Australian Genomics and RCPA Quality Assurance Program

Developing an interpretive module for genomic testing for Childhood Syndromes and ID (Medicare items 73358/9)

March 2024

Australian Genomics

Acknowledgement of Country

In the spirit of reconciliation Australian Genomics acknowledges the Traditional Custodians of country throughout Australia and their connections to land, sea, and community.

We pay our respect to their elders past and present and extend that respect to all Aboriginal and Torres Strait Islander peoples today.

Artwork Alkina

by Yorta Yorta artist, Edwards, for Australian Genomics.

Australian Genomics and RCPA Quality Assurance Program

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Executive Summary

Project Overview

Australian Government is expected to increasingly approve funding for items on the Medicare Benefits Schedule (MBS) for genomic tests (based on whole-genome or -exome sequencing). With multiple private and public laboratories involved in service delivery across Australia, there needs to be quality assurance (QA) processes in place.

This project is a collaboration between Australian Genomics (AG) and the Royal College of Pathologists of Australia Quality Assurance Programs (RCPAQAP) Ltd, a wholly owned subsidiary of the Royal College of Pathologists of Australasia. The project piloted the delivery of a dry interpretive module to test a laboratory's ability to correctly prioritise and interpret variants detected from broad genomic investigations (whole-exome or whole-genome) in the context of a given clinical question. This specific type of investigation is becoming increasingly important, for example, with MBS items such as the 'Characterisation via whole exome or genome sequencing and analysis, of germline variants known to cause monogenic disorders,' (MBS item numbers 73358/9; introduced 1 May 2020) becoming available. Similar investigations will likely become the standard of care in other clinical settings. The project is expected to lead to a sustainable assessment method by which analysis and reporting processes of Australian laboratories will be standardised.

The primary aim was to develop a sustainable program to ensure reproducibility and quality of variant interpretation and reporting in relation to MBS item numbers 73358/9. Desired secondary outcome(s) included the establishment and publication of components required to deliver QA for this Medicare funded test. This includes establishing a process for assessment, feedback reports, workforce needs, ongoing infrastructure and legal and regulatory compliance.

Methods

Human Research Ethics Committee (HREC) approval was sought to document how any additional findings would be managed, to allow for evaluation of the project and so the findings could be published. One case was selected from the AG Genomic Data Repository (GDR) which matched the MBS item number eligibility criteria. Genomic and appropriate supporting phenotypic data was shared with six Australian laboratories, that analysed and reported on the case using their standard pipelines. The working group developed scoring criteria to assess the diagnostic laboratory reports. Following receipt of feedback reports, participating laboratories were invited to complete an evaluation of the project via a survey.

Key Findings

Establishing necessary ethics, governance and data sharing arrangements was more challenging than anticipated. However, once these challenges had been navigated, all six laboratories were able to participate. Each laboratory identified and correctly classified the target variants for the case provided. Several inconsistencies were identified during assessment, across the scoring criteria and in diagnostic reporting, such as report layout, reporting sample type and ID, genetic counselling recommendations, variant interpretation and assay limitations. Almost all laboratories (n=5/6) would

consider ongoing involvement if the QA program was implemented in practice, and most laboratories (n=4/6) would like to see this scheme implemented across different phenotypes/organ systems. Suggestions from participating laboratories to improve the QA program included reduced time between analysis and providing feedback reports, providing clearer instructions related to how to approach data-based QA programs and labelling of data samples, and making the scheme more representative regarding approaches to variant interpretation.

Impacts

This project provides assurance to Australian Government, Medical Services Advisory Committee (MSAC), and anyone ordering, or consumers of genomic testing publicly funded via the MBS of the quality of interpretation and reporting of results. This project contributes to standardisation of external QA for genomic testing, in turn ensuring results are comparable between laboratory's, therefore reducing the risk of reporting incorrect results and potential harm to consumers.

Recommendations

A second pilot round is already planned and will be led by RCPAQAP. This will be important to ensure sustainability of the program. The second pilot aims to use synthetic genomic data and meet timeframes reflective of a standard external QA calendar. It is also recommended to consider consumer involvement, particularly of Aboriginal and Torres Strait Islander people to ensure analysis and reporting pipelines provide accurate reports for all consumers. As the program evolves, members should engage with National Pathology Accreditation Advisory Council (NPAAC) to discuss recurring issues observed in diagnostic reporting. Expanding the program to include more laboratories, nationally and internationally, should also be considered.

Conclusion

This project completed one pilot round of dry interpretive module to test a laboratory's ability to correctly prioritise and interpret variants detected from broad genomic investigations.

All laboratories correctly identified and interpreted the target variant, although there were several differences in diagnostic reports, such as approaches to variant interpretation and clinical recommendations. Participating laboratories want to be involved in similar future QA programs, provided there are modifications to the current format, and overall participation was viewed as a valuable learning opportunity in an important area of quality assurance.

Plain Language Summary

More genomic tests are expected to be added to Medicare - meaning Medicare will cover all or some of the cost for a larger number of genomic tests for patients in future. With multiple laboratories across Australia involved in delivering genomic testing services, it is essential to have quality assurance processes in place to make sure all laboratories find and report the same result. This project piloted the delivery of a quality assurance module to test a laboratory's ability to correctly identify the genetic change/s causing a childhood syndrome with intellectual disability (MBS item numbers 73358/9). The project is expected to lead to a sustainable assessment method that can be used to regularly assess the analysis and reporting processes of Australian laboratories.

Background

External QA is a way to objectively check a laboratory's performance using an external agency. It is an essential part of the laboratory accreditation process through the National Association of Testing Authorities, Australia (NATA) and requirements from the NPAAC mandate participation in external QA. RCPAQAP offer a range of QA programs in Australia and internationally, across all areas of pathology. Figure 1 illustrates the some of the existing external QA programs run by RCPAQAP and highlights the gap this project aims to address.

