participate in the focus group meetings. All eligible participants were prospectively recruited at the three Partnering Centers of HKGI, Hong Kong Children's Hospital, the Prince of Wales Hospital, and the Queen Mary Hospital. All eligible participants were informed of the study's objectives and data confidentiality standards and were invited to participate in the focus group interviews conducted by the independent agency. The Pilot and Main Phases of the HKGP and all related studies in the HKGP were covered by ethics approvals granted by the Central Institutional Review Board, the University of Hong Kong/Hospital Authority Hong Kong West Cluster, the Joint Chinese University of Hong Kong/New Territories East Cluster, and the Department of Health (HKGP-2021-001, HKGP-2022-001, UW 21-413, 2021.423, LM 257/2021). All focus group participants provided written informed consent to participate and be audio-recorded for publication.

An experienced facilitator conducted all focus groups from an independent agency that was not involved in the planning and execution of the HKGP. Each focus group consisted of a two-hour session, including a five-minute break. The discussion was conversational, guided by a semi-structured Focus Group Discussion Guide with a theme list. A set of carefully determined questions were included in the Focus Group Discussion Guide to assist the facilitator in leading the discussion, which included nine sections: introduction, participant's diagnostic odyssey, understanding of and perspectives toward WGS, HKGI, and HKGP, participant's views toward the Project's campaign, marketing and launching strategies for the HKGP, campaign ambassadors, promotional materials and sources, and public engagement. PowerPoint prompts facilitated focus group discussion to inform the participants on the purpose of the discussion, the HKGP, and the project campaign. Participation was anonymized to preserve confidentiality.

Data capture, coding process, and thematic analysis of qualitative data

The focus groups were conducted in Cantonese Chinese, audio-recorded after obtaining written informed consent, and transcribed verbatim by a technical assistant at the HKGI. Another research assistant reviewed and validated the audio recordings and transcripts independently. Data collected from the focus group

meetings were analyzed following the principles of qualitative analysis by Clarke *et al.* and were in alignment with the six phases of thematic analysis by Nowell *et al.*[7,8].

Initially, the familiarization step was achieved by reviewing the transcripts to form the initial coding framework, which was structured according to the Semi-Guided Question List. Coding was guided by inductive reasoning. Additional themes and subcodes were identified and added to the initial coding framework. Inductive reasoning relies on assessing the allocation of coded words, phrases, and paragraphs to the top-level codes and subcodes. The data within each theme were then continuously analyzed to identify variations and stop until thematic patterns saturated. ATWC and CCYC performed coding. Discrepancies were discussed between ATWC, CCYC, and BHYC, and disagreements were resolved by consensus.

RESULTS

Twenty participants were recruited to participate in the three focus group meetings, including eight undiagnosed and rare disease patients or their family members (40%), five hereditary cancer patients or their family members (25%), and seven healthcare professionals (35%). A range of rare and undiagnosed diseases and hereditary cancers were covered, including Costello syndrome, Alstrom syndrome, Angelman syndrome, Rett syndrome, undiagnosed disease, hereditary breast and ovarian cancer, and rare brain cancer. Gender and age were reasonably well balanced among the groups, with eight male (40%) and 12 female (60%) participants. Among the 15 participants who reported age at recruitment, the mean age was 41.2 years, ranging from 18 to 56.

Four major themes were derived from thematic analysis: "decisional considerations of undertaking genetic testing: perceived benefits and motivators", "concerns and worries: personal, familial, and societal concerns", "a quest for a patient-oriented, transparent, and decommercialized WGS campaign", and "communicating genomics efficaciously: the importance of informational support and literacy enhancement". Selected quotes based on these four themes are summarized in Supplementary Table 1.

Decisional considerations of undertaking genetic testing: perceived benefits and motivators

Integration of genomics into medicine raises ethical and psychosocial challenges. These include public acceptance and decisional considerations. Several qualitative studies from Western countries have shed light on the perspectives and attitudes of parents and patients with rare diseases toward genomic sequencing, highlighting common motivational factors; these include the desire for a diagnosis and potential treatment implications[9-12]. Resembling their Caucasian counterparts, the possibility of genetic testing in confirming personal diagnoses and informing clinical management is also a significant motivator introduced by Hong Kong Chinese patients and families with undiagnosed and rare diseases and hereditary cancer.

Focus group participants (patients and their family members) felt that their decision to undertake genetic testing was affected mainly by "*whether the genetic diagnosis would impact clinical management*" (son of hereditary cancer patient P11). According to their personal and family experiences in going through genetic counseling and testing process, such attempts made them feel relieved after receiving the genetic diagnosis, as it has allowed them to "*explore about the disease prognosis and plan for the next steps in life*" (father of rare disease patient P2).

Long diagnostic journeys often plague patients with rare diseases and hereditary cancers[13]. WGS is often offered as a last resort to obtain a diagnosis; this process has substantial potential to impact clinical management, although benefits are often challenging to achieve in the short term. Nevertheless, patients

and families are willing to participate in genomic research for "the greater good" despite a lack of immediate direct personal benefit; this sentiment was a common theme from the present thematic analysis.

"*Perhaps there is not much help for my son, but I believe eventually one day, it will benefit many children, I mean our next, next, next generations*". (mother of rare disease patient P5)

"*I think since my kid is affected already, why don't we contribute to the society or the world? I believe it* (WGS testing) *has its purpose. Well, let me tell you frankly, at this moment, even if a pharmaceutical company could identify a drug for my daughter, it must go through phase 1, phase 2, phase 3 trials, you can foresee that. My daughter is a grown-up now, the reason for us to advocate that much is obviously not simply for my daughter. I hope that there is something we can do to contribute to the world*". (father of rare disease patient P3)

"*Well, if I contribute my DNA for* (WGS) *sequence, or my body tissues* (samples)*, I hope that I will be able to help other people in the future, and I believe all patients are willing to do so*". (hereditary cancer patient P10)

Echoing the "greater good" narrative, the emphasis on participants' contributions to research in the local setting supports previous research evidence in this area, highlighting participants' altruistic motivations[9,11,12,14]. Our focus group participants emphasized the importance of establishing a local clinical genomic database for personalized medicine and disease risk prevention. By establishing a flexible platform with a rich database for genomic technologies and multi-omics studies, and promoting disease-focused research networks in local and international settings, genomic sequencing is anticipated to facilitate genomic science and discoveries.

"*Well, if you ask me, we should have done this much earlier… as WGS helps society a lot, this is a very effective tool… currently if you send the patient to a hospital to look for the underlying reason, many of the times the doctors will not be able to give you an answer, because they have no tools. But if you set up this genomic database, the doctors will have the platform, then at least he will be able to investigate what is going on with the patient. This also saves a lot of societal resources*". (father of rare disease patient P3)

"*I think that it is always good to have one more set of data, if everyone refuses to join, if you are not willing to accept it, then we will never be able to contribute to scientific advancement*". (patient with undiagnosed disease P1)

"*I support my mother to undergo testing as it didn't only give her an answer* (why cancer was running in her family)*, but it could also help the Chinese population and the world to understand this syndrome better*". (son of a female patient with hereditary breast and ovarian cancer syndrome P11)

On the other hand, local healthcare professionals in Hong Kong underscored the clinical benefits of genomic sequencing for patients, especially the importance of a genetic diagnosis for ending the long diagnostic journey. Above all, clinical geneticists accentuated the importance of big data through genome projects to "discover treasure" for medical development in the long run, reducing uncertainties during the diagnostic process.

"*Our initial intention as doctors is to diagnose.* (…) *At least most of the patients and families feel relieved when there is a genetic diagnosis* (…) *I guess most of them would like to know what condition they are suffering from because some of them lived with it for over 30 years without knowing what it is*". (clinical geneticist P18)

"*Even when there is no cure, it is important to have a diagnosis.* (…) *in many of our adult patients, the diagnosis actually helped to avoid unnecessary investigations and follow-ups, which is beneficial, I believe, to both the general public and health system in terms of saving resources*". (medical specialist P16)

Concerns and worries: personal, familial, and societal concerns

Despite having positive attitudes toward genomic sequencing, participants also expressed ambivalence about genome projects and the translation of genomics into routine clinical practice. Resembling studies conducted in the United Kingdom (UK), the United States, and Canada, patients and families in Hong Kong are concerned about potential psychological distress in themselves and their family members[9,10,12,14].

"*If I went to test when I was 18 and found out that I've got a disease that will present when I am in my 40s, what am I going to do in the upcoming 30 years?*" (son of hereditary cancer patient P11)

"*To be honest, I think it doesn't really matter if it is just for myself because you agreed and gave consent, and you wanted to know the answer. But at the end, my family did the test too. So what did the test bring them into? Well… my mum was very worried. As in, she might have been okay initially, but what if you found something wrong? What should we do? No one could answer us this question*". (undiagnosed disease patient P8)

"*Let's say if I did WGS at 20 years old and found out that I have many problems, so in my future life, I may need to make decisions such as whether to get married and have kids. It feels like I am carrying bombs around. How can I get through it? This is something I am very worried about*". (hereditary cancer patient P10)

Concerns and skepticism regarding data privacy and data-sharing related to genomic research and genome projects were introduced by focus group participants[2,9,10,12,14-16].

"*How would I know who is in charge of this genomic research project? And I would think whether that person would collect our genetic data for personal use or to conduct another research study? Like, I don't know. Is there a way I will be notified when someone accesses my data? Such as having an alert on my phone?*" (mother of rare disease patient P6)

"*When I first heard about this* (project)*, that we are building a genomic database, a genomic database for the Hong Kong population, my first thought was about data privacy issues*". (hereditary cancer patient P10)

All the clinicians interviewed emphasized the importance of making the process as "transparent" and "fully informed" as possible to tackle concerns regarding data privacy and enhance public trust. Importantly, clinical geneticists shared their experiences and thoughts on highlighting patients' rights in the informed consent process, especially regarding data confidentiality and project withdrawal.

"*You have to be transparent; otherwise, people will not understand what you are trying to do in your project. If you hide the data, and if the participants are not able to find answers from the project's website, then people will start to make guesses*". (clinical geneticist P15)

"*I think the biggest concern among patients and families is how are they protected. That's their genomic data; of course, they would like to know how they are protected. So if that paragraph* (paragraph on patients' rights in the information booklet) *includes more information in this area, with the details clearly written, then they will have less things to be worried about*". (clinical geneticist P14)

A quest for a patient-oriented, transparent, and decommercialized WGS campaign

A previous meta-analysis indicated that broad audiences observe and emulate celebrities' actions and decisions. Their endorsements can remarkably impact knowledge, attitudes, and behaviors and affect the community sentiments on health-related projects and services[17]. Celebrity endorsers include "digital influencers" who can achieve recognition through various communication channels[18]. In the social media era, social media influencers can broadcast images and messages that are accessed and reposted instantly.

In Hong Kong and Asian cultures, it is not uncommon to identify celebrities - often actors or actresses to act as "ambassadors" for government-spearheaded health initiatives or health-related campaigns. It is beyond the scope of this study to examine the complicated and interrelated economic, marketing, neuroscience, psychological, and sociological mechanisms of celebrity impact. However, deciding to use celebrity ambassadors and how to engage them has become a valid decisional consideration for many health-related campaigns. In the initial planning stage for the launching strategies for HKGP, there was a series of discussions within the CEC on adopting celebrity ambassadors in promotion strategies.

Concerning marketing strategies, a common theme shared by patients and family members of hereditary cancers and rare and undiagnosed diseases was that a health project like HKGP is legitimate and intends to benefit the community; it should speak for itself without the need to have excessive and dramatic marketing strategies. A common theme opposing using celebrities as ambassadors of the HKGP was unequivocal: Focus group participants preferred spokespeople who had shared the journey to speak on their behalf.

"*In this generation, do we still need celebrities, so-called celebrities, to be ambassadors? Doctors can be* (ambassadors). *And would it be possible to find someone, who are not necessarily famous, but someone who carries a meaning, a meaning that will stand out? Because I think this idea* (celebrities) *really does not work* (in promoting HKGP), *frankly speaking, I think it is a waste of money*". (hereditary cancer patient P10)

"*That is to say, I think there is no benefit to use an artist for this campaign. Things will look fake and commercialized. (…) Because artists give people an impression of commercialization, and when this becomes commercial… since this* (HKGP) *has an ambitious mission, as soon as you use an artist to speak about it, you just have a feeling of "wow, how much do you charge for this advertisement?*" (hereditary cancer patient P10)

"*We must find someone who is relevant* (to be the ambassador). *That is, he/she must have experienced it. Then, as an audience, when I see someone who has experienced this to talk about this matter, even if I don't know this person, I will trust him/her because of his/her experience*". (hereditary cancer patient P10)

"*In fact, I think if you need to find one* (ambassador) *if it is necessary… like if you want to target a certain issue, I think you need to find someone who is relevant. Because if the host or somebody* (i.e., patients and caregivers) *questions you* (at a seminar/talk), *and if you can't answer the question, it becomes rather embarrassing. As in, if you don't even know about it* (genetic diseases), *how do you understand me? Like, sorry, first of all, if I sit here to listen, I think I will be more willing to listen to you if you understand what I am going through*". (undiagnosed disease patient P8)

"*In fact, I think we should just be straightforward and find a patient* (to be the ambassador). *I would know that it was a genetic disease at a glance. It was simple, straightforward, and had an even more profound impression*". (father of rare disease patient P3)

"*I would think it would be much powerful if you use a real case*". (father of hereditary cancer patient P9)

"*This* (campaign) *needs to be very serious, so I guess public promotion cannot be too entertaining. That is, it is not a plan to entertain the public; it's just that you want to call for an iconic person* (…) *so that people will know about this. But this person must be politically neutral*". (clinical geneticist P14)

Communicating genomics efficaciously: the importance of informational support and literacy enhancement

In an ideal world, governments would embark on extensive "genomic literacy" campaigns, insisting that genetic information and related technologies be introduced in the formal curriculum as early as possible. In reality, promoting the subject is usually left to scientists.

It is expected that in science (like genomic medicine), the speed of innovation and service dramatically outstrips public awareness and capacity. Specific challenges in genomic education include tailoring complex topics to diverse audiences ranging from the public and patients of different ages to highly educated professionals.

All focus group participants (from clinical geneticists to patients and their family members) emphasized one crucial point that aligns with an imperative the Steering Committee highlighted. To successfully integrate genomic medicine into mainstream health services in Hong Kong, the genomic literacy of the general public and the medical field has to be substantially enhanced.

Hereditary cancer patients, rare and undiagnosed disease patients, family members, clinical geneticists, and medical subspecialists in Hong Kong agreed that genomic medicine is challenging for non-specialists to understand. A common theme identified from these focus groups is that most participants agree that the Hong Kong public is not well-equipped to understand the complexity of genomic science. They highlighted the importance of providing solid informational support by designing simple presentations of clinical information and genomic materials for various age groups.

"*Well, I think the main reason why it* (the diagnosis) *was delayed, or* "*wrong*"*, was due to the lack of genomic education. Be it to the public or to us doctors, if we are more educated* (in genomic medicine), *then this will be improved*". (clinical geneticist P18)

"*Even if you ask people who are educated, there aren't many of them who can tell you what is* "*Human Genome Project*". (clinical geneticist P15)

"*Honestly speaking, whether it is in Hong Kong or in Mainland China, majority of the doctors have never seen and have never heard of it* (the specific hereditary disease). *Well, he/she can't help, and you can't blame him/her*". (rare disease patient P3)

"*I need to use a medical dictionary to identify the jargons. I need to spend a lot of time to read and to understand one journal* (one paper) *because I have to simultaneously read and look up for the definitions from the dictionary…*" (rare disease patient P5)

DISCUSSION

After months of collaborative and tireless efforts, the launch of the HKGP Pilot Phase adopted the findings of the present study, focusing on the entire participant's journey with an emphasis on (1) transparent and

secured data collection procedures; (2) ethical and patient-oriented informed consent; (3) decommercialized promotional methods; (4) stress-free withdrawal initiated by participants anytime; and (5) provision of accurate and colloquial genomic information.

The obstacles of any population-based health project's marketing and publicity campaigns consist of the uncertainties and contributions of sudden and outsider effects, such as drastic changes in the public atmosphere and sentiment due to unprecedented social events. Fortunately, the HKGP Pilot Phase was launched when Hong Kong was in a phase of political and societal calmness. Although it occurred during the COVID-19 pandemic, the community had been immersed in personal health and well-being priorities. As stated in our previous publication, a dedicated project website with user-friendly information, videos, and publications on genomic medicine (as participants' information and welcoming package) was developed to complement and promote the launch of HKGP and to attract the public's interest in genomics and enhance genomic literacy (Available from: https://hkgp.org/en/)[5,19]. Based on the findings from the current study, promotional materials for the "See the Unseen" campaign are patient-oriented, with the two promotional videos adopting the stories of two patients (one adult and one minor). The background of the HKGP is featured in educational videos by clinical geneticists and medical professionals. Cartoon videos ensure simple, colloquial, and fun explanations for children [Figure 1].

With the project focusing on the entire patient journey, along with a robust and ethical informed consent process, a highly secured and transparent data processing platform, and a colloquial and simple information package, the Pilot Phase of HKGP was implemented successfully. Clinician and patient feedback were positive, with a withdrawal rate of 0.08% in the first year (as of November 2022). All media coverage was, in general, positive and supportive.

The HKGP now provides a valuable opportunity to learn how patients and their families respond when offered WGS in a hybrid clinical and research context. Our local findings align with previous research findings, showing that decisional considerations to participate in large-scale genome study (e.g., the HKGP) is not based solely on a rational choice following a weighing the personal benefits and concerns but also on the complex interactions between personal, psychosocial, and economic considerations, and the institutional context where consent is sought. Transparency and openness are highly valued and recognized as crucial elements of the HKGP public engagement strategy to encourage involvement and recruitment.

The importance of developing long-term strategies for enhancing genomic literacy and raising public awareness for the general population is increasingly recognized by governmental, non-governmental, and international organizations. Compared to Western countries, Hong Kong delivers genomic education at a much later learning stage[20]. Focus group patients, family members, and healthcare professionals highlighted that the general population of Hong Kong is not well-equipped for the complexity of genomic science and that overall genomic literacy must be significantly enhanced. As such, HKGP must provide robust informational support by designing simple and colloquial presentations of clinical information and genomic materials for different age groups. More importantly, strategies to enhance genomic literacy (e.g., funding of educational institutions, incorporation of genomic topics into formal education, and establishment of training programs for healthcare professionals) should be implemented[21].

Recognizing the importance of enhancing genomic literacy, several national genome projects have included education as one of their significant objectives[21]. In particular, Canada, Finland, France, and the UK have begun integrating genomics into primary and secondary education by updating the education curricula, upgrading the textbook contents, offering online educational platforms, and providing teachers with

Figure 1. Selected information booklets and promotional videos for Hong Kong Genome Project. (A) Information booklets and leaflets; (B) Hong Kong Genome Project Details - Animated Stories; (C) "See the Unseen" campaign - The Unseen Stories by patients; (D) Educational videos by clinical geneticists and medical professionals.

specialized training[21]. Similarly, in Hong Kong, to promote public engagement and enhance genomic literacy, the CEC of the HKGI planned to conduct stakeholder-specific initiates following the Strategic Plan 2022-25 to reinforce awareness of genomic medicine, including (i) engagement with the general public by preparing thematic articles on genomic medicine media outlets and taking part in public and industry events to promote HKGI and HKGP; (ii) engagement with the media to cultivate support for HKGI; (iii) engagement with stakeholders to identify and connect with relevant patient groups and professional bodies, and collaborate with the Hong Kong Academy of Medicine to showcase HKGI's commitment in nurturing talents and fostering research in genomic medicine; and (iv) enhancement of online marketing by making

effective use of digital marketing and search engine optimization tools to promote the HKGI and genomic medicine. The allocation of resources toward genomic education and talent development in Hong Kong is a priority to facilitate the integration of genomic medicine into mainstream healthcare.

Most genome projects highlighted the importance of human capacity, i.e., aptitude, knowledge, perceptions, responsiveness, and commitment to genomic information and campaigns. For example, the UK (a leader in genomic medicine development) stated that none of its success could have been realized without the involvement and participation of the wider UK population. As they pursue their goal to be the most advanced genomic healthcare ecosystem in the world in the coming decade, the UK prioritizes public engagement and assurance that the patient's voice is embedded throughout decision-making, as stated in the Genome UK Implementation Plan 2021-2022[22]. Qatar Genome is also actively increasing its human capacity by initiating several education initiatives from early school to postgraduate levels[23]. Public engagement must be implemented in phases and multifactorial dimensions and platforms.

In conclusion, with the concerted efforts of all members of the HKGI and the support of various stakeholders, the HKGP Pilot Phase had a smooth and successful launch. It is a long road before genomic medicine can become commonplace in Hong Kong and Asia. Public engagement is an ongoing and dynamic process. With age-specific marketing and strategic promotional plans backed by multi-disciplinary health reforms and long-term public education campaigns (supported by tertiary education curriculum and genomic knowledge outcome studies, to name but a few), we hope that via the HKGP and the related initiatives that geared up by its momentum, genomic literacy in Hong Kong and other Chinese-speaking cultures can be significantly advanced in the coming decade. Our study findings shaped the publicizing strategies of the Pilot Phase of the HKGP and laid a patient-oriented foundation for its Main Phase.

DECLARATIONS

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Authors' contributions

Conceptualization; project administration: Chu ATW, Chung BHY
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Supervision: Lo SV, Chung BHY
Writing-review & editing: Chung CCY, Lo SV, Chung BHY

Availability of data and materials

Upon a reasonable request, Focus Group Discussion Guide, theme list, PowerPoint prompts, and focus group transcripts reported in this article, after de-identification, will be made available to investigators whose independent review committee has approved the proposed use of the data. Data will be available from the corresponding authors up to five years following publication.

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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
The Pilot and Main Phases of the HKGP and all related studies in the HKGP were covered by ethics approvals granted by the Central Institutional Review Board, the University of Hong Kong/Hospital Authority Hong Kong West Cluster, the Joint Chinese University of Hong Kong/New Territories East Cluster, and the Department of Health (HKGP-2021-001, HKGP-2022-001, UW 21-413, 2021.423, LM 257/2021). The study followed the principles set out in the Declaration of Helsinki. All participants were informed of the study's objectives and data confidentiality standards and provided written informed consent to participate in the focus group meetings.

Consent for publication
Written informed consent for publication was obtained from all participants.

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Review

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Genomics in leukaemia in clinical practice: past, present and the future

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Abstract

Acute myeloid leukaemia (AML) is a heterogeneous group of diseases with diverse genetic drivers. The conventional one-size-fits-all approach with chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT) has reached an impasse, and only about 40% of patients can achieve long-term survival. Disease heterogeneities have also hampered the development of effective therapy applicable to the multitude of AML subtypes. Recent advances in cancer genetics and genomics have shed light on the genetic underpinnings of AML and both inter-individual and intra-tumoral heterogeneities. These new pieces of knowledge have begun to impact the management and prognostication of AML. They also provide the foundation for personalized treatment for this group of diseases.

Keywords: Acute myeloid leukaemia, next-generation sequencing, measurable residual disease, personalized medicine

INTRODUCTION

Advances in genome sequencing technologies in the past two decades have resulted in an unprecedented increase in knowledge of cancer genetics and genomics. The information arising has shed important light on the genetic underpinnings of oncogenesis and the complexity of inter-individual and intra-tumoral

heterogeneity that tends to evolve during the course of cancer treatment[1]. This information has also formed the theoretical basis of personalized cancer treatment based on the unique mutation compositions of cancers at diagnosis and relapse.

Leukaemia has long been the foundational paradigm for new concepts in cancer biology and innovation in therapeutic targeting. Phenomenal observations of chromosomal translocations in chronic myeloid leukaemia (CML)[2] and acute promyelocytic leukaemia (APL)[3], and the subsequent identification of pathogenic fusion genes in these diseases, have led to the development of tyrosine kinase inhibitors and differentiation agents. Both of these therapies have transformed the outcomes of patients, who can now be cured based on chemotherapy-free regimens.

Acute myeloid leukaemia (AML) is a devastating disease worldwide, for which treatment outcomes are unsatisfactory overall. The considerable inter- and intra-tumoral heterogeneities in AML have hampered the development of effective treatments applicable to the multitude of AML subtypes. However, advances in cancer genetics and genomics in recent years have empowered clinicians and scientists with the ability to analyze leukaemia heterogeneities and clonal evolution at different treatment stages. This information has begun to influence the clinical management of AML, improving outcomes for some of these patients[4].

We review the history of genomic research in AML in the past and how this knowledge has led to improvements in the treatment outcomes of patients. We also discuss the future development of a personalized approach to AML management.

ACUTE MYELOID LEUKAEMIA IN THE CLINICS – CURRENT STATE OF AFFAIRS

AML is a group of heterogeneous diseases with diverse morphologies, immunophenotypes, cytogenetics, and genetics, sharing in common an abnormal increase in blasts in blood and bone marrow (BM). It occurs in 3-5 patients per 100,000 individuals every year, and its incidence has increased in the past few decades. It is a highly lethal disease and is the fifth deadliest cancer of all kinds, particularly in elderly patients who are unfit to receive standard treatment. About 50% of AML cases carry normal cytogenetics, and they show on average 2-4 recurrent mutations in different combinations, some of which are considered drivers and others passengers in leukemogenesis[5]. Some of these mutations are of prognostic significance. In particular, *NPM1* mutation and bZIP in-frame mutation of *CEBPa* are associated with a favorable response to conventional chemotherapy, whereas *FLT3*-ITD is associated with a less favorable response. About 10%-15% of AML cases carry translocation t(8;21) (*RUNX1::RUNX1T1*) or inversion of chromosome 16 (CBFβ::MYH11), both of which involve components of the core-binding factor (CBF), which is a heterodimeric transcription factor comprising the non-DNA-binding CBFβ chain and the DNA-binding CBFα chain RUNX1. Half of these patients carry KIT mutations, which confer an inferior prognosis in this AML subtype, which hitherto had a favorable response to conventional chemotherapy[6]. Another 10% of AML cases carry complex (≥ 3 karyotypic abnormalities) or monosomy karyotype (≥ 2 monosomies or 1 monosomy and one structural abnormality), and this subtype portends an extremely poor prognosis, particularly those carrying *TP53* mutations, which happen in half of the patients with this subtype[7]. The rest of AML cases are made up of diverse diseases with different karyotypic and genetic abnormalities [Figure 1].

Despite the heterogeneity, AML has been managed by a one-size-fits-all approach in the past 4 decades. In young and fit patients, intensive chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT) are the mainstays of treatment. However, this approach has reached an impasse, and only 40% of patients can survive long-term. A number of agents, when added to the chemotherapy regimen, were shown

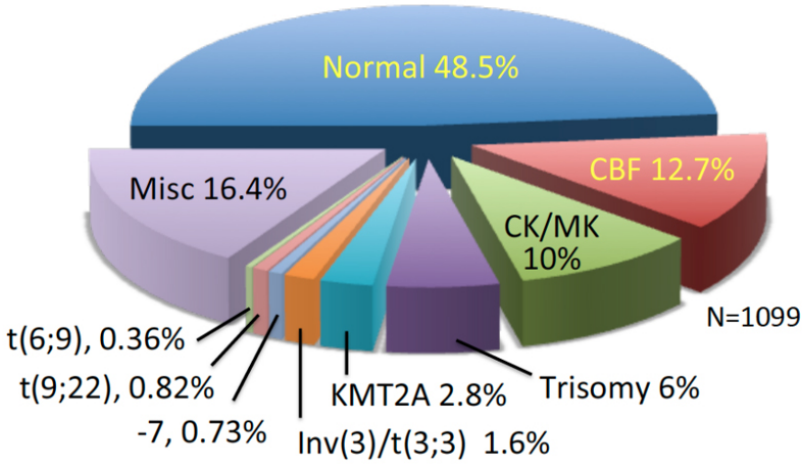

Figure 1. Cytogenetic landscape of acute myeloid leukaemia (AML) patients younger than 60 years old in Hong Kong. Adapted from Leung *et al.*[7]

to improve overall survival based on randomized control trials or meta-analyses, including *FLT3* inhibitor midostaurin[8] and monoclonal antibody against CD33[9], and were approved by the US FDA for such indications. Old and frail patients who are ineligible for standard treatment are primarily treated by low-intensity treatment of palliative intent, including hypomethylating agents or low-dose cytarabine. More recently, the addition of BCL2 inhibitor venetoclax to these agents was shown to improve overall survival, with 30% of these patients able to live beyond 30[10].

AML GENOMICS - A HISTORICAL PERSPECTIVE

Modern genomics in leukaemia research owes much to the phenomenal observation of recurrent chromosomal translocations in patients with AML[11], APL[3], and CML[2] by Dr. Janet Rowley and others about 50 years ago, leading to the identification of oncogenic fusion genes, i.e., *RUNX1-RUNX1T1*, *PML-RAR*, and *BCR-ABL1*, respectively. These discoveries have been instrumental in our understanding of the molecular mechanisms of leukaemogenesis and formed the foundation for the subsequent development of targeted therapies, including all-trans retinoic acid and tyrosine kinase inhibitors for APL and CML, both of which have transformed the outcome of these patients. Since then, more chromosomal translocations in AML have been described, many of which were predictive of clinical outcomes upon conventional treatment. In addition, specific mutations have been identified, including those of *CEBP* and *NPM1*, which were associated with a favorable prognosis, and those of *RUNX1* and *FLT3-ITD*, which were associated with an unfavorable prognosis[12].

The advent of next-generation sequencing (NGS) has made it possible to rapidly discover genetic mutations in the cancer genome. Since the first report of whole-genome sequencing in AML in 2008[13], the list of novel gene mutations has expanded at an unprecedented rate [Figure 2]. In particular, mutations of genes encoding for isocitrate dehydrogenase 1 and 2 were identified by NGS in patients with cytogenetically normal AML[14,15], and specific inhibitors are now available in clinics for AML carrying these mutations, attesting to the power of genomic information in the development of target-specific therapy[16,17]. The technologies have been generalized to solid cancers, forming the basis of The Cancer Genome Atlas (TCGA). TCGA comprises more than 20,000 primary and matched normal samples spanning more than 30 cancer types and has become an important reference for cancer genome research(https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga).

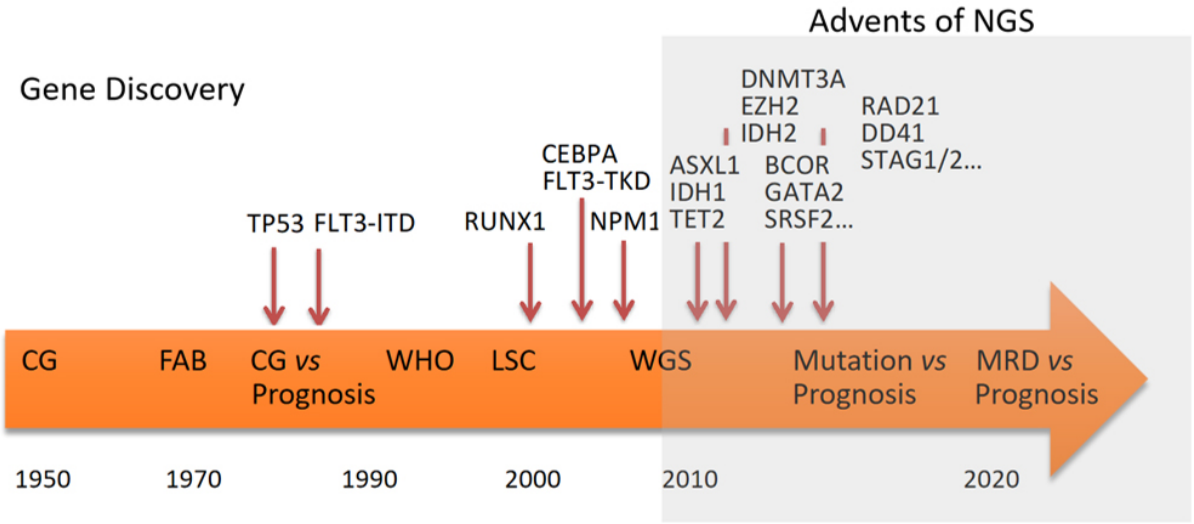

Figure 2. Timeline of gene discovery and evolution of concepts and management principles in acute myeloid leukaemia. NGS: Next Generation Sequencing, CG: cytogenetics; FAB: French, American and British classification; WHO: World Health Organization classification; LSC: leukaemia stem cells; WGS: whole genome sequencing; MRD: measurable residual disease.

CURRENT APPLICATION OF GENETICS AND GENOMICS IN AML

NGS has now become the standard of care for AML in tertiary institutes, where facilities and expertise are available. In most clinical practices, targeted DNA sequencing is used to identify mutations at diagnosis. This method focuses on a panel of 50-70 recurrent mutations in myeloid malignancies. Briefly, the first step involves library preparation, which entails the fragmentation of the genome into DNA fragments of about 150 base pairs and the addition of specialized adaptors to both ends of the fragments. The second step involves DNA sequencing, in which the library is loaded onto a flow cell and DNA fragments are amplified, resulting in millions of copies of single-stranded DNA. The third step involves data analysis, in which the nucleotides in the amplified products are confirmed through base-calling, and the presence of genetic variants is identified [Figure 3]. Gene mutations at diagnosis often predict patient outcomes upon treatment with conventional therapy, and hence inform clinical decisions on allogeneic HSCT at first complete remission (CR1). Patients who are at high risk of relapse should receive HSCT, which is of curative intent, and those with predictably favorable outcomes could be observed, to avoid the morbidity and occasional mortality associated with HSCT. Using deep learning algorithms, predictive modeling has been developed in cytogenetically normal AML based on genetics and clinicopathologic characteristics, which could be helpful in guiding the HSCT decision[18]. Furthermore, patients whose diseases show a high likelihood of relapse, even after HSCT, for instance, *TP53* mutated AML, should be given the option of clinical trials. The importance of genetic mutations and the growing number of mutations identified in AML and myelodysplastic syndrome (MDS), often a harbinger of AML, were underscored by the increased recognition of mutation-defined AML subtypes in the new WHO 2022 classification. Many of these subtypes were shown to define prognosis upon conventional treatment [Table 1][12,19].

In addition to gene mutations at diagnosis, the detection of measurable residual disease (MRD) has become the standard of care in the management of AML, providing real-time information about the depth of remission during the course of treatment[20,21]. In newly diagnosed AML, patients typically carry 10^{12} leukaemia cells in the body. Traditionally, morphologic assessment of BM and peripheral blood post-chemotherapy has been used to define disease remission based on a cutoff of blasts < 5%, amounting to approximately 10^{9} leukaemia cells in the body, which is substantial. MRD detection based on flow cytometry

Table 1. 2022 European LeukemiaNet (ELN) risk classification by genetics at initial diagnosis[21]

Risk category	Genetic abnormality
Favorable	RUNX1::RUNX1T1 [t(8;21)]
	CBFB::MYH11 [Inv(16)]
	Mutated NPM1 without FLT3-ITD
	bZIP in-frame mutated CEBPA
Intermediate	FLT3-ITD
	MLLT3::KMT2A [t(9;11)]
	Cytogenetic or mutations not classified as favourable or adverse
Adverse	DEK::NUP214 [t(6;9)]
	KMT2A rearranged (other than MLLT3::KMT2A)
	BCR::ABL1 [t(9;22)]
	KAT6A::CREBBP [t(8;16)]
	MECOM(EVI1) rearranged [incl. Inv(3)]
	ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2, TP53
	Complex and/or monosomy karyotype
	-5/del(5q); -7; -17/abn(17p)

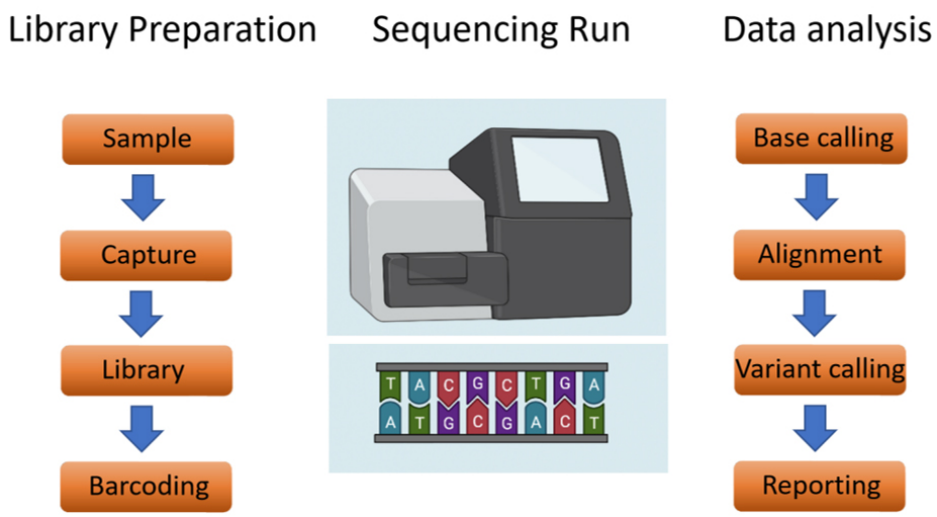

Figure 3. Workflow of myeloid focused next-generation sequencing.

or molecular means can achieve a sensitivity of at least 10^{-3}, and a persistently negative MRD during the course of treatment can predict disease eradication and long-term survival. In most tertiary centers, MRD is evaluated by either one of two methods. Flow cytometry offers a more rapid evaluation of patient samples but requires a higher level of technical expertise and standardization. Molecular methods are widely used and encompass quantitative RT-PCR, droplet digital PCR, and NGS platforms, choices of which often depend on institutional experience and expertise and the genes of interest.

PERSONALIZED APPROACH OF AML TREATMENT – FUTURE PERSPECTIVE

An important question in AML management is whether personalized treatment based on genomic information of individual patients, if available, would confer benefits to patients over the one-size-fits-all approach based on the "standard of care" (SOC). This is being addressed by the Beat AML trial, in which untreated patients older than 60 years received either the SOC or clinical studies founded on the mutation

profiles of these patients. Reportedly, the latter resulted in less frequent 30-day mortality and overall survival compared with those patients receiving SOC[22]. While the impact of this study remains to be seen, it highlighted the potential application of genomic information in guiding the upfront treatment of AML.

Another approach to personalized treatment of AML involves *in vitro* testing of drug sensitivity, with the goal of using the results to guide the clinical treatment of patients in real-time. This approach is akin to culture and sensitivity testing that guides antibiotic treatment for infectious diseases in clinics. Interestingly, leukaemia blasts grow very slowly *ex vivo* once they are taken out of their hosts, and optimization of culture conditions becomes critical for their maintenance and evaluation of drug sensitivity. When coupled with genomic information of the samples, *in vitro* drug sensitivity provides a powerful platform for identifying novel biomarkers predictive of drug response and personalized treatment through drug repurposing. Using these platforms, homoharringtonine was shown to be effective in FLT3-ITD AML through protein translation inhibition[23]. Importantly, clinical response to FLT3 inhibitors and homoharringtonine in patients receiving such treatments were correlated with the *in vitro* drug sensitivity of their samples, attesting to the potential application of the latter in predicting clinical responses. More recently, results from *in vitro* drug sensitivity testing of blood and BM samples from AML patients with relapsed and refractory disease against 515 anticancer drugs were used to guide the treatment of 37 patients using a customized combination of 2-3 drugs based on patient-specific sensitivity to single drugs and molecular data. Reportedly, nearly 60% of evaluable patients showed clinically meaningful responses, of whom the majority achieved complete remission (CR) or CR with incomplete hematologic recovery (CRi)[24]. A similar approach was also reported in pediatric AML patients[25].

Recent advances in transcriptomic analysis at the single-cell level have enabled the evaluation of cellular heterogeneity and hierarchy in AML, as well as the simultaneous examination of non-leukemic immune cell populations. It is now technically possible to enhance its analytic power by simultaneously measuring immunophenotype using barcoded antibodies and epigenetic state based on single-cell ATAC-sequencing. Bioinformatic analyses can enable the clustering of distinct cell populations based on their transcriptome profile[26]. Serial monitoring of the BM transcriptome upon leukaemia treatment at the single-cell level may shed light on the therapeutic responses in the leukaemia and microenvironment compartments.

CONCLUSION

Recent advances in genome sequencing technologies have empowered scientists and clinicians with the ability to examine inter-individual and intra-tumoral heterogeneities in acute myeloid leukaemia in great detail. The information has begun to influence the clinical management and prognostication of the disease. Transcriptome analyses at a single cell level are ideally suited to examine cellular heterogeneity in leukaemia samples and will shed important light on the dynamic changes of the microenvironment in the course of leukaemia therapy.

DECLARATIONS

Authors' contributions

Performed the literature search and wrote the manuscript: Leung HC
Outlined the scope of the review and wrote the manuscript: Leung AYH

Availability of data and materials

Not applicable.

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Case Report **Open Access**

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A case with prenatal molecular diagnosis of X-linked transient antenatal Bartter syndrome

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Abstract

Early-onset polyhydramnios during pregnancy can be caused by X-linked transient antenatal Bartter syndrome. Most of the reported cases were molecularly diagnosed after birth, whereas few cases were diagnosed in the fetus period. We received a pregnant woman who had polyhydramnios detected by ultrasound imaging at 25 weeks of gestation, and treated with magnesium sulfate, indomethacin and an amnioreduction at 30 weeks of gestation, whereas amniotic fluid decreased spontaneously since 32 weeks of gestation. Prenatal molecular testing showed the fetus carried *MAGED2* hemizygous variant c.967C>T [p. (Asp323*)] inherited from the mother. The preterm boy did not present with polyuria and electrolytes and acid-base imbalance in the early neonatal period, and had good development without polyuria at the age of 20 months. We presented the phenotypes of a Chinese case with a prenatal diagnosis of X-linked transient antenatal Bartter syndrome and his response to prenatal indomethacin treatment. Early identification of the condition helps to provide appropriate prenatal genetic counseling and postnatal management.

Keywords: Polyhydramnios, Bartter syndrome, *MAGED2* pathogenic variant, prenatal diagnosis

INTRODUCTION

Polyhydramnios is a common complication of pregnancy, with an incidence rate of 1 to 2 percent and is

associated with increased morbidity and mortality[1-3]. It was reported that 12 percent of these cases were diagnosed as severe polyhydramnios[4]. The most common mechanisms for polyhydramnios are decreased fetal swallowing and fetal polyuria[5], which may be idiopathic or caused by a variety of diseases. Approximately 40% of polyhydramnios is idiopathic[6]. However, 25 percent of infants with a prenatal diagnosis of idiopathic polyhydramnios are diagnosed with an abnormality after birth, such as Bartter syndrome (BS)[7]. Antenatal BS typically presents with severe polyhydramnios, premature delivery, hypokalemic alkalosis, and secondary hyperaldosteronism. Most antenatal BS patients require lifelong treatment with mineral supplementation and nonsteroidal anti-inflammatory drugs, and some may have severe chronic kidney disease progression[8]. In 2016, Kömhoff *et al.* reported that pathogenic variants in the *MAGED2* (melanoma-associated antigen D2) gene result in mislocalization of both NKCC2 (sodium-potassium-2-chloride cotransporter) and NCC (sodium chloride cotransporter)[9]. The mutant MAGED2 proteins cause a severe but transient X-linked antenatal BS (BS type 5), which mainly affects male infants[10]. Up to now, fewer than 50 cases with BS type 5 have been reported all over the world. Although some patients died in utero or shortly after birth[10,11], most of them had a normal estimated glomerular filtration rate at last follow-up[10-18]. Individual cases of BS type 5 with positive outcomes after serial amniocentesis therapy have been reported[11,15,17,18], and one fetus has spontaneous remission of polyhydramnios after two amnioreductions[18]. Prenatal indomethacin therapy has been reported to decrease amniotic fluid in some fetuses with antenatal BS[19-21], whereas its effects on fetuses with BS type 5 are still uncertain. To date, only a male infant with BS type 5 treated with prenatal indomethacin has been reported[13]. He had severe progressive polyhydramnios since 21 weeks of gestation (WG); despite several courses of indomethacin, amnioreductions on five separate occasions were required, and he was born at 29 WG after premature rupture of membranes. However, the dose of indomethacin and the genotype have not been described. Although antenatal BS is quite rare, it should be considered in the differential diagnosis of polyhydramnios if a structural abnormality and maternal diabetes are excluded. Accurate prenatal diagnosis may help to provide appropriate prenatal consultation and postnatal management.

CASE REPORT

A previously healthy 33-year-old G2P1 female was referred at 29 WG due to polyhydramnios detected by ultrasound imaging since 25 WG. Ultrasonography at 28 WG indicated severe polyhydramnios, with amniotic fluid index (AFI) of 43 cm (normal AFI: 5-24 cm)[22]. There were no structural abnormalities in the fetus. Screening for Down's syndrome was negative. The result of 75 g oral glucose tolerance test (OGTT) was normal. She gave birth to a healthy full-term girl in 2018. There was no family history of severe polyhydramnios or hereditary diseases. Ultrasonography at 29 WG in our hospital also indicated polyhydramnios, with the single deepest pocket (SDP) of 15.2 cm (normal SDP: 2-8 cm), and cervical shortening (13.3 mm). Owing to premature rupture of membranes at 30 WG, the woman received a short course of magnesium sulfate infusion and oral indomethacin (a dose of 25 mg six times daily for the first week and four times daily for the second week) for tocolysis and its amniotic fluid-reducing effects. A course of dexamethasone was also administered to reduce the possibility rate of neonatal respiratory distress syndrome. An amnioreduction was performed under continuous US guidance at 30 + 2 WG, and the volume drained was 2,200 mL. Amniotic fluid leakage ceased within a week after the amnioreduction. The SDP at 32 WG+, 33 WG+ and 34 WG+ were 13 cm, 9.4 cm and 8.7 cm, respectively [Figure 1]. After obtaining the couple's informed consent for the genetic analysis, genomic DNA of the fetus was extracted from amniotic fluid, and the couple's DNAs were extracted from peripheral white blood cells. No chromosomal abnormalities were detected using Karyotype analysis and chromosomal microarray. Whole-exome sequencing (WES) followed by Sanger sequencing analysis showed the fetus carried *MAGED2* (NM_177433.1) hemizygous nonsense variant c.967C>T [p. (Asp323*)] inherited from the mother [Figure 2]. This variant has been reported previously[11], and was classified as pathogenic (PVS1, PM2, PP4) according to the American College of Medical Genetics and Genomics guidelines[23].

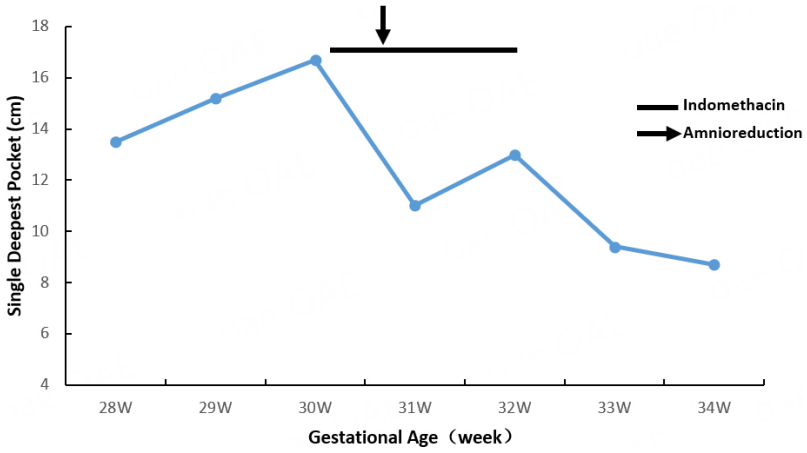

Figure 1. Treatment and changes in single deepest pocket during pregnancy.

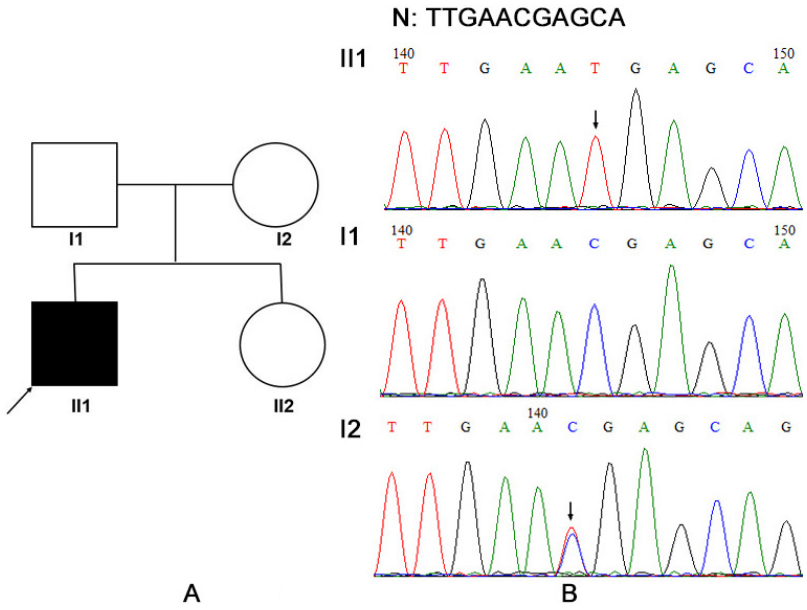

Figure 2. Pedigree of the case in this report (A) and Sanger sequencing of exon 6 in the *MAGED2* gene (B). The proband is indicated by a slope arrow, and the changed nucleotide is indicated by the vertical arrows. N: normal sequence.

The female gave birth to a preterm male baby infant born via vaginal delivery at 34 + 6 WG with birth weight of 2,900 g (P85) and length of 50 cm (P50). The newborn baby's 1 min Apgar score was 9 (muscle tone-1) and the 5 min Apgar score was 10. He did not develop obvious polyuria. Although he had a high blood level of renin (> 500 mU/L, reference range 2.8-39.9 mU/L) and aldosterone (> 100 ng/dL, reference range 3.0-23.6 ng/dL), his postnatal blood pressure (60/39 mmHg) and biochemical blood data were normal [Table 1]. Head ultrasound showed mild bilateral intraventricular hemorrhage. Kidney ultrasound and newborn hearing screening did not detect any abnormalities. At the age of 20 months old, he showed good development (height was 91.5 cm and weight was 13 kg) without polyuria.