This project will complement existing QA programs for wet-lab procedures (such as sample processing, DNA extraction and sequencing) and raw data quality, by establishing a standardised process for an external QA focussing solely on dry-lab procedures, such namely variant prioritisation, interpretation and reporting.

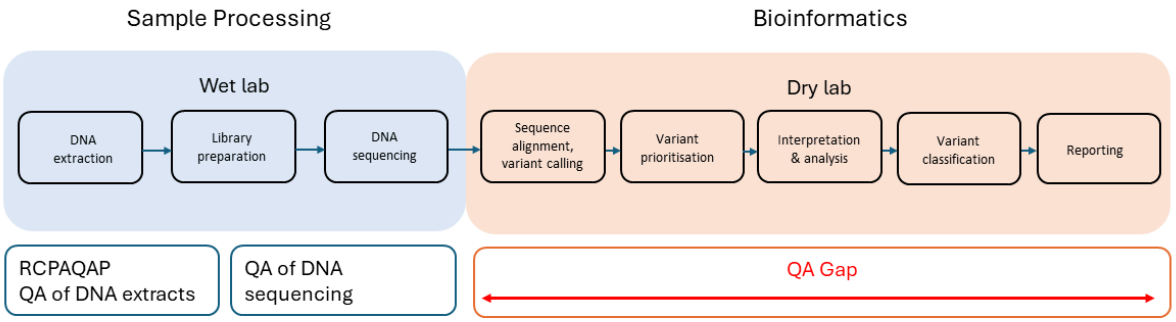

***Figure 1**. Laboratory pipeline and corresponding RCPAQAP quality assurance programs and gap. Note the gap primarily exists for testing using whole-exome or whole-genome sequencing for broad investigations.*

Introduction

There are currently no QA processes with a primary focus on variant interpretation in place in Australia. Therefore, this project piloted the delivery of a dry interpretive module to test a laboratory's ability to correctly prioritise and interpret variants from broad genomic investigations (whole exome or whole genome) in the context of a given clinical question.

The pilot focussed on genomic analysis relating to the MBS item numbers 73358/9 for 'Characterisation via whole exome or genome sequencing and analysis, of germline variants known to cause monogenic disorders'. It is highly likely that similar genomic investigations will also become standard of care in other clinical settings, for example, in the context of prenatal genomic testing and genomic testing for patients in acute care settings.

The project was a collaboration between RCPAQAP and AG. This project will assure the Commonwealth Government and MSAC of the quality and standardisation of genomic test interpretation and results for tests being publicly funded. It will also allow individual services to demonstrate competency and benchmark their processes against other laboratories offering the same tests. The module may also eventually be made available to international laboratories abiding by the same professional standards. For example, this may be made available in New Zealand, as RCPAQAP is a major supplier of QA programs to New Zealand pathology laboratories.

The working group oversaw all aspects of the operation of an end-to-end pilot and then implement a sustainable approach to QA for dry-lab processes in genomic testing. The module was designed to incorporate key steps in variant interpretation: prioritisation, curation, classification and reporting.

Aims

- To develop a QA program specifically targeted toward the process of variant interpretation from whole-exome or whole-genome investigations and evaluation of variants against a clinical referral.
- To facilitate benchmarking between laboratories offering the same tests.
- To help inform best practices for the prioritisation and evaluation of variants in broad genomic investigations (whole-exome or whole-genome).
- To investigate a sustainable model for QA program delivery to enable ongoing QA of interpretation and reporting in genomic testing.

Objectives

The objective of the project is to test the end-to-end provision of a QA program for analysis, interpretation, and reporting of genomic sequencing information. It will:

- Identify and work towards solutions to challenges in the delivery of such a program.
- Navigate the practicalities and legal, regulatory and privacy issues associated with sharing data across diagnostic NATA accredited laboratories.
- Develop a standard scoring criteria for assessing diagnostic reports generated for the QA program.
- Determine the workforce commitment required for the program beyond the pilot.
- Determine whether participating laboratories find the process acceptable.

- Identify discrepancies in reporting between laboratories, which is expected to inform the importance of a QA program.
- Publish the findings of this project.

Inputs

Project Leads: Bruce Bennetts (Sydney Children's Hospital at Westmead)

Project Coordinators: Matilda Haas, Ami Stott, Dani Webber (Australian Genomics)

Working Group Members: John Christodoulou (MCRI), Dimitar Azmanov (PathWest), Karin Kassahn (SA Pathology), Ben Lundie (Queensland Pathology), Sebastian Lunke (VCGS), Bryony Thompson (Royal Melbourne Hospital), Alicia Byrne (Broad Institute), Sze Chai (RCPAQAP), Tony Badrick (RCPAQAP).

External Collaborators: RCPAQAP, diagnostic laboratory services, CSIRO, European Molecular Genetics Quality Network (EMQN).

Consumer Involvement and Engagement with First Nations Communities: Consumer and First Nations communities were not directly involved in this project due to the standardised, procedural nature of external QA and pathology testing programs. Additionally, as only one round of the pilot was completed, much of the focus was on testing the feasibility of such a QA program. As this QA program evolves, consumer involvement could be considered for assessing the utility of reporting practices used by laboratories, particularly as pathology reports become more readily available via My Health Record. Importantly, completing a QA with Aboriginal or Torres Strait Islander data and community representation could provide insight into how the current low availability of reference genomic data impacts interpretation and reporting.

Stakeholders: AG Community Advisory Group, patients and patient organisations. Australian Government, including MSAC and Quality Use of Pathology Programs (QUPP) grant scheme. These stakeholders were not directly involved in this project. However, indirect impacts of improving standardisation of genomic tests and laboratory services provides assurance to those stakeholders that provide, fund or consume such testing.