Table 1. Clinical and postnatal blood biochemical characteristics of the boy

Parameters	Normal value	Age		
		1 day	4 days	45 days
Urine volume-mL/kg/h	3-4 (First week)	4.1	3.8	normal
Potassium-mmol/L	3.5-5.3	4.91	4.01	4.81
Sodium-mmol/L	137-147	138.80	137.37	137.7
Chloride-mmol/L	99-110	109.9	108.6	106
Bicarbonates-mmol/L	22-30	24	15.9	26.4
Magnesium-mmol/L	0.75-1.02	0.84	1.01	0.83
Creatinine-μmol/L	44-133	81.8	78.1	32.6
Urea-mmol/L	1.8-7.1	2.97	1.17	2.56

DISCUSSION

Severe polyhydramnios has been associated with an increased risk of several adverse maternal and newborn outcomes, such as maternal respiratory compromise, premature rupture of membranes and preterm birth[24]. The specific underlying etiology of the polyhydramnios guides intrapartum management and timing of birth. For example, polyhydramnios of fetal origin should raise the clinical suspicion of BS[8]. It was reported that BS accounts for 6% of cases with isolated polyhydramnios[25]. Because of different prognoses of BS, it is important to perform prenatal genetic testing to confirm the diagnosis. When prenatal genetic testing is not available or not sufficient to make a definite diagnosis, the assessment of the "Bartter index" (total protein × alfa-fetoprotein) is suggested[25]. In addition, the use of serial amniocentesis with or without prenatal indomethacin therapy has been reported to prolong gestational age at birth in a few cases of antenatal BS[26,27]. However, there was no report of successful prenatal treatment of fetal BS type 5 with indomethacin alone. Indomethacin is a potent inhibitor of prostaglandin synthesis and decreases salt wasting. This, in turn, can reduce fetal urine output and thereby controls the polyhydramnios[28]. Moreover, indomethacin can delay preterm birth by suppressing uterine contractions[29]. After an amnioreduction and two weeks of maternal indomethacin therapy, the recovery of polyhydramnios was observed in our case, which may be related to prenatal indomethacin therapy. Meanwhile, no serious side effects, such as fetal ductus arteriosus constriction and neonatal necrotizing enterocolitis, were observed. In our case, the response of indomethacin is better than that reported by Meyer *et al.*, which may be related to the older fetal age or *MAGED2* different genotypes[13]. In addition, the polyhydramnios symptoms can be relieved spontaneously with the increase of gestational age in patients with BS type 5[18]. This is the first report on the successful application of maternal indomethacin therapy in the fetus with BS type 5 to prevent the progression of polyhydramnios.

The most common form of antenatal BS is the autosomal recessive inheritance pattern. The infants exhibit postnatal polyuria and persistent renal salt wasting, requiring lifelong treatment. Some parents may terminate pregnancy due to concerns about poor prognosis. In contrast, favorable prognosis of BS type 5 caused by *MAGED2* pathogenic variants results in pregnant women choosing ongoing pregnancy. *MAGED2* pathogenic variants explained 9% of cases with antenatal BS[11]. In BS type 5, the onset of severe polyhydramnios (18-27 WG) and gestational age at birth [median (IQR): 29 (21-37) WG] are typically earlier than in other types, but signs and symptoms of renal impairment resolve spontaneously postnatally[30]. Polyhydramnios was detected in our case during the routine antenatal ultrasonic examination at 25 WG, which is similar to the previous report[11]. Since earlier routine antenatal ultrasonic examination in our hospital is performed in early pregnancy and 11-13 WG, respectively, and the proband's mother had no complaints of discomfort, we speculated that polyhydramnios might develop at 14-24 WG. As an inherited salt-losing tubulopathy, the transient nature of BS type 5 remains unclear. Two mechanisms may

be involved[10]. First, the sensitivity of adenylate cyclase activity to vasopressin gradually increases with fetal age, making the expression of NKCC2 and NCC independent of MAGED2 beyond a certain period of renal development. In addition, MAGED2 is required for cAMP generation and induction of the transcription factor HIF-1α under hypoxia[31]; the significant increase of oxygenation after birth may promote the synthesis of NKCC2 and NCC. Whether *MAGED2* pathogenic variants interfere with the expression of other proteins and cause extra kidney manifestations or may have some impact on female carriers[11] is still unknown.

The dominant presentation of our case was severe polyhydramnios, which led to the mother presenting with premature rupture of membranes at 30 WG. However, after treatment with amnioreduction and indomethacin, the amniotic fluid decreased since 32 WG. These dynamic changes in amniotic fluid volume indicated polyhydramnios was not related to fetal structural abnormalities. As we were initially unaware of antenatal BS due to its quite rare feature, biochemical analyses of amniotic fluids were not performed, which was a limitation of our report. Prenatal genetic testing demonstrated that polyhydramnios was caused by BS type 5, which provided evidence for timely genetic counseling and postnatal management of the patient. In addition, our case's postnatal mild symptoms may be associated with his gestational age at birth relatively close to term. A French boy with antenatal BS postnatally got a genetic diagnosis by identified *MAGED2* De novo variant c.967C>T, while the detailed manifestations were not described[11]. Our case complemented the phenotype of *MAGED2* variant c.967C>T. Besides our case, there were three cases prenatally diagnosed as BS type 5 caused by *MAGED2* splice site variant, deletion of the entire *MAGED2* gene and frameshift variant, respectively[14,15,18]. Different variant types of *MAGED2*, including nonsense, missense, splice site, frameshift, and large deletion, had been reported, but fortunately, most of the patients had a favorable outcome[10-13,15-18]. Prenatal genetic diagnosis and subsequent amnioreduction and prenatal indomethacin therapy seem to have a beneficial effect in the fetus with BS type 5, especially on the progression of polyhydramnios; therefore, extreme prematurity and related complications could be prevented.

In conclusion, we report a case of prenatal molecular diagnosis of BS type 5, which extends the phenotypic spectrum. Meanwhile, we share our experience of prenatal indomethacin therapy and discuss the optimal management approach for fetuses with BS type 5. Timely prenatal identification of BS type 5 could guide the management of polyhydramnios and postnatal symptoms.

DECLARATIONS

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Authors' contributions

Conception and design of the work, interpretation of the results, editing of the manuscript, and final approval of the version to be published: Wang F

Drafting of the manuscript, data acquisition, and final approval of the version to be published: Xu K

Administrative, technical, and material support, critical revision of the manuscript, and final approval of the version to be published: Zhang Y, Hou X, Yang H, Ding J

Availability of data and materials

All datasets generated for this study are included in the manuscript material.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study involving human participants was reviewed and approved by the Ethical Committee of Peking University First Hospital (2021 Scientific Research 074). Written informed consent to participate in this study was provided by the participants.

Consent for publication

Written informed consent was obtained from the participants for the publication of any potentially identifiable images or data included in this manuscript.

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Original Article

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Genomics education for medical specialists: case-based specialty workshops and blended learning

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Abstract

Aim: To develop and evaluate genomics education programs for health professionals to expedite the translation of genomics into healthcare.

Methods: Our co-design team of genetic specialists, expert medical specialist peers, and genomics educators developed two continuing genomics education programs for health professionals: stand-alone, specialty-specific workshops and a generic blended learning course, combining online learning with workshops. Both programs referenced adult learning theories; workshops included case-based learning and expert peer-led discussion. Longitudinal surveys evaluated changes in confidence and understanding of genomic testing processes and clinical practice.

Results: We delivered eleven specialty workshops (414 attendees) and a blended learning course comprising four self-directed online modules (61 users) and workshops (71 attendees) for mixed-specialty groups with adult, pediatric, or oncology cases. Surveys (214 workshops; 63 blended) showed that both programs significantly increased confidence and understanding of genomic testing processes. Blended learning participants showed

additional gains in confidence after attending a workshop following online learning. Workshop discussions with experts were valued, particularly regarding interpreting and applying results. At follow-up, gains in confidence and understanding were maintained for both programs and 81% of respondents had performed a new genomics activity in clinical practice.

Conclusion: Scalable education is needed. Our results suggest that specialty-specific genomics education may not be required to meet the needs of multiple specialties across a health system. Online learning can meet foundational learning needs but may not be sufficient to apply learning to practice. Blended learning offers flexible, continuing education pathways for dispersed national audiences as genomics becomes increasingly used across varied specialties.

Keywords: Workforce, genomic medicine, physician, continuing education, professional development, evaluation, case-based learning, blended learning

INTRODUCTION

Incorporating genomics into healthcare requires a health professional workforce that can apply genomic technologies translated from research settings to medicine[1,2]. Medical specialists without genetics training ("non-genetics" specialists) and other health professionals (such as nurses) need genomic literacy, skills and competencies appropriate for their professional role as genomics becomes relevant to the care of their patients[3]. Efforts to ensure graduating medical professionals have fundamental skills in genomic medicine[4,5] do not address the immediate need of practicing medical specialists to develop appropriate genomic skills. Medical professionals report a need for specialty-specific continuing medical education (CME) in genomics[6]. This approach has been adopted by genomics educators globally, with efforts focused on a single clinical area or specialty, such as oncology or primary care[7,8]. However, a specialty-specific approach to address the continuing professional development needs of "non-genetics" specialists may not be feasible across an entire health system.

In Australia, doctors complete a medical degree and then undertake specialty training through a medical college to become a Fellow (Consultant) of that college, e.g., the Royal Australasian College of Physicians. To maintain their medical registration with the national Australian Health Practitioner Regulation Agency, medical practitioners with specialist registration must self-report CME activities. Educational activities can count for up to 60% of the minimum CME requirements established by their college, with activities offered by a range of education providers. The funding and management of public health care delivery is the responsibility of each state or territory[9]. The State Government of Victoria funded the Melbourne Genomics Health Alliance ("Melbourne Genomics") to address barriers to the use of genomics in the Victorian health system. This included identifying and testing approaches to foster the understanding and skills in genomic medicine of practicing non-genetic specialists. At the time of our education programs, access to genomic testing in a clinical setting by non-genetic specialists was largely through clinical implementation research[10,11], with some having access to hospital-funded panel tests, and the role of genetic counselors varied[12].

Melbourne Genomics developed and implemented a multifaceted education strategy. The first phase of the strategy (reported elsewhere[10,13]) was to foster specialty-specific "peer" experts, potential opinion leaders who could lead change within their discipline[14] through experiences in genomics-rich workplaces[3]. Typically, these funded positions included rotation through a clinical genetics service or laboratory, or projects at the point of clinical implementation (clinical service design "flagship" projects). This resource-intensive approach is arguably best used at an early stage of translation when a cadre of early adopters needs

to be developed to support subsequent stages of implementation. To achieve the reach needed across a health system, scalable CME is needed[15]. Therefore, the second facet of our strategy was to co-design and deliver structured continuing education programs for non-genetics medical specialists to increase their understanding of genomic medicine and its application[3].

We developed and implemented two structured CME programs in genomics: face-to-face, stand-alone workshops tailored to specialist groups and a blended learning course with specialty-agnostic online content and adult, pediatric or cancer clinical cases.

Here we describe the development of these two CME programs and present the results of their evaluation. The place of specialty-specific and generalized genomics education to upskilling medical professionals across a health system is considered in light of these results.

METHODS

Program design and delivery

Audience

The target audience for both our genomics education programs were Victorian medical doctors training or qualified in specialties other than medical genetics ("medical specialists"). There were no pre-requisite genomics knowledge or skills. The specialty-specific workshops were designed to meet needs that emerged as the use of genomic testing was being evaluated in clinical care through the Melbourne Genomics program. The blended learning course was informed by the specialty workshops and designed to meet the needs of a broader range of specialists. In each case, educational programs were promoted through member hospitals and existing communication channels, including the Melbourne Genomics newsletter, website, and/or social media accounts. Advertising for specialty workshops was targeted to the relevant medical specialty. The periods of advertising for each program did not overlap; specialists could attend either program.

Theoretical frameworks, design principles and processes

All learning activities were based on theories of adult learning, that is, learners have varied prior knowledge and are self-motivated to learn when content is most relevant to their professional practice[16]. The learning design was case-based learning (CBL), with both programs including small-group discussion and learning from peer experts. The CBL approach is commonly used in medical education to link theory with practice[17]. The blended learning course also incorporated self-directed use of interactive, online resources.

The programs focused on core background knowledge and clinically-relevant practical application. This approach was based on adult learning theory and our experience that applied clinical learning, such as identifying patients suitable for genomic tests and interpreting test results, is more immediately relevant to most non-genetic specialists than technical aspects of genomics, such as test methodology and variant interpretation processes[18,19].

We used a co-design approach to develop both education programs, where the end-users of a "product" collaborate with designers and relevant stakeholders to contribute to the design process[20]. Learning objectives for the specialty workshops were developed by a co-design team that included Melbourne Genomics education staff (TC, FM, and EL), genetic specialists (clinical/medical geneticists, genetic counselors) and non-genetic "peer expert" medical specialists from the relevant specialties, many of whom had gained some expertise in genomics through Melbourne Genomics clinical flagship or immersion activities. The co-design team for the blended learning course comprised the same Melbourne Genomics

staff (TC, FM, and EL) plus a subset of peer experts to represent adult, pediatric and/or somatic (cancer) specialties. These peer experts were an integral part of the co-design process as they were able to identify key concepts to develop understanding and skills in genomic testing relevant to their specialty, with learning objectives worded in a way that was accessible to their peers. To ensure authentic, clinically-relevant learning through cases based on real-world experience, they helped develop clinical cases for the specialty workshops so that learners could apply new knowledge in relevant clinical contexts; for the blended learning course, the peer experts helped select cases to illustrate adult, pediatric or somatic application of genomic testing. They then also facilitated the CBL components of the program (details provided in Supplementary Table 1). As clinical members of the co-design team were not principally educators, the development phase included building skills in facilitating small-group discussion[21], including how to establish and build on learners' prior knowledge through guided questioning and using step-wise explanations.

Program evaluation

Each program was evaluated separately to assess effectiveness; we did not aim to compare the effectiveness of the two programs against each other or a control group. Evaluation questions aimed to assess program objectives as well as inform the design of future effective and scalable education:

1. Do the programs increase confidence, understanding and skills, and impact attitudes to practicing genomic medicine?

2. What is the value of facilitated case-based workshops for participants who have completed self-directed online modules, in addition to any gains in confidence or understanding?

We compared the results of the two program evaluations to inform broader, enduring genomics educational efforts.

In a longitudinal evaluation study design [Figure 1], we deployed surveys at up to four time points, with at least one reminder to complete each survey:

- Baseline: to all registrants, before pre-reading was provided (specialty workshop) or online modules opened (blended learning course)

- Post-online: to blended learning course participants who completed the online modules

- Completion: to all workshop attendees, after the workshop

- Follow-up: to all workshop attendees, at least 15 months later

Survey domains and outcome measures included changes in confidence, understanding, skills and attitudes relating to genomic medicine, common constructs when evaluating continuing genomics education[8] [Supplementary Table 2]. Measures were designed to assess the application of new knowledge and skills within the Australian health system context. Questions were categorical, open-text, or Likert-scale responses. To assess the ability to apply new knowledge, respondents reviewed an excerpt of a genomic test report that identified a variant of uncertain significance (VUS) and answered a question about whether predictive testing could be offered to family members [Supplementary Figure 1]. All surveys are available on request.

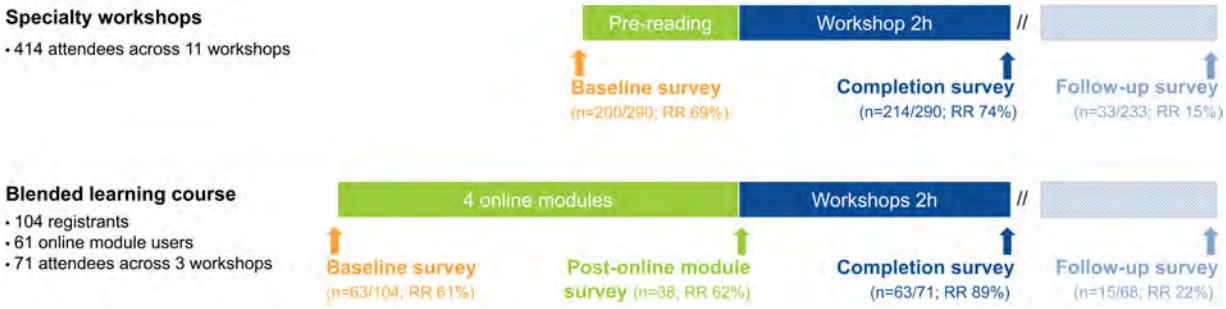

Figure 1. Longitudinal program evaluation. Specialty workshops were conducted over a 15-month period (August 2018-November 2019). The blended learning course (online and workshops) occurred in August 2019. Follow-up surveys were deployed in 2021, with responses for specialty workshops, a median of 23 months (range 16-32) and for blended learning course, a median of 20 months (range 19-20). RR: response rate. Surveys could not be deployed for two neurology workshops, so denominators differ.

Survey data were collected using REDCap[22] electronic data capture tools hosted at MCRI. Data were exported, cleaned, and analyzed using STATA 16.1[23]. All surveys and all questions were optional, so totals differed due to missing data. Percentages are reported to the nearest integer. Data were analyzed separately for each program. To assess self-rated confidence, means were calculated and data were compared between time points within each program using one-sided unpaired t-tests (normally distributed data), or Wilcoxon rank-sum tests (non-normally distributed data). Self-rated understanding of a genomic test report was assessed using chi-squared tests of pooled data at each time point to assess the change in distribution of responses across categories over time. Understanding whether predictive testing can be offered for a VUS was tested using a one-sided proportion test comparing correct with incorrect/unsure proportions across time points. A P value < 0.05 was considered significant. Open-text responses were reviewed by at least two authors both inductively and deductively for themes, then categorized[24].

RESULTS

Educational programs

When developing the learning objectives for each specialty workshop in 2018-2019, the Melbourne Genomics staff observed a common subset of learning objectives that emerged across all specialties. These objectives [Table 1] reflect concepts considered fundamental for all medical specialists to develop basic understanding, skills and confidence in genomic testing, which can then be supplemented by specialty-specific concepts for specialty workshops. The co-design team for the blended learning course deemed these common learning objectives appropriate for the blended learning course. The content of both education programs aligned with these learning objectives and was designed to relate participants' existing knowledge and clinical expertise to new learning of genomic testing relevant to their patients.

Specialty workshops

Eleven specialty workshops were held in 2018-2019 in genomics for germline (heritable) conditions in cardiology, pediatric neurology and development (twice), adult neurology (thrice), pediatric acute care, deafness, bone marrow failure, immunology, and dermatology. These specialties were targeted as they aligned with the local availability of specialty "peer" experts and priorities. Two-hour workshops for up to approximately 40 individuals included optional brief pre-reading material, a short presentation on key concepts, then 3-4 specialty-specific clinical cases presented by a genetic or peer expert, interspersed with facilitated small group case discussion (6-10 people) that addressed the learning objectives [Table 2 and Supplementary Materials].

Table 1. Common learning objectives in clinical genomics

- Identify patients suitable for genomic testing
- Understand the importance of family history
- Recognise the importance of detailed phenotyping
- Discriminate between different genomic tests, e.g., chromosomal microarray, multigene panel, NGS exome or genome sequencing, and select the right test for the patient, based on their purpose (indication), advantages and limitations
- Interpret a genomic test report
- Review the variant classification scheme (ACMG and AMP Guidelines) and the evidence-based foundation of the scheme (pathogenic; likely pathogenic; uncertain significance ("VUS"); likely benign; benign)
- Apply appropriate clinical action based on a genomic test result, e.g., changes to patient management, segregation testing, prenatal screening, or cancer therapy

Table 2. Format of the specialty workshops and blended learning course. All content included text and visuals. Online content also included animations, videos, and knowledge checks. Registrants for both programs also had access to online interactive resources and downloads available at https://learn-genomics.org.au

Content	Specialty workshops	Blended learning course
Foundational genetics and genomics theory	Pre-reading ~30 min • Introduction • DNA, chromosomes, and genes • Reading the code to make proteins • Genomic variants • Genetic and genomic tests	Module 1 ~25 min • *Genomics-Background Biology* e-book, including links to animations and glossary • Video of workshop Introductory presentation • What is genomic testing? • What is the utility of genomic testing? • Downloadable infographic of genetic and genomic testing pipelines
Foundational concepts of genomic medicine	Introductory presentation 20 min • DNA, chromosomes • Genes and proteins • Genetic and genomic tests • Genomic sequencing • Classifying variants • Interpreting reports • Clinical action	
Pre-test aspects		Module 2 ~35 min • Knowing your test options • The right patient • The right test • Pre-test counseling • Ordering genomic tests
Post-test aspects		Module 3 ~25 min • Variant identification, curation, and classification • Interpreting microarray reports • Interpreting exome reports • Post-test counseling
Somatic (cancer) genomics	-	Module 4 ~30 min • Introduction • Pre-test aspects of somatic (cancer) genomics • Variant interpretation (germline) • Variant interpretation (cancer) • Post-test aspects: interpreting somatic (cancer) reports; actionability; genetic counseling
Case study applications of genomic medicine[a]	Specialty-specific 20-30 min/case • 3-4 specialty-specific cases per 2 h workshop, interspersed with expert-facilitated small group discussions	Generic ~30 min/case • 4 cases per 2 h workshop, interspersed with expert-facilitated small group discussions

[a]More details of the case presentations are provided in Supplementary Materials.

Blended learning course

The course commenced with four online modules that covered foundational biology and genetics, and principles of genomic testing and variant interpretation in greater detail than that provided in the specialty workshop pre-reading [Table 2]. The modules addressed both germline and somatic (cancer) genomic testing. Registrants were encouraged to complete online modules over a four-week period (August 2019), with face-to-face workshops held after two weeks. Registrants could choose to attend one or two of three two-hour workshops. Each consisted entirely of CBL using "generic" cases designed to be accessible to a range of specialists while illustrating the principles of genomic testing: germline pediatric genomics, germline adult genomics, and/or somatic (cancer) genomics.

Education participants and evaluation sample

All education activities reached capacity.

Specialty workshops

A total of 414 health professionals attended a specialty workshop (20-43 per workshop). Respondents to the baseline workshop survey (n = 200) were mainly Consultants (48%) or Trainees (37%) in each specialty [Supplementary Table 3]. Two-thirds (67%) reported having no formal genetics training; those who did cite lectures at university (65%) or during basic medical training (26%). Over two-thirds (69%) had ordered or interpreted genetic or genomic tests in their practice, most often ordering single gene (78%) or chromosome tests (66%), with 46% ordering an exome/genome test. Of those who had not previously used genetic or genomic testing, 70% anticipated doing so in the future.

Workshop participants also responded to surveys at the completion of education (n = 214) and follow-up (n = 33; Figure 1).

Blended learning course

One hundred and four health professionals registered for the blended learning course, with all 86 engaging with at least one part of the program. Sixty-one registrants accessed the online modules, averaging 26 min per module; 29 participants completed all four modules. Seventy-one registrants attended workshops (pediatric germline, n = 32; adult germline, n = 23; cancer, n = 27), with 11 of those attending two workshops (pediatric plus cancer; adult plus cancer). Those who completed baseline surveys (n = 63) were mainly Consultants (52%) and Trainees (46%) from varied specialties [Supplementary Table 3]. In contrast to specialty workshop attendees, most (98%) blended learning course participants reported no formal genetics training. Approximately two-thirds (68%) had previously used genetic or genomic testing in their practice, most often ordering single gene tests (74%) or multigene panel tests (74%), and 33% had ordered exome/genome tests. 80% of those who had not previously used genomic testing anticipated doing so in the future.

Blended learning course participants also responded to surveys after accessing the online modules (post-online, n = 38), completion of education program (n = 63), and follow-up (n = 15; Figure 1). As paired survey responses were only completed by 29 participants, unpaired analyses were used. (Paired survey data are provided in Supplementary Figure 2 and showed the same trends as unmatched responses, suggesting that our findings may reflect individual gains.)

Impact of the education programs on confidence, understanding, skills and attitudes

Confidence in understanding and following genomic processes

Respondents rated their confidence in the understanding of five aspects of genomic testing processes [Figure 2]. Confidence increased from baseline in all aspects after both the specialty workshop and blended learning course. For specialty workshops, confidence increased from baseline to completion ($P < 0.001$ for all processes). For the blended learning course, confidence increased from baseline to post-online ($P < 0.001$ for all processes), with further post-workshop gains in confidence for all processes ($P < 0.02$) except "referral pathways" ($P = 0.057$). At follow-up, mean confidence remained above baseline levels for all processes for both programs (workshops $P < 0.001$; blended $P < 0.02$). Comparing change in confidence from completion to follow-up after specialty workshops, there was no significant decrease in confidence for phenotyping ($P = 0.208$) and referral pathways ($P = 0.280$; Figure 2A). For blended learning, confidence was maintained only for referral pathways ($P = 0.193$; Figure 2B).

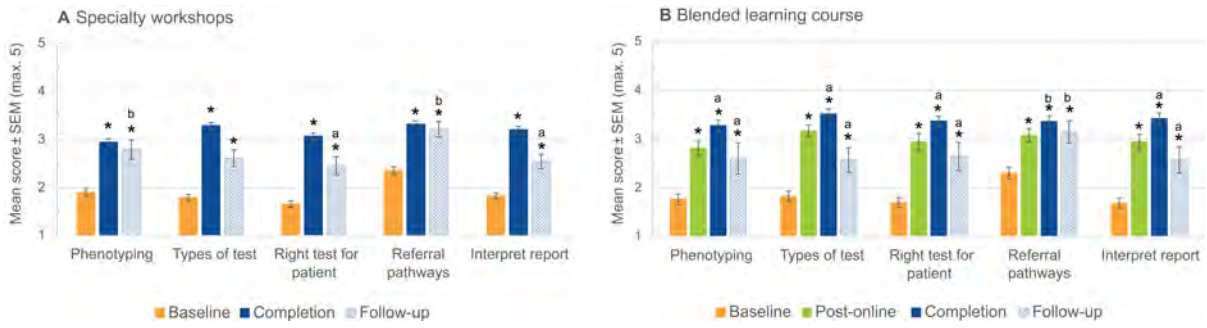

Figure 2. Changes in confidence in genomic processes. (A) Specialty workshops, (B) Blended learning course. 1 = "Needs improvement", 3 = "Good", 5 = "Excellent". SEM: Standard error of the mean. Sample size for each item differs. For specialty workshops, n at baseline, completion, and follow-up, respectively: Phenotyping, 198, 210, 33; Types of test, 198, 211, 33; Right test for patient, 198, 211, 33; Referral pathways, 197, 180, 33; Interpret report, 195, 212, 33. For blended learning course, n at baseline, post-online, completion, and follow-up, respectively: Phenotyping, 63, 38, 62, 15; Types of test, 63, 38, 62, 14; Right test for patient, 63, 38, 62, 14; Referral pathways, 63, 38, 61, 14; Interpret report, 63, 38, 62, 14. *Increased above baseline, $P < 0.02$; [a]increased from previous time point, $P < 0.02$; [b]no significant difference from previous time point (Wilcoxon rank-sum or one-sided t-test as appropriate).

Understanding of genomic testing and skills in interpreting test results

Survey respondents in both education programs showed a marked increase in self-rated understanding of the genomic test report from baseline to completion [Figure 3]. "Good" or higher self-ratings tripled for both specialty workshops (from 28% to 85%; Figure 3A) and blended learning (27% to 87%; Figure 3B), with substantial declines in "Fair" and lower ratings. "Very good" self-ratings increased incrementally through the different components of the blended learning course [Figure 3B]. At follow-up, self-rating of "Good" was maintained for specialty workshop respondents, while self-rating of "Good" or higher declined overall compared to completion (85% to 63% workshops; 87% to 58% blended).

Objective understanding of the clinical implications of a VUS result improved after both programs [Figure 4]. The proportion of respondents that correctly identified that predictive testing cannot be offered based on a VUS finding increased substantially through both programs (workshops 66%, $P < 0.001$, Figure 4A; blended 82%, $P = 0.001$, Figure 4B). Correct responses remained at similar levels at follow-up (72% workshops; 75% blended). However, some respondents remained "Unsure" after program completion (22% workshops; 5% blended).

Changing genomic practice

At program completion, most specialty workshop respondents (90%; 186/206) and all blended learning course respondents (62/62) anticipated incorporating skills into their professional roles:

"*[I can now make a] more informed choice of genetic testing in neuromuscular disease" (Consultant neurologist, blended learning course, completion*)

"*[I can now give] more consideration of the limitations of [genomic] testing" (Consultant pediatrician, blended learning course, completion)*

"*[I now have] confidence to convey information and interpret reports" (Acute care specialist, specialty workshop, completion)*

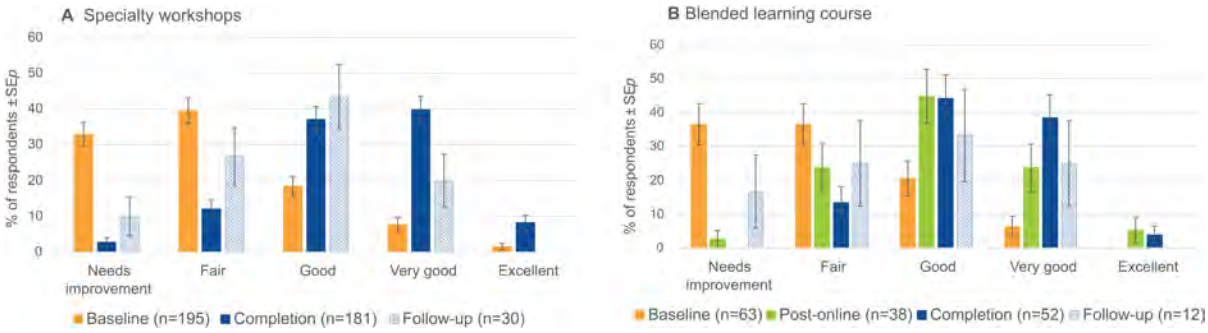

Figure 3. Self-rated understanding of a genomic test report excerpt. (A) Specialty workshops, (B) Blended learning course. Survey question, including a report excerpt with VUS result: "How would you rate your understanding of the information presented in the report excerpt?" Error bars: standard error of the proportion (SE*p*). For both programs, there was a significant difference in the distributions of responses, shifting right from "Needs improvement" towards "Good"/"Very good", from baseline to completion (specialty workshops: $X^2 = 135.35$, df = 4, $P < 0.001$; blended learning course: $X^2 = 46.35$, df = 4, $P < 0.001$), and also from baseline to follow-up for specialty workshops ($X^2 = 17.75$, df = 4, $P = 0.001$) but not for the blended learning course ($X^2 = 6.05$, df = 3, $P = 0.109$).

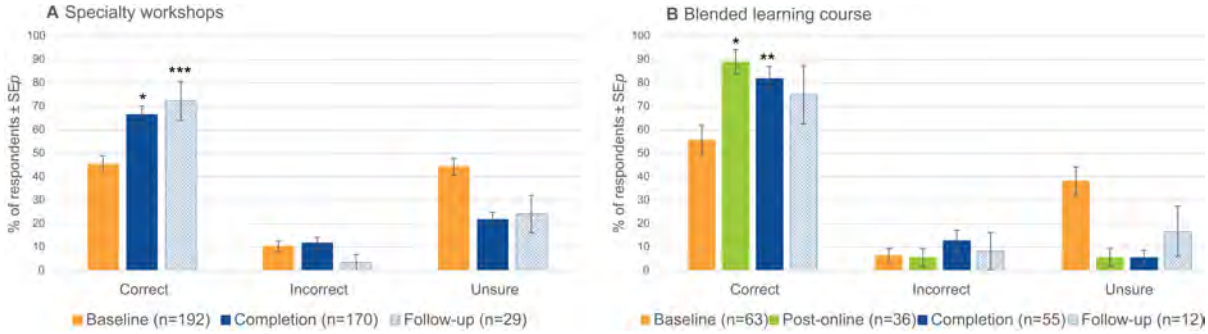

Figure 4. Understanding the implications of a genomic test result of a variant of unknown significance. (A) Specialty workshops, (B) Blended learning course. Survey question, including a report excerpt with VUS result: "Could the referring doctor offer predictive testing in this family?". Error bars: standard error of the proportion (SE*p*). *Increase from baseline (using one-sided proportion test, comparing the proportion correct versus incorrect/unsure combined): *$P < 0.001$, **$P = 0.0012$, ***$P = 0.003$.

Some respondents acknowledged that the education program illuminated gaps in their understanding:

"*I still feel overwhelmed by the amount of knowledge WES [whole exome sequencing] gives and feel I need to improve my knowledge of which panels to focus on. If anything I feel less confident but more aware of gaps to address." (Pediatric neurologist, specialty workshop, completion)*

"*[I have] better knowledge of when to refer to [discuss] with geneticist and interpretation of test results, but still a lot to learn." (Consultant Ophthalmologist, blended learning course, completion)*

An actual change in practice was reported by 81% of respondents to the follow-up surveys ($N = 291$; response rate 16.5%; Table 3); 76% of those who had attended a specialty workshop and 93% of those who had attended the blended course. New activities since attending our education programs included referring patients to a clinical genetics service, consulting a clinical genetics service for advice, requesting exome/genome tests, and educating others about genomics.

Education aims and program feedback

At baseline, respondents were asked what they hoped to gain from completing a workshop or blended

Table 3. New activities undertaken since attending a specialty workshop or blended learning education program[a]

	Specialty workshops	Blended learning course	Total
N	33	15	48
At least one new activity	25 (76%)	14 (93%)	39 (81%)
Type of new activity	**n (% of 25)**	**n (% of 14)**	**n (% of 39)**
Referred patients to a Clinical Genetic Service	15 (60)	12 (86)	27 (69)
Consulted a Clinical Genetic Service for advice	15 (60)	9 (64)	24 (62)
Requested exomes/genomes	9 (36)	8 (57)	17 (44)
Educated others in my discipline about genomics	10 (40)	6 (43)	16 (41)
Requested multigene panel tests	9 (36)	5 (36)	14 (36)
Interpreted multigene panel test reports	10 (40)	4 (29)	14 (36)
Requested single gene tests	7 (28)	4 (29)	11 (28)
Requested single gene tests	7 (28)	4 (29)	11 (28)
Requested chromosome tests	6 (24)	4 (29)	10 (26)
Interpreted exome/genome test reports	5 (20)	4 (29)	9 (23)
Interpreted chromosome reports	5 (20)	3 (21)	8 (21)

[a]If a respondent had attended both a specialty workshop and the blended course (n = 5), they were categorized by the most recent activity. Respondents could select multiple responses, so percentages sum to > 100%.

learning course. The most common responses related to: understanding the clinical utility of genomics in their field; understanding the types, limitations, and appropriate use of genomic tests; ability to interpret genomics test reports; and broader genomics knowledge.

"*[To gain] A better understanding of the genetic testing available, advantages, disadvantages, what some of the latest tests won't pick up etc" (Consultant neonatologist, specialty workshop, baseline)*

"*To gain more confidence in ordering and interpreting relevant genetic tests for my patients and be able to discuss these results with my patients" (Trainee medical oncologist, blended learning course, baseline)*

At completion, most respondents rated the educational activities overall as "Excellent" or "Very good" (87% workshops; 81% blended). Preparatory materials were rated highly, with 91% of specialty workshop respondents and 76% of blended learning course respondents rating these as "Very useful"/"Useful" [Supplementary Table 4]. Case studies were considered the most useful aspect; 99% workshop and 100% blended, rating them "Very useful"/"Useful" [Supplementary Table 4].

When asked to reflect on the value of the different modes of learning, the majority of blended learning respondents indicated a blended approach was more valuable than either online modules or workshops alone (61%-83% across all learning objectives; Figure 5). Respondents considered online modules more valuable than workshops for learning about pre-test consent and counseling (22% *vs.* 12%) and test requests, administration, and referral (25% *vs.* 14%). Workshops were more valued for all other learning objectives, particularly interpreting test reports (29% *vs.* 2%) and post-test counseling (21% *vs.* 7%). The most beneficial aspects of workshops included discussing result interpretation and application with experts.

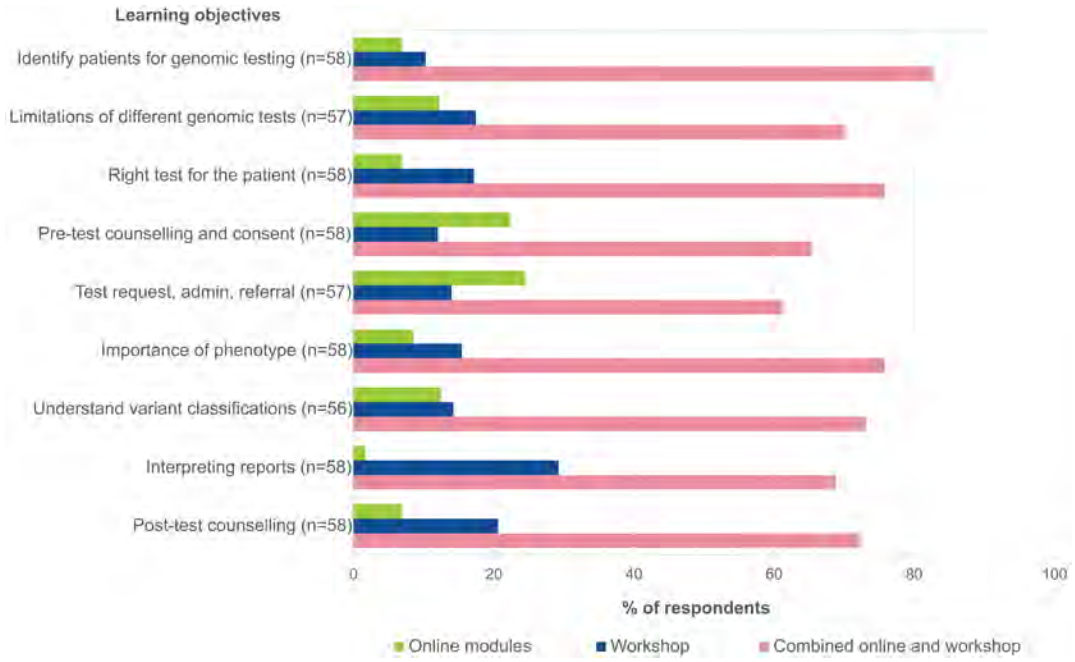

Figure 5. Perceived value of separate or combined components of the blended learning course for each learning objective. Survey question: "Reflecting on both the online content and workshop activities please indicate which component was most helpful in your learning in each of the course objectives (select one option for each objective)".

"*The cases and walking through the reports" (Hematologist, specialty workshop, completion)*

"*Small group discussion with an expert to answer questions and pre-empt discussion after/during each case is very useful." (Consultant cardiologist, blended learning course, completion)*

"*WES explanation interpreting results" (Pediatrician, specialty workshop, completion)*

At program completion, respondents indicated the most valued aspects across both programs were specific content (e.g., understanding genomic tests and appropriate use, interpreting test reports), as well as the CBL format and networking with peer experts.

"*Networking, learning from the best with clinical scenarios, updates in research and clinical management" (Adult neurologist, specialty workshop, follow-up comment)*

The most frequent suggestions for improvement across both programs focused on cases: discussing more cases for longer periods of time or more complex cases.

"*Longer session and more cases" (Neurodevelopmental pediatrician, specialty workshop, completion)*

"*[I would have liked] more complex cases" (Pediatric cardiologist, blended learning course, completion)*

DISCUSSION

Our education programs introduced the principles and processes of genomic medicine to non-genetic medical specialists across a range of disciplines and career levels. Longitudinal evaluation shows real-world changes in practice following our education programs, with an improved understanding of the relevance of genomic medicine, and the types of genomic tests available. The inclusion of non-genetic specialists (peer

experts) in design and delivery was a crucial element of our programs. As a result, we have identified a common set of learning objectives required for "entry level" education, irrespective of medical specialty. These are potentially useful to other education programs, particularly where there are no agreed national standards (e.g., competencies) to guide education for non-genetic medical specialists (e.g., Australia). Our evaluation results provide insights into the place of online learning and workshops as implementation strategies to translate the use of genomics from research settings to health systems.

Medical specialists' preference for CME specific to their specialty and patient group[6] would suggest that education programs need to be designed for a specific discipline. Our results challenge that assumption. Firstly, a common set of core learning objectives could be defined across the specialty-specific workshops, similar to the common "clinical goals" identified across multiple specialties for a continuing genomics education program at a US hospital[25]. Secondly, there was little difference in learner confidence or understanding between survey respondents from our specialty-specific workshops versus generic blended course, with similar ratings on the usefulness of the cases in each program. This suggests that, with an appropriate selection of cases and the inclusion of experts for group facilitation, similar outcomes can be achieved.

There is potentially an inherent tension between continuing genomics education tailored to a specialty and more readily scalable online education approaches to meet the needs of a growing number of many medical specialties across a health system. The reliance on the availability of experts with genomics, specialty, and/or teaching expertise to deliver specialty-specific workshops limits their potential to scale across a health system. However, the step-wise increases observed in the blended learning course respondent data, where participants first completed the online modules and then subsequently attended a workshop, suggest there is an additional benefit in attending a CBL workshop. Workshops can provide additional benefits that support the adoption of genomic medicine by practitioners. Respondents' preferences for the different modes of delivery in our programs suggest that online learning can be satisfactory for education relating to pre-test counseling and test request procedures, which are generally common to all patients. In contrast, workshops may be more suitable for education about post-test counseling, perhaps reflecting the nuance inherent in interpreting and applying the test results for an individual patient. Australian medical specialists prefer a service model of providing genomic medicine with support from clinical genetics services[19]. Multidisciplinary workshops involving genetic specialists as educators can also facilitate the formation of networks with clinical genetics services to foster this support. While workshops are resource intensive, as the practice builds, expert guidance will be increasingly available within the workplace or networks, potentially reducing the need for this approach over time.

Genomics education programs are typically designed and delivered by specialists with up-to-date expertise in genomics[25-28]. A critical success factor for both our genomics education programs was to include non-genetic yet "expert" medical specialists (peer experts), alongside genetic specialists, educators and evaluators. Our peer experts gained specialty-specific genomics experience through periods of immersion in genomics-rich environments and received training in case-based learning before facilitating workshops. Reports of using peer experts to co-design and deliver continuing education are limited, with few relevant to genetics/genomics[15,29-32]. Peer experts view the application of genomic medicine through their discipline-specific lens while also retaining the perspective of a genomics novice[33]. In both our programs, peer experts were crucial in defining learning objectives and content that introduced genomics in a relevant and accessible way to non-genetic medical specialists. Involving genetics specialists as well as peer experts in case-based group discussions at workshops creates an opportunity to establish communication channels between health professionals with different levels of expertise in genomics and to develop new connections

between genetic services and medical specialty services across hospitals. Both these elements support the diffusion of innovation[34]. The extent to which genomics education workshops do support the development of new relationships and thus support the implementation of genomics warrants further investigation.

Assessing the broader impacts of genomics education is a well-recognized challenge[32,35-37]. Evaluation of participants' self-reported changes to practice through follow-up surveys is a common approach[32,38-42]. Survey respondents in our education programs did report undertaking new genomic activities in their real-world practice, including educating others in their discipline about genomics, and patient-related activities. However, we cannot confidently ascertain the magnitude of change to practice. As is common with follow-up surveys, we had a low response rate. In addition, many of our program participants worked in specialties where opportunities to engage with genomic medicine might be limited. If we take a conservative approach and assume all those who made a change responded to the follow-up survey, nearly 15% of participants changed their practice within two years. Audits of clinical practice provide an objective measure of impact[26,41,42]. However, the breadth of hospitals employing participants, diversity of referral pathways, and limited electronic data sources rendered audits following our programs unfeasible. We therefore relied on self-reported behavior change at long-term follow-up.

Although objective measures of application of knowledge following education have been developed for use within a single specialty or setting[25,32,40,43,44], it is challenging to design scenarios and questions that adequately reflect clinical decisions relevant to diverse specialties and contexts, especially in a rapidly-developing field such as genomic medicine. We co-developed a scenario and question to assess the application of genomic knowledge to clinical care to evaluate our workshops. In common with other continuing genomics education programs, we also included subjective measures of evaluation[8,28,29,40,44]. The long-term follow-up survey was deployed during the COVID-19 pandemic. The additional demands on health professionals at this time may have contributed to the lower response rate than the baseline and completion surveys. Survey responses at completion and follow-up may also be biased towards participants who are more confident in their genomic practice.

Education has a key role to play in the implementation and adoption of genomic medicine within a health system. We intentionally describe the theories and design principles referenced during the development of our education programs, and include detailed descriptions and Supplementary Materials for both the education and its evaluation, to provide insights into potentially effective and widely-applicable workforce development strategies[45]. Online modules provide a highly scalable approach to workforce education, including supporting access by health professionals who work in regional towns or remote areas. We hypothesize that wholly online learning may be sufficient preparation for medical specialists to offer genomic testing to patients who meet clear diagnostic criteria, such as hematuria with hearing and vision involvement (Alport syndrome). However, nuanced clinical decision-making is required for pre-testing for complex patient presentations and often post-testing - where test results must be interpreted in the context of the patient's presentation and may influence patient management. Participants in our programs particularly valued workshop discussions with specialty and genetics experts for these aspects. Some respondents also wanted more complex cases, which could be provided as optional program extensions in the future.

Adoption of genomic medicine requires more than just genomic literacy. A key feature of our program was the use of "peer experts" to co-design and deliver education. Peer experts can mediate and "translate" the evidence for the use of genomics in a specialty and adapt clinical genetics practice as appropriate to the specialty; this makes them important mediators of change[33]. Using peer experts in both co-design and

delivery of education, particularly in case discussions, not only builds capability in genomics, but also increases motivation through articulation and illustration of the relevance of genomics to the specialty. Strengthening cross-specialty relationships also provides an opportunity to practice genomic medicine. We would encourage more people to use peer experts purposively in both co-design and delivery of education programs, and explicitly report how they leverage specialist knowledge to bridge gaps between clinical genetics and other medical specialties.

DECLARATIONS

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Authors' contributions

Contributed to all stages of this work and manuscript preparation: Maher F, Lynch E
Made substantial contributions to the design of the work and data interpretation, plus drafting or critically revising the manuscript for important intellectual content: Martyn M, Nisselle A, Gaff C
Contributed to the development of educational materials for the blended learning course and critically reviewed the manuscript: Charles T
Contributed to data cleaning, analysis and manuscript preparation: Tytherleigh R

Availability of data and materials

The surveys and datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. Some education materials are available at https://learn-genomics.org.au.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the Melbourne Health Human Research Ethics Committee (HREC/13/MH/256). All education participants were informed that completing survey questions would be taken as implied consent to participate in the evaluation program.

Consent for publication

All education participants were informed that completing survey questions would be taken as implied consent to participate in the evaluation program.

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The Hong Kong genome project: building genome sequencing capacity and capability for advancing genomic science in Hong Kong

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Abstract

Aim: The Hong Kong Genome Project (HKGP) is the first large-scale genome sequencing (GS) project in the Hong Kong Special Administrative Region. The Hong Kong Genome Institute (HKGI) is entrusted with the task of implementing the HKGP. With the aim to sequence 45,000-50,000 genomes in five years, it is the project's goal to provide participants with more precise diagnosis and personalised treatment, and to drive the application and integration of genomic medicine into routine clinical care.

Methods: The HKGI Laboratory's hardware and software components were customised to tailor to the needs of the project. Sample handling and storage protocol, DNA extraction, and PCR-free GS workflow were developed and optimised. Quality control indicators and metrics for assessing the quality of samples, sequencing libraries and sequencing data were established.

Results: The Laboratory is designed to facilitate a unidirectional GS workflow to minimise the risk of contamination. The Sample Manager system handles laboratory data generated from the HKGP samples and biobank. The

Laboratory handles and analyses approximately 350-500 samples per week, the majority of which are whole blood. During the first 24 months since the launch of the HKGP, 12,937 participants and their family members (6,680 genomes) have been recruited and sequenced. The sequencing capacity of the Laboratory has been further enhanced to include the latest technologies, such as long-read sequencing and multi-omics in order to meet the target of the HKGP.

Conclusion: HKGI Laboratory established a robust GS workflow for the HKGP. The clinical utility of GS will bring precision medicine into routine clinical practice.

Keywords: Hong Kong Genome Project (HKGP), genome sequencing, genomic medicine, precision health, laboratory establishment

INTRODUCTION

Advances in DNA sequencing technologies have fueled the genome sequencing era and led to the initial draft of the human genome sequence and, more recently, the telomere-to-telomere genome assembly[1]. Disease-risk and treatment response studies[2,3] provided clinical interpretation of genomic variants and paved the way for the development of genomic diagnostics and therapies[4]. Substantial improvements in next-generation sequencing and bioinformatic tools have significantly shortened sequencing times, yielded higher sensitivity, specificity, and accuracy, better coverage, and reduced cost[5,6], democratising genome sequencing from the individual level to the population scale. This is reflected in the launch of national genome sequencing initiatives around the world, driving genomic medicine and improving healthcare through the collection, storage, and application of genomic data[7]. In 2015, China launched its first Precision Medicine Initiative[8], followed by neighboring Asian countries such as Japan[9,10] and Thailand[11,12]. Other national genome projects have also emerged in France[13], the United Kingdom[14-17], Denmark[18], Australia[19-21], Canada[22], the United States[23], Saudi Arabia[24], and Turkey[25,26]. The scale of these projects ranges from sequencing twenty-five thousand to one hundred million genomes using various genomic technologies, including DNA microarrays, RNA sequencing, targeted gene panel sequencing, exome sequencing (ES), and genome sequencing (GS)[7,27,28], spanning four to fifteen years. As more genome projects are underway, we expect the catalog of human genomic variations and functional annotation to grow steadily[29,30], marking a major step towards embedding genome sequencing into routine clinical care.

Hong Kong has been a Special Administrative Region of the People's Republic of China since 1997. Hong Kong continues to have its own economic, legal, social, healthcare and welfare infrastructures despite being a recognised financial hub with modern city standards and a high standard of living. The latest population forecasts released by the Census and Statistics Department predict an increase in Hong Kong's population from 7.70 million in 2023 to 8.10 million in 2039[31]. Over 90% of the population of Hong Kong is of ethnic Chinese descent, according to the 2016 by-census[32], with other ethnic groups making up the remaining 8%. Hong Kong is viewed as a prime location to carry out studies on Southern Chinese populations' health due to this relatively homogeneous population. Shouldering about 90% of the inpatient needs of the entire community, the Hospital Authority provides a strong public healthcare safety net through 43 hospitals and institutions, 49 Specialist Out-patient Clinics (SOPCs), and 74 General Out-patient Clinics (GOPCs). With a well-established dual-track healthcare system, the remaining population also has easy access to privately funded healthcare.