Milestones and Timeline

This project was originally anticipated to commence in May 2021 and be completed in December 2021. Once the Royal Children's Hospital HREC advised that the project was subject to an ethics submission, navigating the ethics approvals, determining and complying with individual site governance requirements and review and sign off for data sharing agreements for all participating organisations were significant challenges causing delay for this project against its original timeline. Additionally, the changeover of project coordinator at the end of 2022 represented a period of transition for this complex project.

In early 2023, this project was flagged as a concern for non-completion. Following an operational management meeting with managers at Australian Genomics, timelines were again revised, and strict deadlines and minimal viable outputs were agreed upon to ensure project progress and completion. While the project eventually achieved its original intended laboratory participation

target, the various causes of delay lead to multiple timeline revisions. For this reason, only completion dates for milestones have been included in Table 1.

Table 1. *Project milestones and activities.*

MILESTONE	TIMELINE	ACTIVITIES
Establish working group	Completed: May 2021	• Define scope. • Identify stakeholders. • Appoint chairperson and recruit members.
Ethics approval	Completed: Mar 2023 (ethics amendment)	• Prepare project protocol. • Seek ethics approval from Royal Children's Hospital HREC.
Governance approval and data-sharing and transfer agreements	Completed: May 23	• Progress site-specific governance applications at requesting sites. • Prepare and finalise DSAs with all participating sites.
Develop scoring criteria / matrix	Completed: Jul 23	• Establish sub-working group. • Develop scoring matrix based on EMQN criteria. • Meet with EMQN to discuss approach.
Case selection & preparation	Completed: Apr 23	• Establish inclusion and exclusion criteria. • Identify potential cases with assistance from AG GDR team. • Selection based on expert review of case, available evidence and criteria. • Develop associated referral and phenotype data.
Data sharing with laboratories	Completed: Jul 23	• With assistance from AG GDR team, share data via one-off access securely shared link.

Laboratory analysis and interpretation	Completed: Oct 23	• Participating laboratories use existing pipelines to analyse and interpret data.
Scoring and assessment	Completed: Dec 23	• Develop scoring criteria and variant classification guide. • Compile scores and feedback • Discuss themes and issues.
Develop and share feedback reports	Completed: Dec 23 – Jan 24	• Experts to develop feedback reports including scores and comments.
Evaluation survey	Completed: Feb 24	• Invite participating laboratories to complete an evaluation of the pilot.
Final reporting / publication	Expected: Mar 24 (Publication post final report)	• Prepare final report. • Establish working group to prepare a publication.

Frequency of meetings: meetings were scheduled on a need's basis when feedback or discussion was required. Supplementary progress updates were provided via email. Ten working group meetings were held over 2021-2023.

Several sub-working groups were convened, for:

- Case selection and variant classification (5 meetings)
- Developing approaches to reporting (including developing the scoring criteria) (7 meetings)
- QUPP grant (including developing a pipeline for synthetic genomes (5 meetings)
- Operational management meetings were held weekly.

Budget, Expenditure and Resourcing

There was no allocated budget for this project. The project was coordinated by AG employees and provided in-kind from other organisations.

Methods

Ethics and governance

In line with advice from the Royal Children's Hospital (Melbourne) Ethics Committee, HREC approval was sought for this project. The main reasons for this included 1) to fulfil the ethics requirement for publication of the project in an academic journal, 2) to document how any new (additional) findings would be managed if they arose through re-analysis of research participant genomic data, and 3) to allow for evaluation of the project through participating laboratory surveys.

Case selection

The project plan outlined two options for sourcing genomic and phenotypic data: the AG GDR, or diagnostic laboratories participating in the QA. Figure 2 below outlines the two information source options for the project. There were two potential organisations (Royal Melbourne Hospital (RMH) and Broad Institute of MIT and Harvard) from which to source data for the pilot. RMH only test adult patients and therefore cases would not be suitable as the criteria specified childhood disorders. The Broad Institute data sharing guidelines only permit sharing of cohort data (the pilot required individual data) and while the cohort was consented for use of data in research, QA-related research was not explicitly included. Therefore, the AG GDR was most suitable as it included relevant potential cases that had also provided prior consent for data to be used for research purposes.

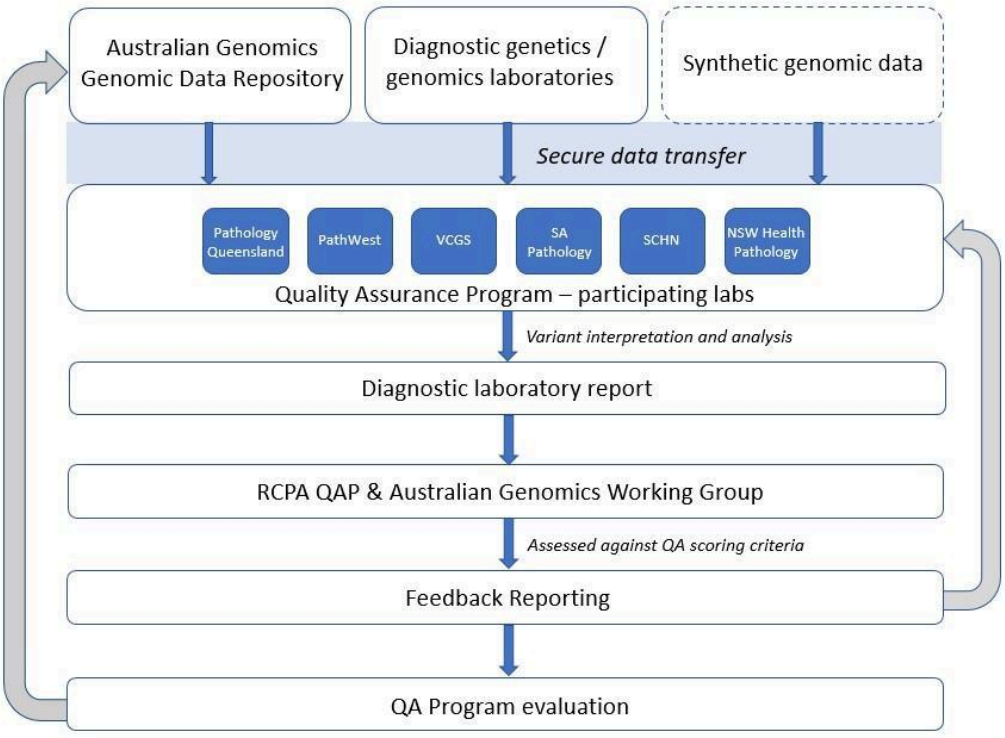

Figure 2. *Process overview for the quality assurance pilot, noting potential sources of data with different data access/request and secure transfer processes (blue arrows), including synthetic data (dotted line). Laboratories participating in the pilot received a Feedback Report benchmarking accuracy against the scoring criteria. Ongoing evaluation mechanisms will inform QA program development (grey arrows).*

The working group agreed that providing laboratories with a trio case would better reflect 'real-world' practice for interpreting similar cases. This also provided the option for laboratories to analyse the case as a singleton if that was the standard practice. Based on this, the inclusion/exclusion criteria outlined in the ethics protocol (Appendix A), and eligibility criteria for MBS items 73358/9 (Figure 3), the Australian Genomics data team filtered potential cases for trios. This narrowed down potential cases to just two. Both cases were considered suitable and met the working group's preference to have a 'straightforward' result for the pilot. Therefore, the working group was comfortable with having one working group member (JC) conduct case selection. Two of the three working group members eligible to be involved in case selection (as they were not personnel from participating laboratories) were unable to return their DSA to take part in this activity.

***Figure 3**: Details for Medicare Benefits Schedule – Item 73358. Source: [Item 73358 | Medicare Benefits Schedule (health.gov.au)](#) 19/2/23*

The selected case had undergone investigations suggestive of a diagnosis of leukodystrophy and phenotypic characteristics including abnormal facial shape, motor regression, and behavioural abnormality. The target variants in the selected case were compound heterozygous and both classified as pathogenic, including a missense paternal variant and a nonsense maternal variant.

Clinical phenotype data for the selected case was prepared to supplement the genomic data and reflect a standard referral, including basic demographic information and artificial personal information. This was limited to the minimum required to perform analysis without the laboratories being able to reasonably identify the participant.

Development of scoring criteria

A scoring criterion to be used for assessment of diagnostic reports (Appendix B) was developed based on an existing QA scoring criteria produced by the EMQN, a community interest company that provides external QA services based in the UK (Appendix C).

A sub-working group reviewed the EMQN scoring criteria for monitoring laboratory performance standards (disease specific and technical external QA programs) and tailored certain aspects to adjust the scoring criteria to be used for the pilot with the purpose of assessing a dry interpretive module. This approach was discussed with and supported by EMQN. The criteria included three sections addressing genotyping, clinical interpretation and patient identifiers/report content. Each section was worth 2.0 marks with a deductive scoring system.

A marking guide (Appendix D), including a detailed summary of the ideal use of the American College of Medical Genetics (ACMG) variant classification guidelines for curation based on the target variant, was also developed to supplement the scoring criteria. The application of ACMG criteria was agreed upon by two working group members (AB and BT). This was primarily aimed at supporting scoring by non-experts.

Data sharing and analysis and interpretation

Access to AG participant data was granted through the AG Data Access Request process, with requisite Data Access Agreements in place.

Genomic, phenotypic and referral data was provided to six NATA-accredited laboratories across Australia. Raw genomic data was shared via a one-off access, securely shared URL link using a Keybase account. Genomic data was provided as FASTQ, BAM, and VCF file formats, to enable laboratories to use their preferred input that worked best with their pipeline and usual practice. Phenotypic and referral data was provided separately via email in a password protected PDF format by the AG data team, upon confirmation of downloading genomic data NATA-accredited laboratories adhere to security, privacy, and confidentiality standards as part of their accreditation and the same standards were adhered to as part of this project.

Suitably qualified laboratory staff analysed the case data using their existing analytical and interpretation pipeline and produced a diagnostic report, in line with their standard reporting practices. Staff involved in analysis at each participating site were determined by the participating working group member from the site.

Assessment and scoring

The diagnostic reports provided by each laboratory were shared with all working group members. The working group agreed to an open scoring process, meaning diagnostic reports remained identifiable and all members were able to access the reports. Working group members included experts (working group members with expertise in variant curation, molecular genetics, etc.) and non-experts (working group members without expertise in such areas, but with a foundational understanding of genomics). Working group members used the scoring criteria and guide to assess and score all diagnostic reports and included comments to provide more detailed feedback where appropriate. Members did not score diagnostic reports from their own laboratory.

Feedback

Two members (SC & BT) with variant curation and QA program expertise compiled scores and comments to produce detailed a feedback report for each laboratory. The format aligned with established reporting practices of the RCPAQAP, use for similar QA programs. Feedback reports included the target variants and associated ACMG classification details, a performance summary including the average score against the three assessment areas (genotyping, clinical interpretation and patient identifiers), specific written feedback and overall performance in comparison to the group of participating laboratories.