Hong Kong has embarked on her journey of developing genomic medicine. Following the release of the Policy Address in 2017, the Hong Kong Special Administrative Region Government established a Steering Committee on Genomic Medicine[33]. Upon reviewing the local landscape, the Committee put forward the

recommendations for the strategic development of genomic medicine in Hong Kong, including the set-up of the Hong Kong Genome Institute (HKGI) in 2020 under the Health Bureau, to implement the Hong Kong Genome Project (HKGP) in 2021[34]. HKGP is the first large-scale GS project in the city to catapult genetic and genomic services and research[34], marking an important milestone in the revolution of the healthcare system in Hong Kong[7,27]. Accelerating the advancement of genomic medicine, HKGP is conducted in two phases: the pilot phase and the main phase. In the pilot phase, from mid-2021 to late 2022, efforts were dedicated to developing and streamlining operational workflows for genomic diagnosis, with the aim to sequence and analyse approximately 5,000 genomes by GS to demonstrate the capability of HKGI and the feasibility of HKGP[7,27]. This represents approximately 2,000 cases of patients with undiagnosed diseases or hereditary cancers and their family members, with the majority of the cases subjected to trio analysis to assist in genomic data interpretation. Valuable experience and insights gained from the pilot phase have laid a solid foundation for formulating directions for the main phase, adding "genomics and precision health" as a new theme, covering other disease and research cohorts, and aiming to sequence 45,000-50,000 genomes (approximately 18,000-20,000 cases) over three years from 2023 to 2025[34].

The entire patient journey is the highlight of HKGP, with considerable efforts dedicated to designing and crafting the protocol and process according to international standards of medical ethics and participants' rights[7]. According to the advice sought from the various professional bodies and personnel, this patient-centered process starts with clinician engagement, and the emphasis that all personnel are fully aware of the importance of a thorough informed consent process. Another primary and unique process is the element of genetic counselling. To facilitate the implementation of HKGP through addressing the needs and challenges of the profession, a representative group of experts and stakeholders in the fields of genetics and genomics was gathered to standardise genomic counselling practice. While pre-test genetic counselling focuses on the process of translating complex genetic information into colloquial and relevant information between the clinicians and the patient, post-test genetic counselling provides an opportunity for participants to understand the genetic diagnosis and discuss the implications of the findings with genetic counsellors or clinical geneticists. The project also has clear protocols for withdrawal procedures, in which withdrawal does not preclude joining the project again, for re-enrollment and consenting procedures were addressed in the project design.

For a seamless GS operation at the beginning of the HKGP, a sequencing service provider was engaged to provide the wet-lab process while the HKGI laboratory was designed and built to provide a bespoke GS workflow with a laboratory information management system. To handle the increasing workload and tight timeline, a NovaSeq 6000 was installed in the HKGI laboratory in mid-2022 to boost the sequencing capacity for the project. A bioinformatics pipeline and variant curation workflow were also developed to assist in the identification of causal variants. Multidisciplinary team (MDT) meeting is an integral part of the HKGP, allowing exchanges from relevant specialists to attain consensus on a molecular diagnosis and clinical management plan. By leveraging the collective knowledge and skills of different specialties, such as clinicians, genetic counsellors, laboratory scientists, genome curators, bioinformaticians, allied health professionals, and trainees, HKGP has been tackling complex genomic challenges and delivering cutting-edge advancements in healthcare. This collaborative environment allows for prime integration of diverse expertise and perspectives, fostering innovative approaches and ensuring comprehensive patient care.

The present paper focuses on sharing the experience in setting up the HKGI laboratory and genome sequencing workflow, and scaling up the sequencing capability and capacity in the laboratory to handle the increasing workload for the HKGP. The HKGI laboratory (Laboratory) comprises four major components: (i) multidisciplinary talents; (ii) laboratory infrastructure tailored for clinical genomics; (iii) scalable semi-

automation sequencing workflow; and (iv) quality assurance/quality control measures, privacy protection, and electronic records. By documenting the experiences and challenges in designing the laboratory and establishing the GS workflow in a tight timeline, lessons learnt might assist international counterparts in steering their own course in the genomic medicine era.

METHODS

Participant recruitment

The HKGP starts its operational workflow with the participants' journey, from engaging referring clinicians in recruitment to ending the diagnostic odyssey with personalised treatment by offering the first end-to-end GS service in Hong Kong [Figure 1]. Eligible patients and families are recruited by a HKGP team set up in each hospital (known as the Partnering Centre; PC). The University of Hong Kong/Queen Mary Hospital (HKU/QMH; HKWC), the Chinese University of Hong Kong/Prince of Wales Hospital (CUHK/PWH; NTEC), and the Hong Kong Children's Hospital (HKCH; KCC) are PCs in the Pilot Phase. In the main phase, recruitment has been extended to other cluster institutions of Hong Kong West Cluster (HKWC, namely The Duchess of Kent Children's Hospital at Sandy Bay (DKCH) and Grantham Hospital (GH) and New Territories East Cluster (NTEC, namely North District Hospital (NDH) and Alice Ho Miu Ling Nethersole Hospital (AHNH). Eligible participants are referred to PCs by clinicians after screening and informed consent is conducted through face-to-face interviews[7].

Sample collection and transfer

For each participant, 6 mL of blood is obtained and stored in two 3-mL EDTA-containing anticoagulation tubes. RapidDri Pouch kit (Isohelix) is used to collect cells inside the cheek for buccal swabs, and saliva samples are collected using the GeneFix Saliva DNA Collection and Stabilization Kit (Isohelix). Specimen collection is performed at the PCs. The specimens are packed in zip bags and stored at 4 °C until being transferred to the HKGI Laboratory, or for a maximum of 72 h. The specimens are packed with cooling packs and temperature-logging devices in the isothermal transfer boxes with combination locks.

Genomic DNA extraction and QC

Following sample registration, blood samples were aliquoted either manually or using the liquid handling system Freedom EVO100 (Tecan). Genomic DNA (gDNA) was extracted from 400 µL of whole blood using the QIAsymphony SP system and QIAsymphony SP DNA Midi Kit (Qiagen). For saliva and buccal swab samples, gDNA was extracted using the EZ2 Connect system and EZ1&2 DNA Tissue Kit (Qiagen). gDNA concentration was determined using the Qubit dsDNA BR assay kit and was measured with the Qubit 4 Fluorometer (Thermo Fisher Scientific). gDNA purity was determined using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific). gDNA integrity was assessed for degradation using a 1% E-Gel precast gel electrophoresis system (Thermo Fisher Scientific). Approximately 100 ng of each gDNA sample was loaded into the precast agarose gel.

Illumina GS sequencing and QC

PCR-free GS libraries were constructed using the KAPA HyperPlus kit for PCR-free workflow and KAPA Unique Dual-Indexed adapter kit (Roche) following the instructions provided by the manufacturer. 1 µg of gDNA is fragmented enzymatically at 37 °C for 15 min, end-repaired, 3′dA-tailed, ligated to dual-index adapters, and size-selected. Reaction cleanup and double-sided size selection steps were performed using KAPA HyperPure Beads (Roche). For the double-sided size selection, 50 µL of beads were added to the adapter-ligated library to remove large-sized DNA fragments in the first cut. To remove small-sized DNA fragments, 8 L of beads were added to the supernatant from the first size cut, resulting in the final library size range of 400-700 bp.

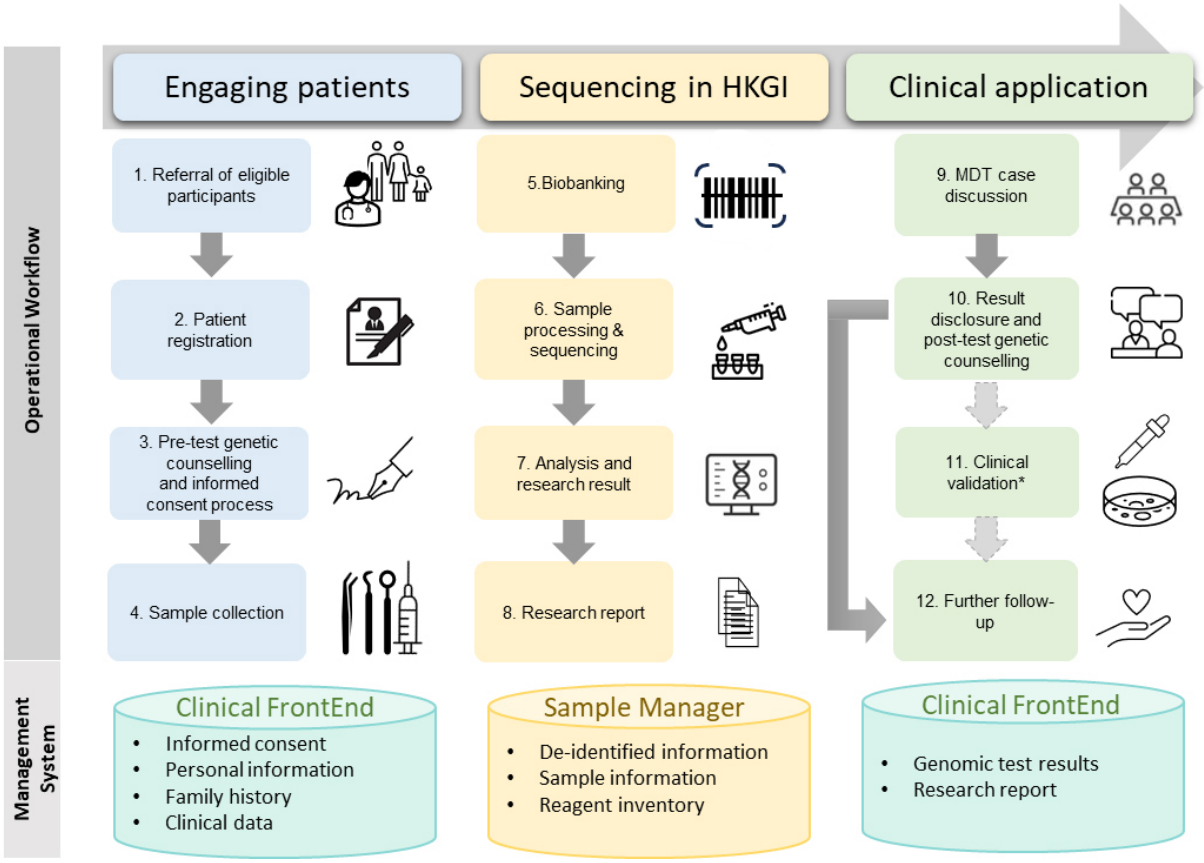


Figure 1. The operational workflow of HKGP using the main data managers: clinical FrontEnd stores all clinical-related data and documents, connected with Sample Manager using de-identified sample IDs. Sample Manager manages the biobank, records the GS journey of the sample, and works as a reagent inventory.

The GS library insert size was determined using the 4200 TapeStation and D1000 ScreenTape assay (Agilent). The library concentration was determined using the dsDNA HS assay kit and measured with the Qubit 4 Fluorometer (Thermo Fisher Scientific). The libraries were quantified by quantitative PCR using KAPA Library Quantification kit (Roche) and QuantStudio™ 5 Real-Time PCR system, 384-well or StepOnePlus™ Real-Time PCR system (Thermo Fisher Scientific). An equimolar library pool containing 24 dual-indexed GS libraries was combined prior to sequencing on the Illumina NovaSeq 6000 sequencer using NovaSeq 6000 S4 Reagent kit v1.5 (300 cycles), with 1% spike-in PhiX control (Illumina).

Sequence data analysis and validation

Base-calling was done using DRAGEN version 4.1.5. The secondary analysis workflow followed the best practice guidelines provided by the Genome Analysis Toolkit (GATK)[35]. Reads were aligned to the GATK-provided reference genome Homo_sapiens_assembly38.fasta using BWA version 0.7.17[36] and duplicates were removed using Picard version 2.27.4[37]. Base quality score recalibration, variant calling, and variant filtering were performed using GATK version 4.2.6.1 and in-house tools. Annotation was performed using Variant Effect Predictor version 104, BCFtools version 1.13, and in-house tools[38,39].

Following sequence data quality control steps, the bioinformatic pipelines identify and filter a list of variants for each GS sample. Candidate variants are prioritised based on the phenotype-based Exomiser[40], and the expert crowdsourced reviewed PanelApp software[41]. Sequence variants are classified according to the

standards and guidelines of the American College of Medical Genetics (ACMG)[42]. Post-analysis multidisciplinary team (MDT) meetings facilitate the exchange of views on the GS findings with respect to patient clinical indications, refining diagnoses and clinical management plans for individual patients. GS findings are validated by appropriate orthogonal methods such as Sanger sequencing, RNA sequencing, long-read sequencing, and digital PCR.

RESULTS

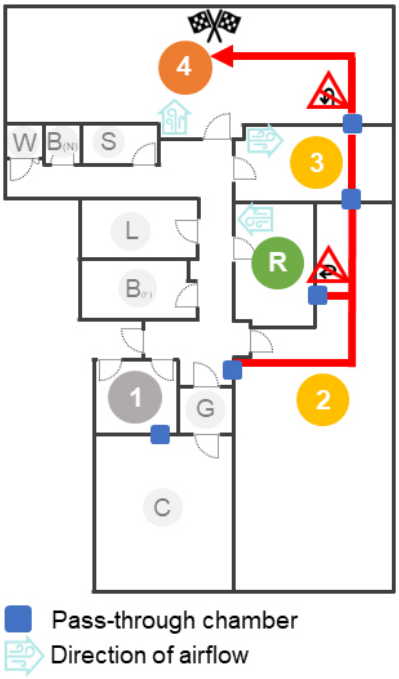
Hong Kong genome institute laboratory

Taking references and guidelines from various professional bodies into consideration, the development of the laboratory data information management system and GS workflow is fine-tuned to meet the recommendations set out by the Medical Genome Initiative for clinical GS[43-46]. One of the critical features of the Laboratory design is the capability of conducting a unidirectional workflow, and in conjunction with proper laboratory practices and operation, can minimise the risk of sample contamination. It requires physical separation of different stages of the procedure, dedicated equipment and supplies for each stage, and a workflow that prevents samples or laboratory personnel from moving "backwards" to potentially contaminate upstream workspaces [Figure 2]. In addition, differential air pressure is maintained to prevent contamination between the rooms. Pass-through chambers with interlocking doors and UV sterilisation capability enable the transfer of reagents or samples between physically separated rooms without compromising isolation and minimising cross-contamination. It ensures that reagents and samples pass through the Laboratory according to the designated route as demarcated in the GS workflow. Briefly, the GS workflow is divided into six wet-bench processes, in the order of: (i) reagent preparation; (ii) biological sample reception and registration; (iii) sample processing and biobanking; (iv) nucleic acid extraction; (v) GS library preparation and quality assessment; and (vi) sequencing. The main steps are performed in six key rooms, including the Reagent Preparation Room, Sample Reception Room, Sample Processing Room, Freezer/Biobank Room, Library Preparation Room, and Sequencing Room[47] [Figure 2].

The Hong Kong genome project biobank

HKGI developed and employed the data manager, Clinical FrontEnd, to facilitate and standardise the patient recruitment process and clinical data collection across different recruitment sites, by properly handling and housing different types of data from the participants [Figure 3]. The interface of this portal includes an e-consent form and a clinical information collection form for supporting patient recruitment, while the specimen collection form and GS report provide efficient information exchange between the HKGI and PCs. The participants' samples are collected at the PCs, and then delivered to the HKGI Laboratory, which acts as a central processing hub for registration, processing, and biobanking. After verification of the participant's identification data in the Clinical FrontEnd, the samples are de-identified to protect participants' personal data and confidentiality, and a HKGI Laboratory ID, a unique alphanumeric identifier, is assigned for downstream processing [Figure 3]. The de-identified HKGI Laboratory IDs are handed over to the LabKey Sample Manager, which is an independent environment for handling laboratory data generated from the HKGP samples and biobank. Its logical data structure, fine-grained security management of data access, and intuitive user-friendly interface facilitate the tracking of samples and reagents in the Laboratory [Figure 3].

The Laboratory routinely handles and analyses approximately 350-500 samples per week of various natures ranging from whole blood, buccal swab, saliva, and tissues. Sample processing uses a hybrid of manual and automated approaches; details of the operations such as date and time, operator identifiers, and location are logged in the Sample Manager system. To minimise repeated freeze-thaw cycles and potential sample degradation and contamination, samples are first divided into aliquots, barcoded, and transferred to the ultra-low temperature archives of the HKGP biobank [Figure 3]. The system maintains audit logs



		Room Name	HKGI (m²)	Min. (m²)
NO DNA	R	Reagent Preparation Room	21	11
	1	Sample Reception Room	14	11
LOW DNA Copy	2	Sample Processing Room	106	33
	3	Library Preparation Room	26	
High DNA Copy	4	Sequencing Room	110	22
	C	Cell Culture Room	52	---
	$B_{(F)}$	Biobank (Freezer Room)	14	---
	$B_{(N)}$	Biobank (Liquid nitrogen)	3	---
	L	Lab Support Room	15	---
	S	Stock Room	6	---
	G	Gowning Room	7	---
	W	Waste Room	4	
		Total Area =	**378**	---

Figure 2. The HKGI genomic laboratory is designed for a unidirectional workflow, with differential air pressure to prevent contamination between the rooms. Pass-through chambers with interlocking doors allow the transfer of reagents or samples between physically separated rooms without compromising isolation and minimising cross-contamination. The movement of biological samples, reagents, and laboratory personnel strictly follows the unidirectional workflow, from the "No DNA" room to "Low DNA copy" rooms, and finally to the "High DNA copy" room. The Reagent Preparation room holds positive pressure, keeping environmental contaminants from entering, while the Library Preparation and Sequencing rooms hold a negative pressure to contain potential contaminants within the rooms and reduce the risk of contaminating corridors and other rooms.

containing detailed linked records of the sample type, number and volume of aliquots, storage location, sample status, derivatives of each aliquot, and associated assay data. As HKGP has a relatively tight timeline with reference to the number of genomes to be sequenced, optimising and enhancing the throughput of high-quality GS is a priority for the laboratory. Automation is integrated into the labor-intensive workflow to maximise productivity, reduce human errors, and increase consistency and reproducibility. Laboratory personnel organise and monitor the automation systems, performing quality assessments of extracted genomic DNA and sequencing libraries, while standard operating procedures (SOPs) and pre-formatted data worksheets guide routine operations. All controlled laboratory documents, including quality manuals, laboratory safety manuals, equipment maintenance records, SOPs, and staff training records, are managed in the Laboratory Document Management System for up-to-date distribution and access by authorised personnel.

The genome sequencing workflow and quality metrics

Taking reference from the Medical Genome Initiative, the Laboratory established a list of stringent quality control indicators and metrics for assessing the quality of the samples and sequencing libraries [Figure 4 and Table 1]. The Laboratory developed and optimised protocols to process different sample types and prepare a PCR-free GS library for the HKGP. To promote genomics research in Hong Kong, the Laboratory further co-developed the GS protocol and established related quality metrics with the DNA sequencing core facility at the University of Hong Kong, Centre of PanorOmics Sciences (CPOS), for the sequencing of HKGP samples. While the role of the sequencing service provider remains critical for the project, the sequencing capacity of the Laboratory has been further enhanced and will take on the responsibility of the

Table 1. Summary of quality metrics for genome sequencing (GS)

Metric	Threshold or expected value[45,48]	Mean of 240 GS data performed by HKGI
Yield of data ≥ Q30[a]	≥ 80 Gb	162 Gb
Mean coverage[b]	≥ 30X	41.0X
Base ≥ Q30 %[c]	≥ 85%	90.0%
Clusters passing filter %[d]	≥ 70%	80.9%
Sample identity[e]	Match/not match	All match
Contamination %[f]	≤ 2%	0.0055%
Mapping rate %[g]	> 95%	99.9%
10X percentage (%)[h]	≥ 95%	95.7%
Gene passed 15X %[i]	≥ 90%	99.3%
Adapter-dimer %[j]	< 0.2%	0.0014%
Duplication %[k]	< 15%	14.4%
Mean insert size[l]	> 300 bp	496bp

[a]Total yield of data with ≥ Q30 scores; [b]Mean coverage across the human reference genome, after all filters are applied; [c]Percentage of bases that meet Q30 scores; [d]A cluster is considered to pass the filter when its chastity value is below 0.6 in the first 25 cycles. Cluster passing filter % is the percentage of clusters with passing filter; [e]Concordance with genotype when family structure is available; [f]Estimated level of sample cross-individual contamination based on a genotype-free estimation; [g]Percentage of unique reads that mapped to the human reference genome; [h]Percentage of bases in human genome with sequencing depth of ≥ 10X; [i]A measure of completeness. Percentage of genes with sequencing depth of ≥ 15X; [j]Fraction of pass-filtered reads that are unaligned and match to a known adapter sequence; [k]Percentage of mapped sequence that is marked as duplicate; [l]Median insert size for all paired-end reads where both ends map to the same chromosome.

sequencing workload in the next few years. During the first 24 months since the launch of the HKGP, 12,937 participants and their family members (6,680 genomes) have been recruited and sequenced by the end of July 2023. As expected, the majority of the cases are from the undiagnosed disease category and a smaller cohort (~12.1%) from hereditary cancer. In order to illustrate the performance of the GS workflow, ten sequencing runs carried out at the Laboratory consisting of 240 samples will be presented in the following sections.

After a series of pilot studies on the method of genomic DNA (gDNA) extraction, two automated magnetic bead-based protocols were established for the extraction of DNA: a high-throughput system for whole blood samples, and a medium-throughput system for saliva, buccal swab, and tissue samples. The extracted DNA is eluted in a slightly alkaline buffer, 10 mM Tris-HCl, pH 8.0, and EDTA is omitted as it interferes with enzymatic reactions in the downstream sequencing library preparation. The extracted gDNA is assessed for degradation using agarose gel electrophoresis. Each DNA sample migrates as a high-molecular weight band without any smearing or signs of degradation, indicative of intact gDNA of high quality and integrity [Supplementary Figure 1]. The purity of extracted DNA is evaluated using the NanoDrop spectrophotometer. A typical pure gDNA has an A260/A280 absorbance ratio of 1.7-2.0 and an A260/A230 ratio of 1.8-2.5. All extracted gDNA samples showed an absorbance ratio of A260/A280 and A260/A230 within the acceptable range, denoting the absence of protein, carbohydrate, salts, and other contaminants. In addition to using UV absorbance, gDNA is quantified using the Qubit fluorometer. Figure 4B shows the concentration and total yield for the 240 gDNA samples. On average, 400 μL of whole blood yields ~6 μg of gDNA, at a concentration ~122 ng/μL. As indicated, all 240 blood samples resulted in high-quality gDNA, sufficient for GS coverage of 30X.

The GS library preparation protocol has been optimised for both manual and automated operations. Figure 4C shows the quality metrics for the 240 GS libraries. Using 1 μg of gDNA as input, the average final library concentration is about 15.5 nM (in 25 μL volume), which is more than sufficient to reach 30-100X genome coverage. The insert size of the GS libraries is analysed on an automated electrophoresis system

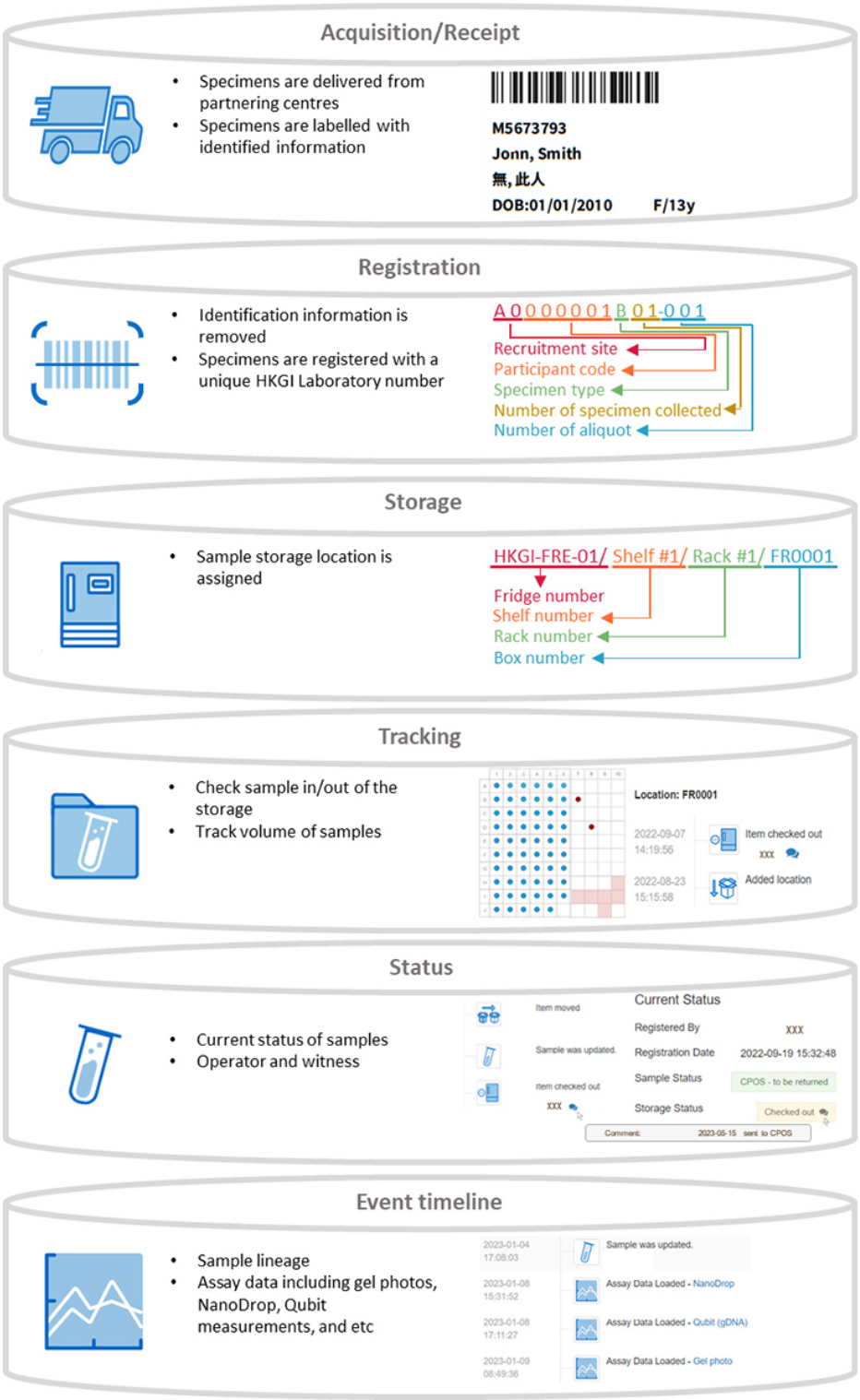


Figure 3. HKGI biobank and data management in Sample Manager for tracing sample lineages, storage, laboratory data, and relevant information during the genome sequencing process.