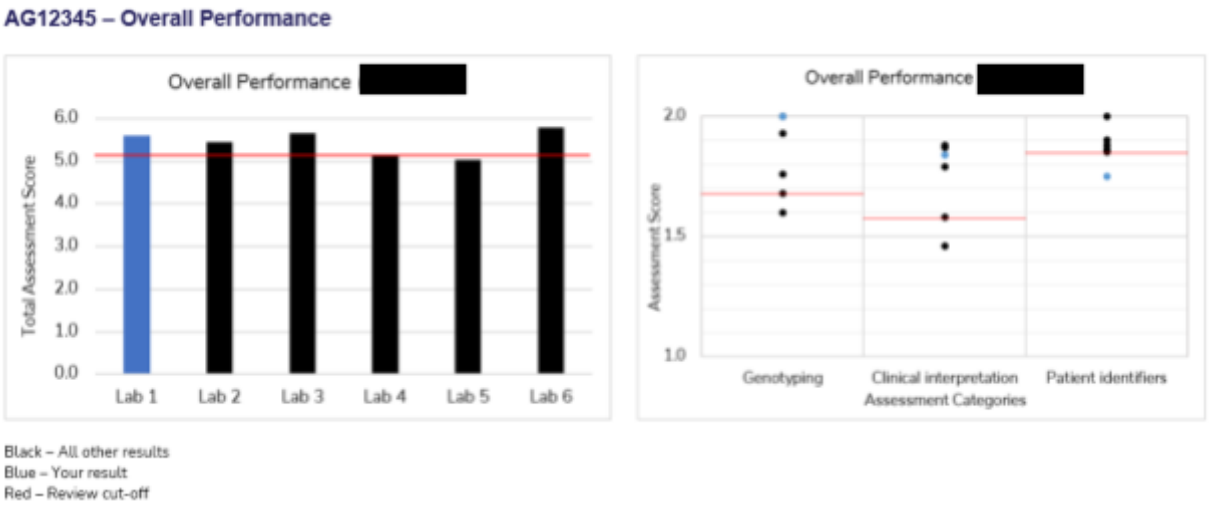

Figure 4: *Overall performance for Laboratory 1 as per Feedback Report.*

Evaluation

Participating laboratories were invited to evaluate the pilot in the form of a REDCap survey (Appendix E) including multiple-choice and free text comments. This approach aimed to understand the acceptability, areas for improvement, and the laboratories experience of the pilot. Survey results will be used to inform a second round of the QA pilot and the publication.

Results

Operationalising the pilot

The working group was established in May 2021, following which work focussed on determining the pathway for, and preparation of the ethics submission. Project coordinators also scoped potential sources of data and the associated data access requirements.

Ethics approval (HREC/81777/RCHM-2022) was originally approved in August 2022 for a single-site study. Subsequently, an amendment to change to a multi-site study was required to facilitate site-specific governance arrangements. This ethics amendment was approved in March 2023.

Six diagnostic laboratories were invited to participate in the pilot, including PathWest, SA Pathology, Pathology Queensland, NSW Health Pathology (NSWHP), Victorian Clinical Genetics Service (VCGS) and Sydney Children's Hospital Network at Westmead (SCHN). There were differing requirements for each participating organisation. For example, three participating laboratories (PathWest, NSW Health Pathology, and Sydney Children's Hospital at Westmead) required site specific governance applications in addition to Data Sharing Agreements (DSA). Each of these three sites had different processes and systems for facilitating site specific governance. The remaining three laboratories (SA Pathology, Pathology Queensland, and VCGS) only required DSAs. Facilitating governance and data sharing processes was challenging, taking several months to be finalised for all six sites.

Upon receipt of fully executed DSAs from each site, data was shared with laboratories in a staggered approach across May-July 2023. The last diagnostic report was returned in October 2023, with an average turnaround time of 9 weeks (range 4-18 weeks). As most laboratories had a representative on the working group and were therefore part of planning the pilot, limited instructions were provided to allow laboratories to follow their usual practice.

Pilot results

All laboratories correctly identified, interpreted and classified the target variants. Working group members, including three experts (KK, DA, BT) and three non-experts (SC, MH, AS), assessed diagnostic reports using the scoring criteria and associated variant classification guide, with an average score of 5.4 (range 5.0-5.8; see Table 2).

***Table 2**. Average non-expert, expert and overall scores for participating laboratories.*

Laboratory	Non-expert	Expert	Combined
1	5.7	5.4	5.5
2	5.9	5.4	5.6
3	5.3	5.5	5.4

4	5.0	5.1	5.1
5	5.5	6.0	5.8
6	5.0	5.0	5.0
Average	5.4	5.4	5.4

Assessment

Assessment of the diagnostic reports revealed several inconsistencies across both the scoring criteria and approaches to reporting. Causes for variation in scoring stemmed from the deductive approach, particularly for criteria that had multiple deduction values. For example, against the scoring criteria for 'insufficient or incorrect evidence for classification of variant', 0.2, 0.5, or 1.0 mark could be deducted, with no further information detailing how much should be deducted. This caused confusion, especially for non-expert scorers, for example when the ACMG criteria used differed from the variant classification guide, and particularly for laboratories that did not use/report ACMG criteria. Determining suitable levels of evidence was difficult for non-experts in the absence of ACMG criteria. Additionally, working group members deducted marks against different scoring criteria (sometimes with different marks) for some areas. Alternatively, some chose to comment only rather than deduct scores. Scoring was also complicated by the fact that there were two variants which influenced approaches to deducting marks, for example some members deducted marks twice if the criteria applied to both variants.

During assessment several differences were observed in the diagnostic reports, including layout, recording of sample type, genetic counselling recommendations, approaches to variation curation, and documenting assay limitations, outlined in more detail in the following sections. Some of these observations contributed to the differences in scoring and scoring criteria have been included to illustrate this where relevant.

Diagnostic report layout

The style of reporting varied across laboratories, with length varying from 3-5 pages (average 3.8 pages). Some laboratories included variant classification information and associated clinical recommendations/indications up front, whereas others distributed this information throughout the report or towards the end after presenting variant classification and interpretation sequentially. Additionally, some presented the key information in box/bolded while some included it the body of the report. As such, information was not always located in the same sections of the report.

Reporting sample type and ID

The working group noted a great deal of variability in reporting the specimen type. Reported specimen types included “DNA extracted from blood”, “DNA”, “Data only”, “EDTA whole blood”, and “Blood”. Additionally, some laboratories did not include the provided patient identifier on their own diagnostic report.

Genetic counselling recommendations

The level of detail provided regarding genetic counselling recommendations was diverse across laboratories and are detailed in Table 3. The relevant scoring criterion was: ‘counselling and/or follow up is relevant but not recommended’ with a deduction of either 0 or 0.5 marks.

***Table 3**. Genetic counselling recommendations included by participating laboratories.*

Laboratory	Genetic counselling recommendation
1	“The parents of this patient are at 1:4 risk of a recurrence of this condition in any subsequent pregnancy. Referral to a clinical genetics service is recommended for clinical review as well as professional genetic and reproductive counselling. Prenatal diagnosis is available for these variants.”
2	“Genetic counselling is recommended. Genetic test results may have significant medical implications for both the patient and genetic relatives. Referral of this patient’s parents to clinical genetics is recommended to discuss reproductive implications of this results. Correlation with the patient’s clinical phenotype, other investigations and family history is recommended.”
3	“Genetic counselling is recommended.”
4	“This result may have important implications for the extended family and genetic counselling should be considered.”
5	“Genetic counselling is recommended. Diagnostic/carrier testing in at-risk family members and prenatal testing, where appropriate, is available through this laboratory.”
6	“Genetic counselling is recommended.”

Variant interpretation

Most laboratories (n=5/6) used the ACMG classification guidelines for variant interpretation. One laboratory used their own classification system, which was not outlined in the report or publicly available. Of the five that did use ACMG, only four included ACMG criteria in the diagnostic report and the remaining laboratory was able to provide this information on request (although it was not requested for the pilot). While the ACMG criteria and strengths were applied slightly differently, this did not impact overall classification and level of pathogenicity of the variants for this case, which was the same across laboratories (Table 4). Additionally, the parent of origin for each variant was not noted in all reports.

***Table 4**. ACMG variant classification criteria applied by laboratories to classify the variants. Green rows represent codes that were part of the expected classification and orange rows represent codes that were not part of the expected classification.*

ACMG	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5** *(ACMG not reported)*	**Lab 6** *(ACMG not used)*
Variant 1						
PVS1	PVS1	PVS1	PVS1	PVS1	-	-
PS4	PS4_supporting	-	-	-	-	-
PM2	PM2_supporting	PM2	PM2	PM2_supporting	-	-
PM3	PM3	PM3_strong	PM3	PM3	-	-
PM5	-	-	-	-	-	-
PP4	-	-	PP4	-	-	-
Variant 2						
PS4	PS4	-	-	-	-	-

PM1	-	-	-	PM1	-	-
PM2	PM2_supporting	PM2	PM2	PM2_supporting	-	-
PM3	PM3	PM3_very strong	PM3_strong	PM3_strong	-	-
PM5	PM5	PM5_supporting	PM5	-	-	-
PP3	PP3_moderate	PP3	PP3	PP3_moderate	-	-
PP4	-	-	PP4	-	-	-

Assay limitations

Description of the assay and its limitations is important for the test requestor, particularly to understand how the sample was analysed if a genetic basis of the phenotype was not identified. There was considerable variability in the documentation of assay details and limitations between the diagnostic reports. One assessor deducted points from laboratories for not providing the scope of the assay, because it was not clear from the report whether exome or panel analysis was done. However, whether this deduction was warranted should be considered, as the scoring criteria says that points are deducted for this criterion only “in cases where no pathogenic variant detected”. In other reports issues such as the test sensitivity not being provided and sequencing details appearing to be stock/standard text were noted. It was very difficult for non-experts to assess this, and they were less likely to deduct points or provide comments in relation to these criteria for all reports.

Evaluation survey results

Governance and data sharing agreements were not seen as being straightforward (n=5/6), however two comments suggested this is not likely to be an issue for non-research QA programs. For the pilot, downloading the data package was straightforward for all labs (n=5) however two laboratories commented on missing metadata/relationships between data. Additionally, providing data in accessible formats was raised as a potential issue for future schemes. It took 1-5 hours for most laboratories (n=5/6) and 6-10 hours for one laboratory to work through analysis and reporting and four staff members were involved at each site. Staff included a bioinformatician (n=5/6), a genetic pathologist (n=5/6), and one or two clinical/medical scientists (n=6). One laboratory involved a senior scientist and two involved a clinical geneticist.

Scoring and feedback in the assessment report was considered transparent by half of the laboratories (n=3/6), the remainder neither agreed not disagreed (n=2/6) or disagreed (n=1/6). Comments suggested that providing clearer instructions would ensure points were not lost unnecessarily, and that further detail in the feedback reports against all criteria (rather than focusing on variant interpretation/curation) would clarify scoring. Comments also suggested that reducing the turnaround time of the feedback and making the scheme more representative of different approaches to variant classification would also improve the QA program.

The majority of laboratories (n=5/6) would consider ongoing involvement if the QA was implemented as an ongoing scheme. One laboratory responded they would not consider ongoing involvement although commented that with modifications, the program would be very valuable.
Most (n=4/6) agreed they would like to see it implemented across different phenotypes/organ systems, with suggestions including other MBS items, or areas such as renal, eye, immunology, neurology or pre-natal genomics.

Only one laboratory said ongoing involvement would mean they would discontinue involvement in other existing schemes. One laboratory commented that while they usually participate in QA programs in a cyclical manner (every three years), the low overhead cost means yearly involvement in a program of this nature would be more feasible. When asked about suggestions for implementing the pilot, comments included making the scheme more representative, avoiding cases reported in Shariant, to consider using synthetic data, and improving the data labelling and instructions around reporting based on data-only analysis. There were also comments that said this type of QA is valuable, and maintaining efforts to continue to improve the program is important.