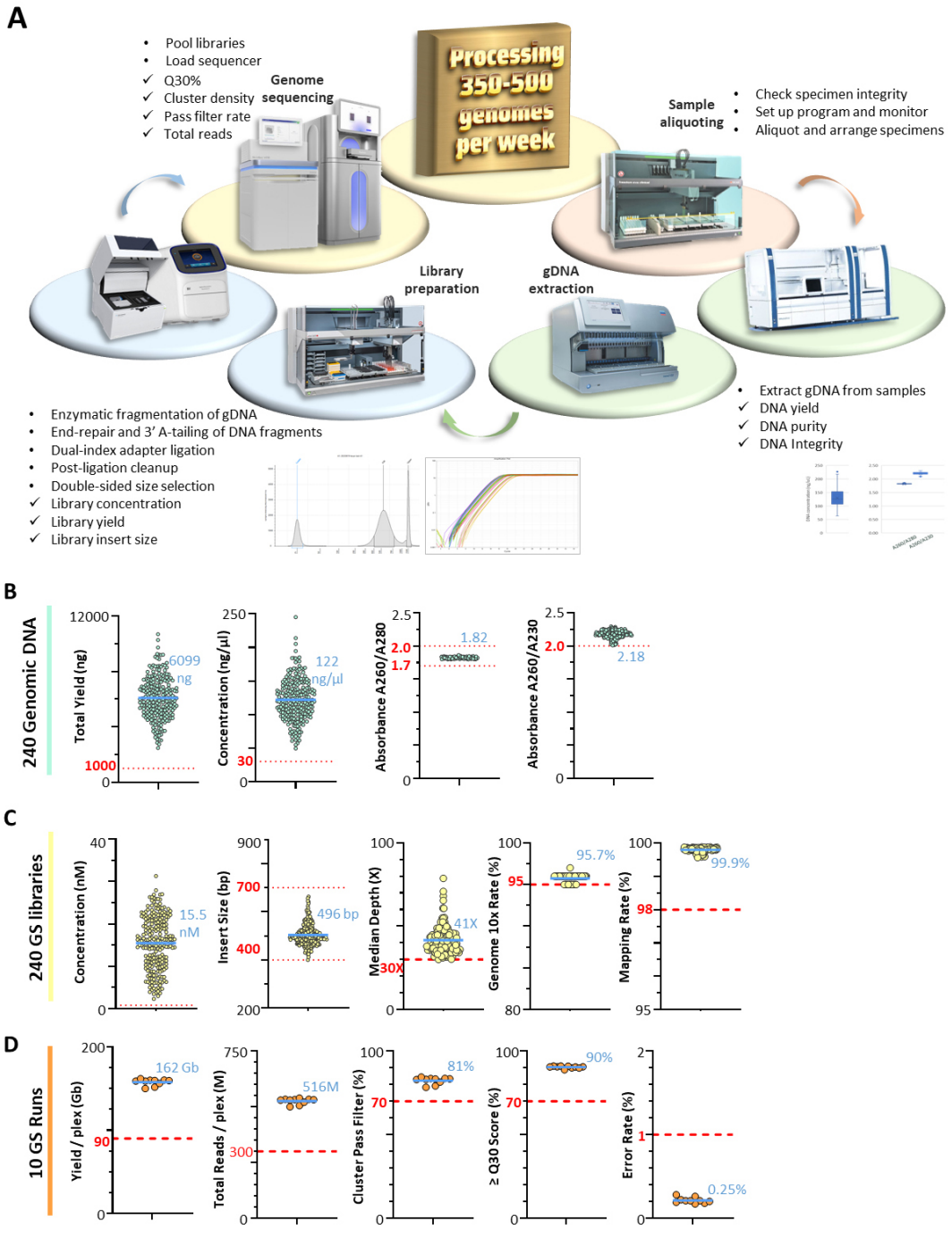


Figure 4. Performance statistics of 240 genome sequencing (GS) conducted by HKGI. (A) Quality indicators and thresholds used in monitoring the quality of different stages in the GS workflow. (B) Plots showing quality and quantity statistics of the 240 extracted gDNA. (C) Plots showing quality and quantity statistics of the 240 GS libraries. (D) Performance statistics of 240 GS libraries in ten NovaSeq 6000 runs. The 240 samples all meet the stringent quality indicators and metrics for assessing the quality of the samples and sequencing libraries.

(TapeStation, Agilent). Amongst the 240 GS libraries shown, the library insert size ranges from 400 to 700 bp, with a median insert size of 496 bp, which is within the range of efficient cluster formation on the Illumina patterned flow cells. GS libraries that pass the above quality controls then proceed to qPCR

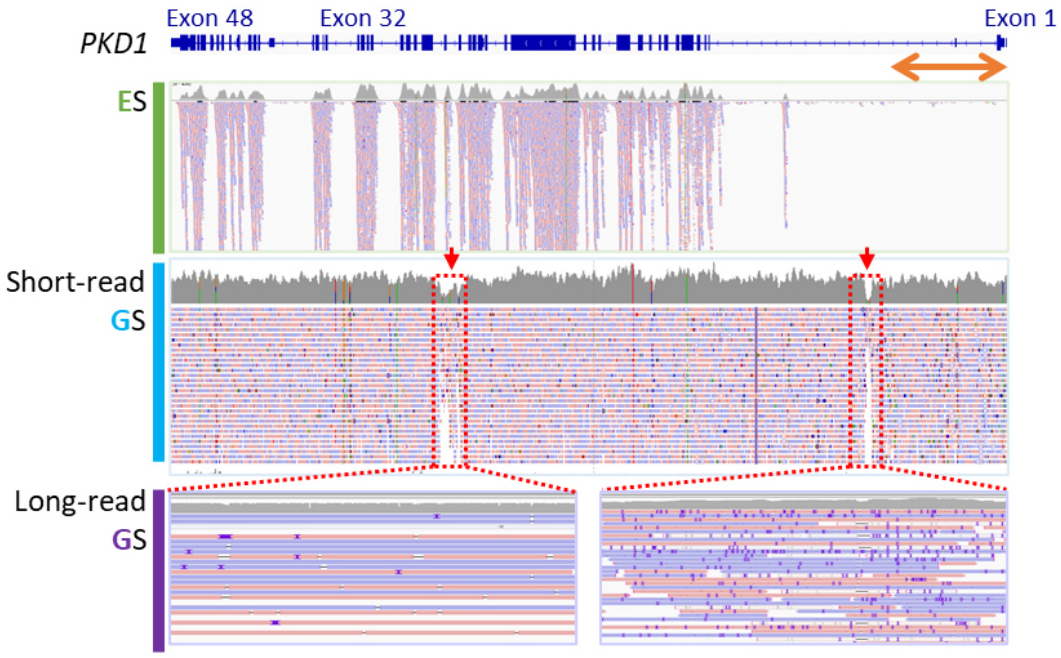


Figure 5. Comparison of exome sequencing (ES), short-read and long-read genome sequencing (GS) in resolving complex regions ("dark regions") of the human genome. An example of such regions is the *PKD1* (polycystic kidney disease 1) gene, where the first 32 exons are located in a segmental duplicated region on chromosome 16p13, with six pseudogenes located 13 Mb proximal to the *PKD1* locus. In addition to high GC content, the sequences of these six pseudogenes are highly homologous to *PKD1* and share 97% sequence similarity, making amplification- and capture-based approaches challenging. The *PKD1* region is visualised with Integrative Genomics Viewer (IGV) using different sequencing approaches. Despite improvements in the capture probe design, ES of exons 1 to 14 of *PKD1* showed lower coverage, while GS achieved a more uniform coverage for the entire locus, including the duplicated region. Long-read GS enables unambiguous alignment of reads, complementing short-read GS, and enhances disease diagnosis. The orange double arrow indicates the "dark region". The red dotted box and arrow indicate regions where short-read GS covers poorly.

quantification, and only libraries that pass all quality indicators are sequenced. An equimolar library pool containing 24 dual-indexed GS libraries is combined prior to sequencing on the Illumina NovaSeq 6000 sequencer using S4 flow cells with 300 cycles (2 × 150 bases).

As the number of nanowells is fixed in the patterned flow cells, the optimal loading concentration is determined by comparing the nanowell occupied rate (%Occupied) and pass filter rate (%PF). The optimal cluster density was attained after several rounds of optimisation on the S4 flow cell. The overall performance of 10 sequencing runs is consistent and of high quality, as shown in Figure 4D. Over 81% of clusters passed the chastity filter; more than 90% of bases had Q30, while the error rate using spike-in PhiX control was less than 0.25%. On average, each GS library yielded 162 Gb of data with 41X depth and over 516 million reads [Figure 4C and D]. Nearly all reads (99.9%) can be mapped to the human reference genome (GRCh38), while cross-individual contamination and adapter-dimer were merely detected. In summary, the overall statistics indicate that the established GS workflow is robust, and the data generated from the HKGI laboratory and CPOS are comparable, and on par with international standards.

The performance of GS in detecting variants in complex regions ("dark regions") of the human genome is illustrated in the example of the *PKD1* (polycystic kidney disease 1) gene. Mutations in the *PKD1* gene contribute to 80%-85% of autosomal dominant polycystic kidney disease (ADPKD) cases. ADPKD is an inherited renal disease characterised by many fluid-filled cysts in the kidneys that progressively impair kidney functions and eventually result in end-stage renal disease. *PKD1* lies in a segmental duplication

region, with six pseudogenes located at 13 Mb proximal to the original *PKD1*. The sequence of these six pseudogenes is highly homologous to the *PKD1* gene, with a sequence similarity as high as 97.7%[47], making the genetic diagnosis of ADPKD challenging when using exome sequencing (ES) or targeted enrichment approaches. Compared to ES, GS showed a more uniform coverage of the entire *PKD1*, including the duplicated region [Figure 5]. Our preliminary data also showed that long-read GS uniformly covered more of the "dark regions" in the genome, including the duplicated regions of *PKD1* that are challenging for short-read GS [Figure 5]. Our findings have demonstrated the capability of GS in clinical applications. The genetic diagnosis informed the patient's clinical management and treatment choices, such as the use of Tolvaptan to slow down the progression of kidney failure.

DISCUSSION

As of today, HKGI is the only organisation in Hong Kong to provide free end-to-end WGS with a goal to cater to participants' diagnostics needs and answer clinical management questions. With the opportunity to customise the Laboratory's hardware and software components to tailor its own needs, the design, and layout of the Laboratory, the monitoring and data management systems were all carefully planned and crafted to serve the specific needs of the HKGP. While local accreditation programs for medical tests involving next-generation sequencing are still under development, the design, construction, and outfitting of the HKGI Laboratory adopt international standards for clinical genomics laboratory for DNA sequencing. The HKGP biobank has the capacity to house more than 200,000 tubes of sample aliquots currently, and can be scaled up as the project extends to various focused disease areas. Together with the genomic database, the HKGP biobank opens new research opportunities in a collaborative environment.

Successful genomics research requires broad public participation and informed collaboration between researchers and society, which relies on trustworthy sharing, effective management, and appropriate privacy and data security protections[47]. Informed consent guidelines, data collection and storage protocols, and responsible sharing policies will be reviewed, improved, and augmented to provide the best practices in genomic research. A research environment is developed to facilitate efficient and effective genomic data sharing and analysis with clinicians and researchers. A well-developed infrastructure that facilitates active genomic research collaborations enables the integration of scientific discoveries and genomic findings into clinical practice. Collaboration between researchers and clinicians could promote the development of standardised protocols for data collection, analysis, and interpretation in genomic medicine. This infrastructure enhances the accuracy and reliability of genomic testing and enables more effective treatment decision-making based on individual genetic profiles. Building a biobank with the inclusion of a population deviated from that of European descent could also drive research and innovation in genetic medicine, leading to the discovery of new gene therapies and interventions to further improve patient outcomes.

The Laboratory adopted a PCR-free GS library preparation for the HKGP. The advantages of an amplification-free GS have been demonstrated by many studies[49,50], including significantly reduced biases introduced by the DNA polymerase, reduced duplication rates and false positives, and greater sensitivity in calling indel and copy number variants. In addition, it provides better mapping and more uniform genome coverage[50,51], allowing comprehensive detection of a wide range of variants, from single nucleotide variants to structural variants. Following the evaluation of different commercial kits for GS library preparation, an enzymatic fragmentation-based approach was selected. It does not only offer greater flexibility in the input gDNA amount, but also adaptability to liquid handling systems for routine library preparation. The Laboratory has devoted considerable investment to fine-tuning the library insert size range and final library yield, specifically the fragmentation and double-sided size selection steps, to achieve longer insert lengths for better coverage and indel variant detection.

The optimal loading concentration of the sequencing libraries is essential for a successful sequencing run. A loading concentration that is too high results in over-clustering and run failure. On the other hand, a loading concentration that is too low results in under-clustering and reduced output and accuracy. The optimal loading concentration is dependent on the library type, sequencing system, and reagent kit, and it requires to be adjusted empirically for each sequencer.

In order to achieve the ambitious target of sequencing 45,000-50,000 genomes by 2025, the Laboratory has recently installed additional automation systems and the latest sequencer, NovaSeq X Plus, which promises an increase of 2.5X throughput with the 25B flow cell that will be released later this year (2023). The higher throughput and lower per unit sequencing cost will benefit population-scale projects like the HKGP. Through standardisation of GS workflow and genomic data, it paves the way for data sharing and collaboration, ultimately advancing the field of genomics and improving patient care[52].

Stepping into the future: the important role of WGS laboratory workflow in enhancing the development of precision medicine in the genomic era

Due to lower assay cost and faster turn-around time, ES and gene panels have been the routine tests employed in clinical genomic diagnosis for the past decade[53,54]. However, this targeted approach requires PCR enrichment of the targeted regions, limiting the detection of small-sized variants found in exonic regions and the overall efficacy. In contrast, GS enables comprehensive interrogation of the entire genome with the option of PCR-free library preparation, allowing the unbiased identification of different types of genetic variants, including protein-coding, regulatory and noncoding regions, as well as regions affecting RNA splicing. Given the superior performance of GS and higher clinical utility[55] compared to other sequencing technologies, it is not only adopted by the HKGP and other international genome projects[8-10,14,15,18,26], but is also replacing ES as a first-tier test in the clinic[53,56].

Advancements in long-read sequencing technologies and broad application have earned it the "Method of the Year 2022"[57]. Long-read sequencing has been shown to complement the shortcomings of short-read technologies, such as directly identifying structural variants and methylation patterns, resolving complex rearrangements, sequencing homologous and repetitive regions, phasing of alleles, and so on[58-60], making previously computationally challenging and inferential approaches more straightforward. Other applications of long-read sequencing, like the characterisation of full-length RNA isoforms while preserving native RNA modifications[61], and the detection of aberrant splicing and gene fusion events[58], have the potential to facilitate functional interpretation of genomic variation[62]. Considering the wide range of applications in clinical genomics[63-65], the Laboratory introduced the Oxford Nanopore Technology (ONT) PromethION system to handle challenging cases such as certain neurological and neuromuscular diseases that are known to be caused by short tandem repeat expansions, and others that are unresolved by short-read GS. The longer reads also allow haplotype phasing of compound recessive variants and the detection of structural variants with precise breakpoint details, as shown in our analysis of *PKD1*. Our preliminary data showed that long-read GS uniformly covered many of the "dark regions" in the genome, including the duplicated regions of *PKD1* that are challenging for short-read GS.

To improve our understanding of the underlying biology of different diseases at the cellular and tissue levels, single-cell genomics, spatial multi-omics, and proteomics technologies have been applied to investigate thousands of individual cells in an unbiased approach[66-71]. In recent years, single-cell sequencing has been widely adopted in cancer research to interrogate tumour microenvironment and heterogeneity, and tumour clonal lineage[72,73]. The Laboratory has also established a single-cell sequencing platform to integrate multi-omics information to enhance the characterisation of disease at the cellular and tissue levels and enable the discovery of biomarkers for therapeutic targets. Integration of clinical information, GS,

single-cell sequencing and other omics data holds the promise of transforming healthcare as it facilitates diagnosis, targeted treatment and prognostic prediction, and may allow better clinical management and inform targeted therapeutic development.

Since commencing its operation, HKGI has overcome many challenges while embracing opportunities to improve the workflow along the way. Tremendous efforts have been invested in establishing its Laboratory from scratch, from recruiting talents and training new bloods, to setting GS quality and QC benchmarks, developing operational workflow and completing the sequencing of over 6,680 genomes, continuous fine-tuning and enhancing laboratory workflow. All within the first two years. HKGI sees the GS Laboratory as an important entry point into the scientific workflow. Our preliminary findings have demonstrated the capability of GS in advancing personalised genomic treatment, as illustrated in the *PKD1* example. While shouldering the important responsibility of offering the first end-to-end GS in Hong Kong, HKGI also values the privilege of paving the way for the career training and development of GS laboratory personnel from medical technologists, laboratory scientists and researchers, laboratory assistants to interns and trainees aspired to embark on the journey. As we move into the main phase, the HKGI GS platform will enable the implementation of precision medicine in clinical research and practice, going beyond genomics.

DECLARATIONS

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Authors' contributions

Made substantial contributions to the conception and design of the study: Chung BHY, Chu ATW, Tong AHY

Drafted the article and made critical revisions: Chung BHY, Chu ATW, Tong AHY, Tse DMS, Lo CWS

Performed data analysis and interpretation: Tong AHY, Lo CWS

Performed data acquisition: Lau CCF, Li CYF, Tai NSY, Wong LW, Choy GKC, Tse BYY

Provided administrative, technical, and material support: Lo SV, Tse DMS, Sung K, Yu M

Resources: Hong Kong Genome Project

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author, [BHYC]. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Ethical approval for this study was obtained from Central Institutional Board, Hospital Authority (HKGP-2021-001 & HKGP-2022-001), The Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (2021.423 & 2023.120) and Institution Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (UW 21-413 & UW23-289). Written informed consent has been obtained from all participants.

Consent for publication

Not applicable.

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Perspective

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Multifaceted nature of young-onset diabetes - can genomic medicine improve the precision of diagnosis and management?

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Abstract

Young-onset type 2 diabetes (YOD), defined as diabetes diagnosis before age 40, has an aggressive clinical course with premature mortality, in part due to long disease duration and lack of evidence to guide diagnosis and management. Autoimmune type 1 diabetes, maturity-onset diabetes of the young (MODY), and latent autoimmune diabetes in adults (LADA) are subtypes of diabetes in young people, which, however, cannot fully explain their complex clinical course. Similarly, family members carrying the same rare genetic variant of monogenic diabetes can have different presentations and outcomes. Ancestral heterogeneity, ecological transition, inter-ethnic differences in genomic architecture, and variations in living environment, lifestyles, access to care, and timeliness of diagnosis and treatment can influence the age of diagnosis and exposure to these cardiometabolic-renal risk factors. Despite the wealth of literature on genetic associations with diabetes, the familial cosegregation of rare variants and their relevance to YOD remains uncertain. This perspective was motivated by decades of clinical observations and learnings from an ongoing randomized controlled trial that uses biogenetic markers to classify patients with YOD for improving outcomes. Apart from highlighting the need to use family-based studies to improve the precision of diagnosis, we discussed atypical causes for diabetic ketoacidosis and the importance of

lifecourse and psychosocial-behavioral factors in patients with YOD. Apart from detailed clinical evaluation, we propose using plasma C peptide, homeostasis model of assessment (HOMA) indexes, autoantibodies, and polygenic risk scores to stratify risk, classify diabetes subtypes, and personalize treatment in YOD. To achieve these goals, we advocate changing the practice environment and team structure to enable physicians to use the insights they learn from patients and their family members to implement precision medicine and improve the outlook of these high-risk individuals.

Keywords: Young-onset type 2 diabetes, YOD, genomic medicine, precision medicine, autoimmunity, type 1 diabetes, monogenic diabetes, MODY, LADA, PRISM, randomized controlled trial

INTRODUCTION

In 2021, diabetes affected 537 million people, with 80% coming from low- and middle-income countries[1]. Age, obesity, and family history of diabetes are major risk factors for diabetes[2]. Thus, people who develop diabetes at a young age, especially if lean, require comprehensive investigations to explain their early metabolic decompensation. In part due to childhood obesity, there is a growing prevalence of young-onset type 2 diabetes (YOD), defined as diabetes diagnosis before the age of 40[3], which affects one in five adults with type 2 diabetes in Asia[4].

Patients with YOD may be exposed to decades of glycemic burden with rapid deterioration of glycemic control[5] and shortened lifespan by 10 years or more[6]. Compared to their peers with late-onset diabetes, patients with YOD had a 2-6 times higher risk of cardiovascular-renal events, recurrent hospitalizations, and premature death, with mental illness and kidney dysfunction being prominent clinical features[7-9].

While there are ongoing large-scale epidemiological, genetic[10], and interventional studies[11] in people with youth-onset diabetes (less than 18 years old), there are research gaps in YOD diagnosed during adulthood to guide diagnosis and management[12]. In high-income jurisdictions with well-developed healthcare systems, such as Hong Kong, despite overall falling trends of diabetes and related death[13,14], the incidence of YOD continued to rise[15]. Individuals with YOD have experienced mortality rates that are 5-8 times higher than those with late-onset diabetes[16].

MOTIVATION, FRAMEWORK AND OBJECTIVES

During the last three decades, the Chinese University of Hong Kong Diabetes Care and Research Team has combined practice and research to gather data and use data to inform practice and policies[3,17]. In the ongoing Precision Medicine to Redefine Insulin Secretion and Monogenic Diabetes Randomized Controlled Trial (PRISM-RCT) in Chinese Patients with Young-Onset Diabetes, we performed comprehensive clinical assessment and used biogenetic information to increase the precision of diagnosis and management of these high-risk patients (https://clinicaltrials.gov/ct2/show/NCT04049149)[18].

In part motivated by learnings from the PRISM-RCT, in this invited perspective not intended to be a review article, we shared our three decades of insights learned from diagnosing, classifying, and managing patients with YOD while pursuing active research in the field of genetics/genomics of diabetes focusing on translation. We first highlighted the minute amount of islets endowment at birth and the many lifecourse factors that may damage their structure and function, ultimately leading to diabetes. We briefly discussed Latent Autoimmune Diabetes in Adults (LADA) and monogenic diabetes as well-recognized subtypes of YOD. In our discussion on autoimmune type 1 diabetes, we highlighted the caveat that atypical diabetes due to acute destruction of islets related to viral infections and immunomodulating therapies may also present

with diabetic ketoacidosis. In our discussion on monogenic diabetes, including Maturity-Onset Diabetes of the Young (MODY), we highlighted the pitfalls of sole reliance on bioinformatics and few reported cases to classify the pathogenic nature of variants, which can lead to missed opportunities for early diagnosis and intervention, especially in populations where common genetic variants may contribute to trajectories of YOD different from that reported in European populations. With the availability of genome sequencing and preconception counseling services, we used clinical examples, albeit rare, to highlight how the detection of mutations with autosomal recessive inheritance in patients with YOD may alert the possibility of syndromic diabetes in homozygous carriers, which calls for genetic and preconception counseling.

We then discussed the clinical application of polygenetic risk scores, now widely accepted by the scientific community to have potential utilities for improving the precision of prediction, prevention, diagnosis, and therapies in complex diseases such as diabetes, and their relevance to YOD. We concluded by summarizing the design of the PRISM-RCT aimed at closing these knowledge gaps but at the same time advocating the need to create an environment conducive to reducing genomic medicine to practice, taking into consideration the unmet psychosocial-behavioral needs in YOD.

Against this background, the motivation underlying this perspective is to encourage more physicians who stand between patients (and their families) and technologies, to gather data systematically to improve our understanding of the nature of this complex syndrome and implement person-centered solutions with ongoing evaluation to inform practice and policies. To non-physicians involved in creating these genomic/genetic data, we emphasize to them the myriad of factors that need to be considered when interpreting these data and translating them into a technology or service aimed at beneficiating the patients and those at risk.

DIABETES AND PANCREATIC ISLETS

Blood glucose is maintained within a narrow range of 4-8 mmol/L most of the time, irrespective of energy intake or expenditure, due to efficient glucose sensing and insulin release by the pancreatic beta-cells[19-21]. Stress hormones, including catecholamines, glucagon, growth hormone, and cortisol, can increase blood glucose, while insulin is the only hormone that lowers blood glucose[19,20]. Other mechanisms of type 2 diabetes include non-suppression of glucagon and hepatic glucose production, excessive lipolysis, insulin resistance in peripheral tissues, dysregulation of appetite control, and abnormal incretin physiology[22]. Recent meta-analyses of multi-ethnic genome-wide association studies (GWAS) discovered hundreds of loci implicated in pancreas, adipose, and muscle tissue biology in type 2 diabetes[23-25].