Outcomes

Collaborative projects/activities

Quality Use of Pathology Program (QUPP) grant: This grant was used to establish an agreement between RCPAQAP and CSIRO to create in-silico datasets to be used for external QA purposes. CSIRO developed a pipeline to generate synthetic genomes using data from the 1000 genomes project database. The final project report and outputs were shared with RCPAQAP in September 2023. This included a pipeline and accompanying instructions developed by CSIRO to enable RCPAQAP to generate synthetic genomes. RCPAQAP is testing the pipeline, with the intention of generating a synthetic genome to potentially be used for a second pilot. The synthetic genome would be ‘spiked’ with a variant(s) generated by working group members. More information about the QUPP project is available at Appendix F.

Other project outputs/outcomes

- An abstract was accepted for an oral presentation at the ASDG SIG Day in November 2023. The project lead, ClinProf Bruce Bennetts, presented initial data and findings, including consistencies and variations across diagnostic reports, variant curation practices, challenges in scoring particularly for non-experts, and areas where additional guidelines or standard of practice could be beneficial.
- An abstract focusing on the variant curations aspects of the project was accepted for a poster presentation by Dr Alicia Byrne at the ACMG Annual Clinical Genetics Meeting to be held in March 2024 in Canada.

Discussion

This project provided laboratories with genomic and supporting phenotypic data for the same case for analysis and interpretation, which, to our knowledge, has not previously been done. This meant that diagnostic reports were easily comparable, including variant prioritisation practices. Additionally, assessment and scoring were open, so working group members from participating laboratories were given the unique opportunity to view diagnostic reports for the same case from other services. This generated valuable and insightful discussion regarding certain aspects of scoring and reporting, in the context of what best practice looks like and the application of existing guidelines. While all six laboratories correctly identified and classified the target variants, several differences below.

Diagnostic report layout

One of the aims of this project was to improve standardisation of laboratory reporting practices. While each laboratory generally followed NPAAC reporting guidelines[1] as well as the Mainstreaming Genomic Pathology Reports project suggestions, there was still a great deal of variability between diagnostic reports. The working group preferred summary information (i.e. clinical recommendations and pathogenicity) that was clearly presented upfront. This approach would make it clear for anyone viewing the diagnostic report, such as consumers and a range of healthcare professionals, to identify and understand the outcomes and recommendations quickly and accurately.

Reporting sample and ID

The supporting clinical data (containing phenotypic and artificial personal/demographic information) provided to laboratories included a sample type and ID number, yet there were different approaches to reporting this information. For this type of analysis (i.e. data-based) there was agreement amongst the working group that specimen type should indicate it is data along with the tissue type the data originated from, and which organisation produced the data. Tissue type was considered important as it may influence the interpretation, for example testing tumour tissue compared to blood in cancer patients. Working group members discussed the importance of clearly stating this information, particularly as data referrals are likely to increase in future. An ideal answer for the pilot would be "Data from DNA extracted from EDTA blood."

Genetic counselling recommendations

[1] Requirements for medical testing for human genetic variation (Third Edition) (safetyandquality.gov.au)

All laboratories included a recommendation for genetic counselling, however they included differing degrees of detail (Table 3). The NPAAC guidelines specify that "The responsible pathologists or scientists, should ensure that the clinical report includes: The implications for genetic relatives and recommendations for genetic counselling" (Section 7, Reporting Standards, pg 37). The working group discussed interpretations of this guideline and how it may vary depending on the result, such as the type of variant and pathogenicity, as well as the potential end user, e.g. consumer, GP, specialist. There was also discussion about balancing duty of care and responsibility of the laboratory and clinical staff. There was a degree of trust by laboratory staff that clinical staff regularly ordering genomic tests understand the implications of diagnostic reports. Regardless, the working group considered that more rigorous discussion was needed outside of this project to develop a consensus. Including examples of recommendations in future guidelines was one potential way to address this.

Variant interpretation

Although all laboratories correctly classified the target variants, the differing approaches were interesting points of discussion. While it would be neat for all laboratories participating in a QA of this nature to use the same criteria, this was not considered feasible to implement or mandate, and would be exclusionary. However, this raises the issue of how to consistently assess laboratories that do not use ACMG criteria, particularly when considering how to streamline scoring of reports, as a lack of ACMG criteria did not equate to incorrect curation. ACMG codes could be inferred from parts of diagnostic reports, although for non-expert assessors this was particularly challenging.

While the differing use of ACMG criteria in this case did not impact overall pathogenicity, the selected case was chosen because there was a 'straightforward' answer. In future rounds of QA and curation of more complex cases, inconsistent use of ACMG guidelines may be likely to result in variants being classified inconsistently. Shariant, a system for real-time sharing of variant interpretations across Australian laboratories, allows laboratories to resolve any discrepancies in variant interpretation. However, reviewing the ACMG criteria used in variant interpretation is not currently a key focus of Shariant, highlighting the utility of this type of QA to consider this aspect of variant curation in more detail.

Only four out of six diagnostic reports included the parent of origin for each variant. The working group agreed that this was important, particularly noting implications for cascade testing in first degree relatives. The scoring criteria did not have a way to capture whether this was included or if variants were accurately attributed (which was incorrect in one report). This will be addressed in future iterations of the criteria.

Assay limitations

At the end of each diagnostic report, all laboratories included sections variously named but covering test method, analysis and test sensitivity/limitations. Providing the assay limitations is an important part of the diagnostic report, as they provide information about what the assay can and cannot

detect (e.g. copy number variants, rearrangements, translocations), sequencing coverage, and the sensitivity of the test specific to the disease/phenotype being investigated. Certain kinds of genetic changes, such as repeat expansions and structural rearrangements are difficult to detect, and it is important to know whether the assay captured these.