In an autopsy series of 100 deceased subjects, the weight of pancreatic islets increased from approximately 0.2 grams at birth to a plateau of 1.0 gram at the age of 21 with marked inter-individual variations [Figure 1][26]. Other autopsy series revealed close correlations between body mass index (BMI) and pancreatic islet mass, with diabetes cases having lower beta-cell mass and larger alpha-cell mass than cases without diabetes[27]. Oxidized proteins, fat infiltration, amyloid deposits, and atherosclerosis were common features in diabetes pancreas[28]. Insufficient islet mass may lead to diabetes and early insulin requirement, so-called type 3c diabetes, due to chronic pancreatitis, pancreatic ductal adenocarcinoma, hemochromatosis, cystic fibrosis, and previous pancreatic surgery[29].

Islet autoimmunity and type 1 diabetes

Autoimmune destruction of islets can lead to progressive and severe insulin deficiency with rising blood glucose. This is accompanied by lipolysis as an alternative fuel with weight loss and ketone formation, culminating in diabetic ketoacidosis and coma[30]. In 2021, 8 million people had type 1 diabetes. Amongst them, 1.5 million were children and adolescents, with the majority diagnosed after the age of 18[31]. There

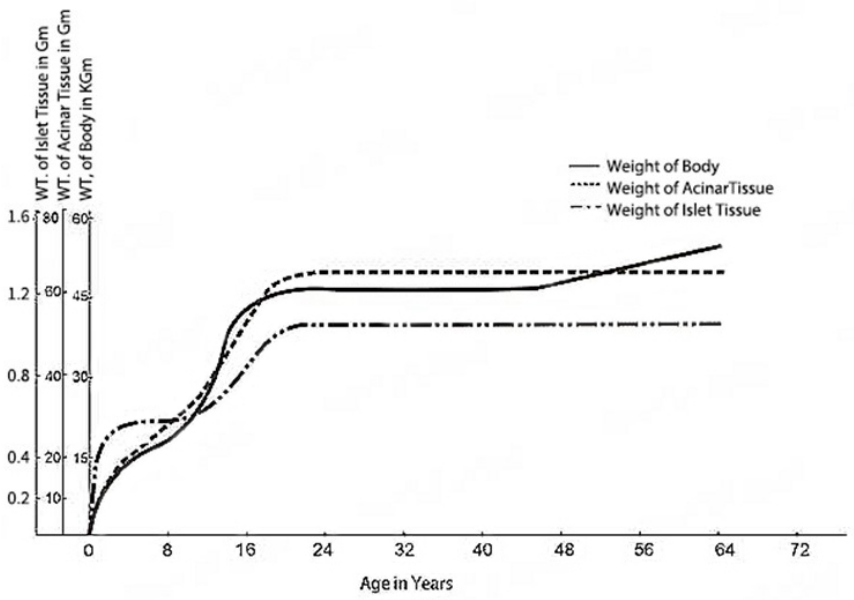

Figure 1. A quantitative analysis of 100 pancreases obtained from individuals in the United Kingdom in the mid-1930, including newly-born infants and adults up to the age of 64 years, all of whom apparently had normal nutrition and died from different causes including pneumonia, cerebral hemorrhage, perforated gastric ulcer burns, showing the proportional weight of islets and their increase in size over time, reaching a plateau in early adulthood. The small islet mass and its plateau in early adulthood might contribute to the marked increase in diabetes prevalence given the average adult body weight of 60-70 kg today compared to 50 kg nearly 100 years ago (reproduced with permission)[26].

was familial clustering of type 1 diabetes with 50% concordance amongst monozygotic twins[32]. Human leukocyte antigen (HLA) haplotypes were associated with increased (e.g., HLA DQ2/DQ8) and decreased (e.g., HLA DQ6/x) risk of islet autoimmunity[33,34]. Genetic risk score incorporating HLA haplotypes and non-HLA genetic variants predicted the onset of type 1 diabetes in European populations[35-37].

In a proof-of-concept RCT conducted in the 1980s, researchers reported that 1-year treatment with cyclosporine, an immuno-modulating therapy, was more effective than placebo in causing remission in patients with type 1 diabetes (10-35 years old)[38]. In 2022, teplizumab, a modified mono-antibody against C3 component on cytotoxic T cells, with a considerably safer profile, was approved by the regulatory agency for delaying the onset of type 1 diabetes[39,40]. In the latest publication, teplizumab was also found to improve beta-cell function in children newly diagnosed with type 1 diabetes[41]. These therapeutic advancements have opened up avenues for using biogenetic markers, such as polygenetic risk scores, to identify high-risk individuals for early prevention and intervention[33,42]. From a treatment perspective, severe insulin deficiency and dysregulation of glucagon secretion put patients with type 1 diabetes at high risk of hyperglycemia and severe hypoglycemia. Advanced insulin formulation and delivery systems[43], supplemented by continuous glucose monitoring devices[44], had improved the safety and effectiveness of intensive insulin therapy, making early diagnosis of type 1 diabetes imperative to improve their prognosis[45,46].

Atypical forms of type 1 diabetes

There are rare forms of type 1 diabetes due to islet destruction. One example is fulminant type 1 diabetes, which occurs within a few days after a viral illness presenting with diabetic ketoacidosis, often accompanied by increased biomarkers of exocrine dysfunction. These patients usually require life-long insulin treatment[47]. These rare examples inspire new insights into alternative pathogenesis for acute diabetic

ketoacidosis other than autoimmunity. Acute infections such as the coronavirus infectious disease of 2019 (COVID-19) have been shown to increase the risk of type 2 diabetes[48]. It remains to be proven whether these viral infections might precipitate acute or subacute forms of type 1 diabetes[49-51].

Our immune system is tightly regulated to control viral infections and suppress cancer growth while avoiding host damage. In developing areas with endemic viral infections, chronic activation of the immune system may lead to immunological tolerance. The latter can contribute to the low prevalence of autoimmune disease but a high prevalence of chronic viral infection and cancer in these areas. Immune checkpoint inhibitor (ICI) increases the activity of cytotoxic T cells of the host to kill cancer cells, but may activate the autoimmune process and cause type 1 diabetes and other endocrine dysfunction[52,53]. Given the increasing trend of young-onset cancer[54] and the use of immune-modulating treatment, physicians should be aware of these atypical forms of ICI-associated type 1 diabetes[55].

Latent autoimmune diabetes in adults

A subacute form of type 1 diabetes, often referred to as LADA, can be missed or undiagnosed, especially in adult patients with obesity or slow disease progression. The availability of many oral glucose-lowering drugs can inadvertently delay insulin treatment. Given the legacy effect of early glycemic control on increasing glycemic durability[56,57] and reducing long-term complications[58-60] in both type 1 and type 2 diabetes, misdiagnosis of LADA should be avoided.

In patients with type 2 diabetes, the presence of one anti-islet autoantibody, for example, glutamic acid decarboxylase antibodies (GADA), and that against IA2 and ZnT8 can be use used to diagnose LADA. There is no agreed cut-off age for diagnosing LADA, although some experts proposed an age below 35. There is a need to standardize the assays of these autoantibodies. The suboptimal quality of these assays hinders the interpretation of low titers with uncertain significance. Additionally, many reports related to the diagnosis and treatment of LADA were of low quality in terms of study design, definitions, and methodologies. That said, the prevalence of LADA appeared to range from 3% to 12% in patients with type 2 diabetes, in whom high titers of antibodies predicted early insulin requirement[61]. Although titers of these autoantibodies tend to decline after diagnosis, GADA could be detected up to 8 years after initial diagnosis. Patients with LADA tended to have an earlier age of diagnosis and lower BMI and were highly responsive to insulin treatment compared to their counterparts without LADA. In patients with LADA and residual beta-cell function, the use of sulphonylureas (SU) might hasten, while that of dipeptidyl peptidase 4 inhibitor (DPP4i) might slow the decline in beta-cell function[62]. These findings underlie the need to assess beta-cell function and autoimmunity, especially in lean patients with type 2 diabetes, to avoid misclassification and inappropriate treatment.

MATURITY ONSET DIABETES OF THE YOUNG AND MONOGENIC DIABETES

The 90% concordance rates for type 2 diabetes amongst monozygotic twins[63] and the 3- to 9-fold increased lifetime risk of diabetes amongst family members of affected individuals supported its strong genetic component[64-66]. Monogenic diabetes is caused by a single gene mutation and maturity onset diabetes of the young (MODY) is the most common form of monogenic diabetes. Traditionally, MODY is characterized by absence of obesity, presentation before the age of 25 years, strong family history suggestive of autosomal dominant inheritance, absence of β-cell autoimmunity, and sustained pancreatic β-cell function. However, many patients with MODY had older age of diagnosis with considerable phenotypic heterogeneity. This complexity calls for more phenotypic and multiomic analysis in family-based cohorts to test for cosegregation of genetic variants amongst affected family members[67].

In 1975, Tattersall first reported a MODY family affecting three generations. The affected members had a young age of diagnosis with non-ketotic presentation and mild clinical course[68]. The use of family-based linkage analysis and sequencing technology has uncovered the cosegregation of mutations of multiple genes amongst affected family members. The "loss-of-function" and "gain-of-function" of these mutations supported the causal roles of these genes in pancreatic islets and other insulin-sensitive tissues. These genes often encode proteins implicated in neogenesis, differentiation, and maturation of islets, transcription factors, enzymes, ion channels, and mitochondria. Collectively, they coordinate the complex processes of glucose entry and sensing as well as synthesis, processing, and secretion of insulin to maintain glucose metabolism[69-71]. Amongst the 40 subtypes of monogenic diabetes, MODY due to mutations in genes encoding glucokinase (*GCK*) and transcription factors including hepatocyte nuclear factor 4α (*HNF4α*), HNF1 homeobox A (*HNF1α*), and HNF1 homeobox B (*HNF1β*) were best described and most frequently reported. These mutations may be *de-novo* or transmitted from one generation to another with varying ages of diagnosis depending on the presence of other risk factors. Other rare mutations with Mendelian recessive mode of inheritance were associated with neonatal diabetes, severe insulin resistance, lipodystrophy, and syndromic features (e.g., deafness, visual impairment, liver/renal cysts, developmental abnormality)[67] [Figure 2 and Table 1].

There are considerable overlaps in the phenotypes amongst individuals with MODY, LADA, type 1 diabetes, or type 2 diabetes in whom autoimmunity, common and rare variants may coexist. In a recent review article, the authors summarized the epidemiology, pathophysiology, diagnosis, and management of monogenic diabetes. As many as 90% of patients with monogenic diabetes were not diagnosed or misclassified in part due to the prohibitive cost of incorporating sequencing in routine service. Depending on the selection criteria, amongst young patients with type 2 diabetes without autoantibodies, the frequency of MODY ranged from less than 10% to more than 50%, with the majority having mutations in *HNF1α*, *GCK*, and *HNF1β*[67].

In a multi-ethnic cohort of 3,333 young patients under the age of 20 with a presumptive diagnosis of type 2 diabetes, whole exome sequencing indicated that 93 (2.8%) patients carried a pathogenic/likely pathogenic (P/LP) variant in genes encoding *HNF4α* (n = 16), *GCK* (n = 23), *HNF1α* (n = 44), pancreatic and duodenal homeobox 1 (*PDX1*) (n = 5), insulin (*INS*) (n = 4) and carboxyl ester lipase (*CEL*) (n = 1). Compared to those without P/LP variants, patients with MODY had a younger age of diagnosis and lower fasting plasma C-peptide (CP) levels. They were less likely to have hypertension and had higher HDL-C levels, although there were no distinct features that reliably distinguished MODY from other forms of diabetes. Importantly, the diagnosis of MODY would have changed clinical management in 89% of them[72]. Although MODY risk calculators using age, age of diagnosis, family history of diabetes, HbA1c, fasting plasma glucose and CP, high sensitivity C-reactive protein, autoantibodies, and syndromic features had been developed in Europeans, their sensitivity and specificity remained suboptimal, especially in non-European populations[73].

Clinical implications of identifying families with MODY or monogenic diabetes

There is good evidence that patients with HNF1α and HNF4α-MODY, as well as K-ATP channel mutations, respond particularly well to SU agents, while adding DPP4i may have incremental benefits[74]. The use of these oral glucose-lowering drugs enabled insulin discontinuation in some patients misdiagnosed with type 1 diabetes. However, in many patients with MODY, notably those with transcription factor MODY, different background genetic profiles might contribute to progressive β-cell failure. Within a MODY family, carriers of the same rare variant might have different presentations and outcomes depending on the presence and control of other risk factors[75,76].

Table 1. List of genes implicated in monogenic diabetes. Adapted from[67]

Maturity-onset diabetes of the young	
ABCC8	ATP binding cassette subfamily C member 8
APPL1	Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1
CEL	Carboxyl ester lipase
GCK	Glucokinase
HNF1A	HNF1 homeobox A
HNF1B	HNF1 homeobox B
HNF4A	Hepatocyte nuclear factor 4 alpha
INS	Insulin
KCNJ11	Potassium inwardly rectifying channel subfamily J member 11
NEUROD1	Neuronal differentiation 1
PAX4	Paired box 4
PDX1	Pancreatic and duodenal homeobox 1
RFX6	Regulatory factor X6
ZFP57	ZFP57 zinc finger protein
Syndromic diabetes	
CEL	Carboxyl ester lipase
CISD2	CDGSH iron sulfur domain 2
DCAF17	DDB1 and CUL4 associated factor 17
DNAJC3	DnaJ heat shock protein family (Hsp40) member C3
DYRK1B	Dual-specificity tyrosine-phosphorylation-regulated kinase 1B
GATA4	GATA binding protein 4
GATA6	GATA binding protein 6
HNF1B	HNF1 homeobox B
PAX6	Paired box 6
PCBD1	Pterin-4 alpha-carbinolamine dehydratase 1
PIK3R1	Phosphoinositide-3-kinase regulatory subunit 1
PPP1R15B	Protein phosphatase 1 regulatory subunit 15B
SLC29A3	Solute carrier family 29 member 3
TRMT10A	tRNA methyltransferase 10A
WFS1	Wolframin ER transmembrane glycoprotein
ZBTB20	Zinc finger and BTB domain containing 20
Neonatal diabetes	
ABCC8	ATP binding cassette subfamily C member 8
CISD2	CDGSH iron sulfur domain 2
GATA4	GATA binding protein 4
GATA6	GATA binding protein 6
GCK	Glucokinase
HNF1B	HNF1 homeobox B
INS	Insulin
INSR	Insulin receptor
KCNJ11	Potassium inwardly rectifying channel subfamily J member 11
NEUROD1	Neuronal differentiation 1
PDX1	Pancreatic and duodenal homeobox 1
RFX6	Regulatory factor X6
SLC29A3	Solute carrier family 29 member 3
WFS1	Wolframin ER transmembrane glycoprotein

Mitochondrial diabetes	
Insulin resistance	
AKT2	AKT serine/threonine kinase 2
INSR	Insulin receptor
LMNA	Lamin A/C
PLIN1	Perilipin 1
POLD1	DNA polymerase delta 1, catalytic subunit
PPARG	Peroxisome proliferator-activated receptor gamma
Lipodystrophy	
AGPAT2	1-acylglycerol-3-phosphate O-acyltransferase 2
BSCL2	BSCL2 lipid droplet biogenesis associated, seipin
CAV1	Caveolin 1
CIDEC	Cell death-inducing DFFA-like effector c
AKT2	AKT serine/threonine kinase 2
LMNA	Lamin A/C
PLIN1	Perilipin 1
PPARG	Peroxisome proliferator-activated receptor gamma
CAVIN1 (PTRF)	Caveolae-associated protein 1
ZMPSTE24	Zinc metallopeptidase STE24

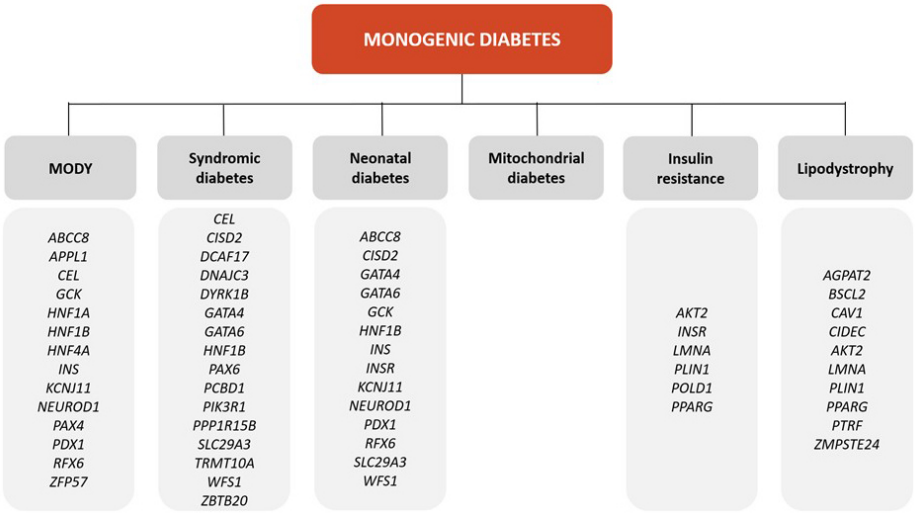

Figure 2. A schematic diagram showing the different types of monogenic diabetes often due to a mutation in a single gene with different clinical presentation, phenotypes, and mode of inheritance.

Hepatic nuclear factor 1 alpha

In the early 1990s, our group reported the first Chinese MODY family. The index patient was a young woman who presented with polyuria and polydipsia in her early 20s. Clinical examination showed severe retinopathy and proteinuria. An inquiry of family history revealed that her mother died of combined heart and kidney failure in her 50s and her grandmother died in her early 60s. Her elder sister presented with blindness due to severe retinopathy in her late 20s. One younger sister was detected to have diabetes during a minor operation at the age of 12 and has been on insulin ever since. The remaining two siblings

underwent oral glucose tolerance tests and the younger brother had a high 2-h plasma glucose level of 17 mmol/L at the age of 17. All siblings were lean. The two younger siblings detected by screening and put on insulin remained complications-free in their late 40s, while the index case died of end-stage kidney disease (ESKD) in her 50s. We later confirmed the cosegregation of a splice site mutation of *HNF1α* amongst all affected members despite their different outcomes depending on the timing of diagnosis and intervention[77].

Glucokinase MODY

Distinct from transcription factor MODY, GCK-MODY is characterized by isolated fasting hyperglycemia due to suboptimal glucose sensing with normal post-prandial insulin secretion once glucose sensing is triggered[78]. In European patients, carriers of *GCK* mutations usually have mild disease and do not require treatment[79]. In Asia, patients with GCK-MODY share features similar to those of their European counterparts, with multiple generations being affected, suggestive of autosomal dominant inheritance. However, the high prevalence of common variants for type 2 diabetes genes and beta-cell dysfunction in young Asian patients, along with other lifecourse factors, may lead to considerable variations in terms of age of diagnosis and subphenotypes both within and across GCK-MODY families[4,80,81].

Additionally, maternal-offspring *GCK* genotype concordance or discordance can affect the pregnancy outcome, which is highly relevant to young women with diabetes. Intensive insulin treatment in a GCK-MODY non-carrier may cause low birth weight in an offspring carrier who requires a high fasting plasma glucose to trigger insulin secretion. On the other hand, high fasting plasma glucose in an affected mother may lead to high birthweight in an offspring non-carrier due to fetal hyperinsulinemia[82,83]. The newly developed GCK activator[84] improved glucose sensing and triggered early insulin secretion. This new class of drug may provide precision therapy in patients with GCK-MODY, although more RCTs are needed to confirm this hypothesis[85].

The discovery of these MODY families due to "gain-of-function" or "loss-of-function" of genetic mutations supports the biological importance of proteins encoded by these genes. Some of their common variants may cause qualitative or quantitative changes in gene expression to increase the risk of type 2 diabetes and its complications. Indeed, some of the loci associated with type 2 diabetes lie within the coding or non-coding regions of MODY genes[24,25,86]. One example is the common variants of *GCK* and *GCK*-regulating proteins associated with glucose/lipid traits and ESKD[87]. Mendelian randomization analysis suggested that genetic proxies of *GCK* were causally linked to cardiovascular disease[88].

Thus, using multiomic analysis including whole exome sequencing focusing on coding regions and whole genome SNP arrays is complementary in identifying high-risk individuals with abnormal biology. Multiomic analysis in prospective cohorts with risk factors, interventions, and outcomes can provide valuable information in our pursuit of genomic medicine. However, the under-presentation of non-European populations, along with differences in ancestry, genomic architecture, and lifecourse factors, means major knowledge gaps in the heritability of YOD remain. Here, supported by other scientists, physicians with knowledge in human biology, pathophysiology, clinical medicine, drug mechanisms, technologies, and care delivery are in a good position to use these technologies and analytical tools to formulate hypotheses for translational purposes.

Wolfram syndrome/DIDMOAD

Currently, there are no guidelines on cascade family screening for heterozygous carriers of MODY genes with recessive mode of inheritance. While the homozygous carriers may suffer from severe disease with

multiple organ damage, the heterozygous carriers may only have "mild" diabetes as a feature. One such example is Wolfram Syndrome, also known as DIDMOAD, the acronym for Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness[89,90]. The latter is a rare, autosomal recessive neurodegenerative disease due to mutations in the gene encoding Wolframin ER transmembrane glycoprotein (*WFS1* and *WFS2*) located in the Chromosome 4p16.1 region. These genes encode a transmembrane protein causing abnormal calcium metabolism and protein misfolding in the endoplasmic reticulum. These patients might also have mitochondrial dysfunction with marked heterogeneity in phenotypes[89,91].

In the 1990s, we reported a Chinese man diagnosed with diabetes at the age of 12 with progressive loss of vision due to optic atrophy. At the age of 17, he presented with severe hyperglycemia and polyuria which was due to diabetes insipidus. Ultrasound imaging revealed severe hydroureters associated with autonomic dysfunction. With the loss of vision, the patient learned to tune the piano but later developed high-tone deafness. Both his parents had mild diabetes. Although genetic diagnosis was not available at the time, his clinical presentation suggested Wolfram Syndrome/DIDMOAD[91]. Given the severe disabilities and premature mortality in these homozygous carriers, preconceptual counseling would have prevented these tragic stories while there are ongoing efforts to discover druggable pathways or use gene editing to correct these genetic abnormalities and their consequences[89,90].

Clinical diagnosis versus prediction by bioinformatics and algorithms

Most of the recommendations on diagnosis and treatment of MODY were based on experiences in Europeans[79]. The current classification of P/LP variants is largely determined by the rarity of variants, functional annotations, experimental studies, and/or reported cases. Many of these variants were predicted to cause amino acid change, protein truncation, premature initiation, or termination of gene transcription. Due to their high frequency and incomplete penetration, they were often classified as variants of uncertain significance (VUS). Although heterozygous carriers of variants with recessive mode of inheritance or carriers of compound heterozygous mutations are not considered to have monogenic diabetes[92], these carriers may develop diabetes in the presence of additional risk factors[51]. The significance of these VUS requires evaluation through detailed phenotyping, systematic family screening, and testing for cosegregation, especially in populations with a high prevalence of familial YOD[71].

Discounting the significance of these VUS may lead to missed opportunities for early diagnosis and intervention. As an analogy, 7% of Asians have thalassemia minor due to a single copy of mutation affecting one of the oxygen-carrying globin proteins in the erythrocytes[93]. The high prevalence of these mutations may have selection advantages. Heterozygous carriers have mild anemia and an increased risk of diabetes[94] due to the close link between glucose and iron metabolism[95]. Given the recessive mode of inheritance, one in four offsprings of parents who are both carriers may suffer from thalassemia major, requiring lifelong blood transfusion, iron overload, multi-organ failure, and premature death[96]. In some countries, preconception counseling for couples who are both carriers has prevented the birth of these homozygous carriers[97,98].

POLYGENIC RISK SCORES, RISK STRATIFICATION AND PRECISION THERAPY

Since the launching of the Human Genome Project in 1993[99], a wealth of knowledge has been amassed confirming the inter-ethnic differences in genomic architecture with hundreds of loci scattered throughout the genome associated with complex diseases including diabetes, cardiovascular-renal disease, and cancer[24,86]. In keeping with their frequent, albeit not invariable clustering, these diseases share common variants, which are mainly non-coding with small effect size, suggesting perturbation of common pathways in these disease clusters[86,100-102]. While some of these genetic loci are potential drug targets[103,104], other

researchers advocate the use of polygenic risk scores to increase the cost-effectiveness of screening for high-risk subjects for intervention and individualized treatment[105-107].

In people with youth-onset diabetes (less than 18 years old), there are ongoing large-scale epidemiological, genetic[10], and interventional studies[11] to discover the causes and evaluate interventions. By contrast, there are major research gaps in adults with YOD often diagnosed by physicians who are less familiar with rare diseases[12]. Amongst patients diagnosed before the age of 40 years, there is heterogeneity in etiologies and phenotypes depending on whether they present during infancy, childhood, adolescence, or early adulthood. Large-scale GWAS have identified different genetic variants associated with the age of diagnosis, with many of them being associated with common forms of type 2 diabetes[108]. These variants can be used to generate polygenic risk scores to stratify risk for prevention and early intervention. Structured lifestyle modification and medications including metformin and alpha-glucosidase inhibitors prevent or delay the onset of type 2 diabetes in people with impaired glucose tolerance[3]. In the United States[109] and China Diabetes Prevention Program[110], metformin was most effective in people under the age of 45. Thus, by combining familial, genetic, and clinical risk scores, it is possible to segment the 20%-30% of individuals at the highest risk of developing YOD with less biological resilience for early intervention[111].

Likewise, while the early use of cheap generic medications such as statin and renin-angiotensin system inhibitors are likely to be cost-effective in preventing cardiovascular-renal events in young patients with type 2 diabetes[3], the cost-effectiveness of new drugs such as glucagon-like peptide 1 receptor agonist (GLP1-RA), sodium-glucose-cotransporter 2 inhibitor (SGLT2i), and non-steroidal mineralocorticoid receptor antagonist[112] in young patients remain uncertain despite their high lifetime risk for complications[113]. In this connection, diagnosis of familial hypercholesterolemia followed by cascade screening has been shown to be cost-effective for preventing premature cardiovascular disease through early detection and intervention[114]. While definitive trials are needed to prove the value of genetic testing, RCTs have demonstrated that the provision of personalized[115,116] and genetic information on the risk of diabetes complications empowered self-management and reduced negative emotions[117-119].

USING BIOGENETIC MARKERS TO INCREASE THE PRECISION OF DIAGNOSIS AND TREATMENT

There are considerable overlaps in clinical presentations and phenotypes amongst people with autoimmune type 1 diabetes, LADA, MODY, type 2 diabetes, and other atypical forms of diabetes in young people. Once diabetes develops, glucolipotoxicity, inflammation, and oxidative stress can cause dedifferentiation and apoptosis of beta-cells and perpetuate glycemic deterioration[22,120]. For the same set of risk factors (e.g., smoking, obesity), there are considerable inter-individual variations in rates of decline of beta-cell function[81]. An estimated 50% of beta-cell function might have been lost at the time of diagnosis of diabetes[121]. Early optimization of glycemic control could restore beta-cell function[122] and delay treatment escalation[56,57]. These data support the use of biomarkers to improve the precision of diagnosis and treatment to preserve beta-cell function[123]. In the ongoing PRISM-RCT, we observed the importance of nature and nurture in these young patients in whom varying combinations of genetic predispositions, lifecourse factors, timeliness of diagnosis, lifestyles, and access to care can result in different presentations, trajectories, and outcomes [Figures 3 and 4 and Table 2].

In Hong Kong Chinese patients with YOD, analysis of stored samples indicated that 8% had LADA, but the majority had not been given early insulin treatment. Compared to their peers with classical type 1 diabetes, patients with LADA had a 2.8 times higher risk of ESKD, likely due to poor glycemic control with delayed insulin treatment. Amongst patients treated with insulin, the mean reduction in HbA1c at 6 months was

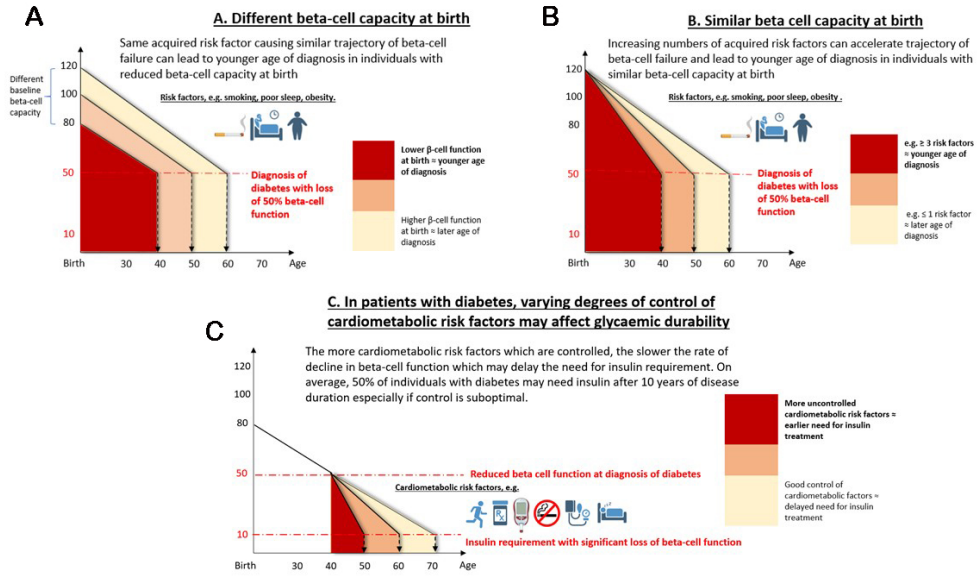

Figure 3. A conceptual framework indicating the decline in beta-cell function due to natural aging, metabolic stress, and inflammation (e.g., obesity, use of tobacco, psychosocial stress, poor sleep, infection), which can influence the age of onset of diabetes. For a given trajectory of beta-cell function, those with reduced beta-cell capacity are more likely to decompensate early. With the onset of diabetes, ongoing lipoglucotoxicity and inflammation can further accelerate beta-cell loss, resulting in insulin requirement. Reducing metabolic stress, especially in people with vulnerable beta-cell function, may delay the onset of diabetes and insulin requirement[128].

2.3% in patients with LADA versus 0.7% in those without autoantibodies, calling for early detection of LADA to avoid delayed insulin treatment[124,125].

Between 1995 and 1998, fasting, stimulated, and random CPs were measured in 280,539 inhabitants in Skaraborg from Sweden, of whom 3.2% had diabetes. Amongst 1,093 patients with well-defined diabetes types, all three CP measurement protocols were robust in discriminating type 1 from type 2 diabetes, based on receiver operator curve (ROC) analysis, with random CP having the best performance. The optimal cut-off value was 0.50 nmol/L for random CP, 0.42 nmol/L for fasting CP, and 0.60 nmol/L for stimulated CP[126]. Other researchers proposed using fasting plasma CP < 250 pmol/L with or without autoantibodies to indicate absolute or severe insulin deficiency[46]. In our prospective analysis, Chinese patients with type 2 diabetes who had GADA positivity and low CP had the fastest progression rate to insulin treatment with a high risk of severe hypoglycemia. These patients should benefit from a basal-bolus insulin regimen to optimize glycemic control. Patients with GADA but residual CP level had a similar risk of insulin requirement as their peers without autoimmune type 2 diabetes. Counter-intuitively, patients with high CP, suggestive of insulin resistance, were more likely to progress to insulin treatment[125]. In a cohort of Chinese of working age, we used fasting plasma CP and glucose to derive Homeostasis model of assessment insulin resistance (HOMA-IR) and HOMA-beta to estimate insulin resistance and deficiency. In keeping with their contributory roles in diabetes[127], both HOMA-IR and HOMA-beta predicted incident diabetes in people with normal glucose tolerance. In patients with YOD, these indexes independently predicted early insulin requirement[128]. Due to these non-linear relationships of CP, the estimation of HOMA-IR and HOMA-beta, which takes prevailing PG into consideration, should be more informative. The use of HOMA indexes and autoantibodies will help physicians make more precise diagnoses and treatments.

The growing number of glucose-lowering drugs with different mechanisms of action calls for better patient segmentation to prioritize treatment selection[22]. Using HOMA indexes, GADA, age, BMI, and age of diagnosis, researchers classified patients with type 2 diabetes into five subtypes. Patients with severe insulin resistance had a high risk of chronic kidney disease, while those with autoimmune or severe insulin deficiency required early insulin treatment[129]. These studies had been replicated in other populations, including Chinese, in whom severe insulin-deficient type was more common and mild age-related diabetes was less common than their European counterparts. In all five subtypes of diabetes, Chinese patients had an earlier age of diagnosis, lower BMI, HOMA-beta, and higher HbA1c[130,131]. In a cross-sectional analysis, Chinese patients with YOD had lower beta-cell function with a steeper negative relationship between beta-cell function and disease duration than that in their late-onset counterparts[81].

NEED FOR RANDOMIZED CONTROLLED TRIAL GUIDED BY PHENOTYPES TO INFORM PRACTICE

Despite the organ-protective effects of SGLT2i and GLP1-RA, such evidence mainly came from high-risk patients with complications. Optimal glycemic control remained the cornerstone in diabetes management, and given the variable phenotypes suggestive of contribution from different etiologies, more studies are needed to guide treatment based on phenotypes or genotypes[132]. For example, in the Trimaster Study, patients with type 2 diabetes and an estimated glomerular filtration rate of 60-90 mL/min/1.73m^2 were more responsive to DPP4i than SGLT2i. Patients with low BMI (< 30 kg/m^2) were also more responsive to DPP4i than thiazolidinediones[133]. Apart from its proven benefits in preventing diabetes, metformin also reduces the risk of vascular, renal, cancer, and pneumonia events and all-cause death in type 2 diabetes[134]. There is also evidence suggesting that patients with predominant insulin deficiency may benefit from the addition of insulin secretagogues (e.g., SU), prandial insulin regulators (e.g., GLP1-RA, DPP4i, AGI), or insulin treatment, while those with insulin resistance, often due to obesity, might benefit more from weight-neutral or weight-reducing therapies such as GLP1-RA and SGLT2i[135,136]. In a proof-of-concept analysis, we stratified patients with type 2 diabetes by CP and insulin treatment and reported that patients with low CP and treated with insulin had the lowest mortality rate[137].

In the VERIFY Trial, newly diagnosed patients with type 2 diabetes treated with combination therapy of metformin and DPP4i had more durable glycemic control and 30% reduced risk of progression to insulin treatment compared to those treated with metformin monotherapy followed by DPP4i only with rising HbA1c[138]. These effects were particularly evident in patients with YOD[123]. In a real-world database, patients with type 2 diabetes, the majority of whom were on metformin and/or SU, those with additional DPP4i within 2 years of diagnosis had a 30% reduced risk of insulin treatment compared to those with additional DPP4 after 3-5 years of diagnosis[57]. These findings highlight the importance of early diagnosis, early intervention, and early control in preserving beta-cell function, especially in patients with compromised beta-cell function. Against a backdrop of the declining use of SU, increasing popularity of SGLT2i, and advocacy of using GLP1-RA to replace insulin, the importance of better classification cannot be emphasized enough to ensure timely and appropriate treatment for maximizing efficacy (e.g., SU in patients with HNF1α- and HNF4α-MODY) and reducing side-effects (e.g., insulin analogs in patients with LADA or severe insulin insufficiency to avoid diabetic ketoacidosis and severe hypoglycemia). Despite their heterogeneous phenotypes and treatment responses, there is a lack of RCT data to guide diagnosis and treatment in these high-risk patients with YOD[12].

PSYCHOSOCIAL-BEHAVIORAL NEEDS AND LIFECOURSE MANAGEMENT

Perinatal development, environmental exposure, socioeconomic status, migration, education, and health behaviors[139] can interact in a complex manner to cause childhood obesity, which can track into adulthood

Table 2. Lifecourse factors that may influence the onset, trajectory, and consequences of diabetes (also refer to Figure 3)

Infancy and childhood	Adolescence	Middle age	Old age
• Ethnicity and migration • Common and rare genetic variants • Other familial factors (e.g., hemoglobinopathy, chronic hepatitis B infection) • Epigenetics and perinatal development • Low birth weight	• Childhood illness (e.g., malignancy and steroid use) • Childhood obesity • Formation of habits and lifestyles • Education • Socioeconomic status	• Obesity, metabolic syndrome, and fatty liver • Lifestyles (e.g., unhealthy diet, physical inactivity, poor sleep) • Psychosocial stress •Other risk conditions (e.g., depression, gestational diabetes, PCOS, ...) • Endocrinopathy and drug use	• Micro/macrovascular complications • Chronic kidney disease • Heart failure • Cancer • Dementia • Frailty

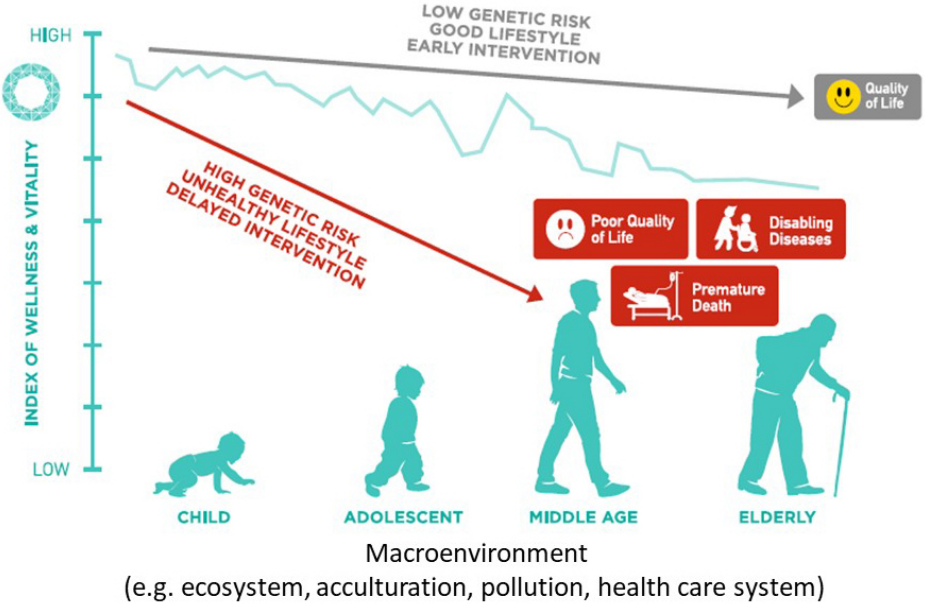

Figure 4. A conceptual diagram showing the complex interactions between nature and nurture where multiple lifecourse factors can predispose, precipitate, and perpetuate the onset and trajectories of diabetes resulting in markedly different outcomes, strongly influenced by genetic factors, early childhood development, environmental exposures, lifestyles, and access to care and education (reproduced with permission from GemVCare)[151].

and bring forward the age of onset of diabetes. Other familial factors, such as chronic hepatitis B infection and hemoglobinopathy, have been associated with diabetes in some ethnic groups[94,140]. Medical histories such as pancreatic disease, gestational diabetes, polycystic ovary syndrome, thyroid disease, tuberculosis, and mental illness may provide clues regarding the predisposition, precipitation, and perpetuation of YOD[71,141,142]. A family history of diabetes affecting multiple generations with or without syndromic features may alert the possibility of MODY or monogenic diabetes[67]. Understanding patient-reported outcome measures (PROMs) such as quality of life, competing priorities, psychosocial stress from work or family, interpersonal relationships, and life events[143] may help care providers address negative emotions, maladjustment, and poor adherence frequently encountered in young patients with diabetes[101,144-146] [Figure 3 and 4, Table 2].

PRACTICE ENVIRONMENT, CLINICAL ACUMEN AND PERSON-ORIENTATED CARE

A correct diagnosis is key to a meaningful dialogue between doctors and patients for informed and shared decision-making[147,148]. This can only be achieved through comprehensive profiling, good doctor-patient relationships, regular reviews, and quality care. However, the busy working environment, short consultation

time, and frequent changes of care providers had made this approach challenging. The many technological advances focusing on procedures have further contributed to the increasing organ-based and fragmented healthcare practices[149].

Reducing genomic medicine to practice for improving the precision of diagnosis and therapy[150,151] must be aligned with reform in undergraduate/professional education and practice environments to facilitate implementation[117]. Genomic medicine is only one of the many facets of person-orientated care which begins with good history taking, physical examination, and value-based investigations. This should allow physicians to prioritize a list of differential diagnoses followed by definitive or empirical treatment with anticipated outcomes, and action plans if the outcome is not achieved. Doctors interested in the field of diabetes need to stay abreast of the advances in genomic medicine, data analytics, and drug development and learn how to use lay language to communicate probabilities, uncertainties, and complexities. They are in the best position to assess the utility of using clinical/genetic risk scores or algorithms to segment patients for targeted treatment, exclude hormonal or drug-induced forms of diabetes, and order comprehensive genetic profiling to diagnose rare or syndromic forms of diabetes. For research-orientated physicians, setting up registers[152] will provide a powerful tool to assess the values of using new technologies and approaches aimed at addressing the many needs of a young person with diabetes[17,101,153-155]. The adoption of this person-orientated approach will bring back the science and arts of clinical medicine which is particularly relevant to patients with YOD given the implications of misdiagnosis, misclassification, and mismatched treatment.

PRISM: precision medicine to redefine insulin secretion and monogenic diabetes (PRISM) in Chinese patients with young-onset diabetes

Complexity is a key feature in internal medicine. For the same disease, different people can have different clinical presentations. For the same clinical presentation, different people can have different underlying causes. For the same treatment, different people can have different responses. It is against this background that the authors embarked upon a pragmatic 3-year RCT [Precision medicine to redefine insulin secretion and monogenic diabetes (PRISM)] where 884 patients with type 2 diabetes diagnosed before the age of 40 and aged less than 50 years underwent structured clinical assessment and comprehensive biogenetic profiling including measurement of HOMA-indices, CP, and GADA to diagnose LADA and assess beta-cell function. These patients had genome-wide genotyping for computing polygenic risk scores for beta-cell function and complications. They also had targeted gene-sequencing to detect mutations of genes for MODY and monogenic diabetes. Other PROMs included psychosocial-behavioral factors and quality of life. Half of these patients were randomized to receive 1-year intensive counseling and personalized treatment guided by their biogenetic profiles and psychosocial needs, delivered by a specialist-led multidisciplinary team in a diabetes center away from busy clinics aimed at attaining multiple treatment targets. After this 1-year multi-component management[154,156], these patients will return to their usual clinics for follow-up with yearly review at the diabetes center while the other half receive usual care. All patients will undergo re-evaluation at 3 years. The primary outcome of PRISM is the incidence of all diabetes-related endpoints and the secondary outcome is control of cardiometabolic risk factors https://clinicaltrials.gov/ct2/show/NCT04049149. The results will be analyzed within the RE-AIM framework (Reach, Effectiveness, Adoption, Implementation and Evaluation)[157] to inform planners, practitioners, and policymakers about the resources, infrastructure, personnel, logistics, and technology needed to reduce precision medicine in YOD to practice and their cost-effectiveness. This project commenced in January 2020 and completed recruitment in September 2021, and the 3-year study period will end in September 2024[18].

CONCLUSION

Diabetes is a societal, public health and personal challenge. The rapid changes in our ecosystem, physical and food environment, cultures, lifestyles, values, and perspectives have unmasked biological defects in vulnerable individuals at risk of developing diabetes at a young age. These individuals need to be diagnosed, treated, and controlled early to prevent complications, maintain earning power, and preserve quality of life. Decision makers including governments, payors, and healthcare planners are tasked with creating a health-enabling environment, building capacity, and ensuring access to affordable medications, care, and support in collaboration with industry. Likewise, care providers have the responsibility to identify unmet needs and discover new knowledge to improve outcomes. Against this backdrop, physicians standing between patients and technologies, equipped with knowledge in human biology, pathophysiology, clinical medicine, drug mechanisms, and care delivery, should spearhead the use of genomic medicine and holistic care to reclassify diabetes and implement personalized solutions in people with or at risk of developing YOD. By combining research and practice, there is a real possibility that we can use personalized and genomic data to transform care and save patient lives[3].

DECLARATIONS

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Authors' contributions

Conceptualized and wrote the first draft with contribution and finalized the paper for submission: Chan JCN
Provided critical comments and approved the final manuscript: all authors

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Chan JCN and Ma R hold patents for using genetic markers to predict diabetes and its complications for personalized care. Chan JCN, Ma R, and Lim C are cofounders of a start-up biotech company partially supported by the Technology Start-up Support Scheme for Universities (TSSSU) of the Hong Kong Government Innovation and Technology Commission. All other coauthors have no conflict of interest to declare.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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AUTHOR INSTRUCTIONS

1. Submission Overview

Before you decide to publish with us, please read the following items carefully and make sure that you are well aware of Editorial Policies and the following requirements.

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The topic of the manuscript must fit the scope of the journal. Please refer to Aims and Scope for more information.

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The journal adopts Gold Open Access publishing model and distributes content under the Creative Commons Attribution 4.0 International License. Please make sure that you are well aware of these policies.

1.3 Publication Fees

Before June 30, 2023, there are no fees for submission, processing, and publication.

1.4 Language Editing

All submissions are required to be presented clearly and cohesively in good English. Authors whose first language is not English are advised to have their manuscripts checked or edited by a native English speaker before submission to ensure the high quality of expression. A well-organized manuscript in good English would make the peer review even the whole editorial handling more smoothly and efficiently.
If needed, authors are recommended to consider the language editing services provided by Charlesworth to ensure that the manuscript is written in correct scientific English before submission. Authors who publish with OAE journals enjoy a special discount for the services of Charlesworth via the following two ways.
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1.5 Work Funded by the National Institutes of Health

If an accepted manuscript was funded by National Institutes of Health (NIH), the author may inform editors of the NIH funding number. The editors are able to deposit the paper to the NIH Manuscript Submission System on behalf of the author.

2. Submission Preparation

2.1 Cover Letter

A cover letter is required to be submitted accompanying each manuscript. It should be concise and explain why the study is significant, why it fits the scope of the journal, and why it would be attractive to readers, *etc.*
Here is a guideline of a cover letter for authors’ consideration:
In the first paragraph: include the title and type (e.g., Original Article, Review, Case Report, *etc.*) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;
In the second paragraph: concisely explain what was done, the main findings and why they are significant;
In the third paragraph: indicate why the manuscript fits the Aims and Scope of the journal, and why it would be attractive to readers;
In the fourth paragraph: confirm that the manuscript has not been published elsewhere and not under consideration of any other journal. All authors have approved the manuscript and agreed on its submission to the journal. Journal’s specific requirements have been met if any.
If the manuscript is contributed to a special issue, please also mention it in the cover letter.
If the manuscript was presented partly or entirely in a conference, the author should clearly state the background information of the event, including the conference name, time and place in the cover letter.

2.2 Types of Manuscripts

There is no restriction on the length of manuscripts, number of figures, tables and references, provided that the manuscript is concise and comprehensive. The journal publishes Original Article, Review, Meta-Analysis, Case Report, Commentary, *etc.* For more details about paper type, please refer to the following table.

Manuscript Type	Definition	Abstract	Keywords	Main Text Structure
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Review	A Review paper summarizes the literature on previous studies. It usually does not present any new information on a subject.	Unstructured abstract. No more than 250 words.	3-8 keywords	The main text may consist of several sections with unfixed section titles. We suggest that the author include an "Introduction" section at the beginning, several sections with unfixed titles in the middle part, and a "Conclusion" section in the end.
Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
Meta-Analysis	A Meta-Analysis is a statistical analysis combining the results of multiple scientific studies. It is often an overview of clinical trials.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Systematic Review	A Systematic Review collects and critically analyzes multiple research studies, using methods selected before one or more research questions are formulated, and then finding and analyzing related studies and answering those questions in a structured methodology.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Technical Note	A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applied in research.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Editorial	An Editorial is a short article describing news about the journal or opinions of senior editors or the publisher.	None required	None required	/
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Perspective	A Perspective provides personal points of view on the state-of-the-art of a specific area of knowledge and its future prospects. Links to areas of intense current research focus can also be made. The emphasis should be on a personal assessment rather than a comprehensive, critical review. However, comments should be put into the context of existing literature. Perspectives are usually invited by the Editors.	Unstructured abstract. No more than 150 words.	3-8 keywords	/

2.3 Manuscript Structure

2.3.1 Front Matter

2.3.1.1 Title

The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

2.3.1.2 Authors and Affiliations

Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

2.3.1.3 Abstract

The abstract should be a single paragraph with word limitation and specific structure requirements (for more details please refer to Types of Manuscripts). It usually describes the main objective(s) of the study, explains how the study was done, including any model organisms used, without methodological detail, and summarizes the most important results and their significance. The abstract must be an objective representation of the study: it is not allowed to contain results which are not presented and substantiated in the manuscript, or exaggerate the main conclusions. Citations should not be included in the abstract.

2.3.1.4 Keywords

Three to eight keywords should be provided, which are specific to the article, yet reasonably common within the subject discipline.

2.3.2 Main Text

Manuscripts of different types are structured with different sections of content. Please refer to Types of Manuscripts to make sure which sections should be included in the manuscripts.

2.3.2.1 Introduction

The introduction should contain background that puts the manuscript into context, allow readers to understand why the study is important, include a brief review of key literature, and conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved. Relevant controversies or disagreements in the field should be introduced as well.

2.3.2.2 Methods

Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

2.3.2.3 Results

This section contains the findings of the study. Results of statistical analysis should also be included either as text or as tables or figures if appropriate. Authors should emphasize and summarize only the most important observations. Data on all primary and secondary outcomes identified in the section Methods should also be provided. Extra or supplementary materials and technical details can be placed in supplementary documents.

2.3.2.4 Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study. Future research directions may also be mentioned.

2.3.2.5 Conclusion

It should state clearly the main conclusions and include the explanation of their relevance or importance to the field.

2.3.3 Back Matter

2.3.3.1 Acknowledgments

Anyone who contributed towards the article but does not meet the criteria for authorship, including those who provided professional writing services or materials, should be acknowledged. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgments section. This section is not added if the author does not have anyone to acknowledge.

2.3.3.2 Authors' Contributions

Each author is expected to have made substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data, or the creation of new software used in the work, or have drafted the work or substantively revised it.

Please use Surname and Initial of Forename to refer to an author's contribution. For example: made substantial contributions to conception and design of the study and performed data analysis and interpretation: Salas H, Castaneda WV; performed data acquisition, as well as provided administrative, technical, and material support: Castillo N, Young V.

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Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. BJOG 2018; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
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Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm. [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
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