These findings led the working group to consider in more detail why limitations are reported. They may be important to the clinician, to determine the probability of a disorder being present despite a non-diagnostic result and to guide clinical management or further testing, such as MLPA or WGS. The working group also considered whether assay limitations are reported for the clinical geneticist, or requesting clinician, other healthcare professionals or for medico-legal reasons, and whether minimal requirements or standards are needed. NPAAC guidelines 'Requirements for medical pathology services (Third Edition 2018)[2]' and 'Requirements for medical testing for human genetic variation (Third Edition)[3]' do not provide specific guidance on assay limitations. As genomic testing is mainstreamed, the communication of this information will need to be considered further.

Evaluation survey results

Noting the time taken to establish ethics and governance for this project, it is understandable that the process for establishing data sharing agreements was not seen to be straightforward. As governance processes vary based on site-specific requirements, this issue is not unique to this project and has been reported elsewhere. Governance and data-sharing agreements are not currently required as part of non-research QA programs, and this issue may be unique to the research setting for QA programs. Using synthetic data for future rounds may be a way to circumvent this issue. However, the long-term implications of creating and using synthetic data in this way are not yet understood and may evolve as the use of such data becomes more common.

There was a lengthy time between analysis, scoring and reporting. Part of this relates to the fact that scoring was also a research component of this project, and the working group members are not experts in scoring for QA programs, many of whom scored for the first time. The second pilot, facilitated by RCPAQAP, will be best placed to adhere to QA calendar, likely using scorers more familiar with the process, while also applying lessons learned through this pilot. Developing a framework that enables assessment of laboratories that do not use ACMG guidelines will be an important part of ensuring representativeness of the program.

Laboratories had constructive feedback for implementing the pilot such as improving instructions relating to handling data-only analysis. This will be documented separately and addressed in future

[2] https://www.safetyandquality.gov.au/publications-and-resources/resource-library/requirements-medical-pathology-services-third-edition-2018

[3] https://www.safetyandquality.gov.au/publications-and-resources/resource-library/requirements-medical-testing-human-genetic-variation-third-edition

rounds as the program is iteratively updated. The low cost of this type of program, noting that only data is used (i.e. no wet lab procedures) and relatively small-time commitment (for 5/6 laboratories it took 1-5 hours), is a valuable insight. Additionally, positive feedback from laboratories, willingness to continue to participate, and most laboratories seeing the benefit of implementing this type of QA across different phenotypes/organ systems reflects the is a testament to further developing this QA program.

Impacts

Significance of the project:

- This project provides assurance to the Australian Government, MSAC, and anyone ordering or consuming genomic testing being publicly funded via the MBS of the quality and standardisation of interpretation and reporting of results. For laboratories providing the testing, this project will facilitate benchmarking of processes against aggregate data from other services offering the same tests. This will be important as more laboratories start to offer MBS testing, and as the frequency of data only referrals increase.

Key impacts:

- The project established a national working group with experts from diagnostic laboratories and research or QA organisations. Additionally, discussions have expanded to work with international experts from EMQN and learn from their expertise in providing QA for a vast range of diverse services.
- This project contributes to standardisation of external QA for genomic testing, in turn ensuring results are comparable between laboratories, therefore reducing the risk of reporting incorrect results and potential harm to consumers.
- Many of the differences observed during assessment, such as descriptions of assay limitations and genetic counselling recommendations, led to discussions about the current guidelines and potential gaps or areas for refinement. As further rounds of the pilot continue and more laboratories become involved, this QA provides a valuable opportunity to inform guidelines for reporting genomic test results and ensuring standardisation.

Implementation plans: sustainability or longevity of the project and its outputs:

- This project was a collaboration between AG and RCPAQAP to ensure sustainability of the QA program from its inception. RCPAQAP are leaders in providing QA in Australia and provided expertise in guiding the pilot to align with standard practices. This approach ensured the QA was familiar to participating laboratories and more importantly, the work for round one lead by AG can be handed over to RCPAQAP who will shortly commence a second round of the pilot. The long-term plan is to continually improve and expand the QA as an ongoing

program, as illustrated in Figure 2. This includes expanding to include more laboratories across Australia and potentially internationally.

- Alongside the pilot, RCPAQAP were awarded a QUPP grant. This grant was used to establish an agreement between RCPAQAP and CSIRO to create in-silico (i.e. synthetic) datasets to be used for external QA purposes. CSIRO developed a pipeline to generate synthetic genomes using data from the 1000 Genomes Project database. The final project report and outputs were shared with RCPAQAP in September 2023. This included a pipeline and accompanying instructions developed by CSIRO to enable RCPAQAP to generate synthetic genomes. RCPAQAP is testing the pipeline, with the intention of generating a synthetic genome which may be used for a second pilot. The synthetic genome would be 'spiked' with a variant(s) generated by working group members. More information about the QUPP project is available at Appendix F.

Limitations

The project was initially scheduled to be completed by December 2021. As part of NHMRC reporting in February 2023, the project was identified as being at risk for non-completion. Strategies to mitigate the risk of non-completion by the end of 2023 were discussed and agreed upon, in consultation with the project lead and Australian Genomics managers. Specifically, the requirements and timelines for the pilot were readjusted, and it was agreed that DSAs must be returned by 1 April 2023 to participate in case selection, that pilot QA data would be shared no later than 1 July 2023 and that a minimum of three (out of a potential six) participating laboratories would meet the requirements for the pilot.

Implementing stricter timelines for returning DSAs and data-sharing had a positive impact by encouraging more active progression with each participating organisation. Additionally, this momentum resulted in all six participating laboratories completing governance and data-sharing agreements in time and were able to continue full involvement in the project.

The intention of the project was to establish a sustainable QA program, and with only one pilot round completed over an extended timeframe, evaluation feedback will only be able to be addressed beyond the pilot round. However, RCPAQAP have been involved throughout the project, and will have access to evaluation feedback, which will facilitate further refinement of the QA program.

As outlined in the protocol, one of the objectives is to build upon existing agreements to navigate data sharing requirements to regularly transfer genomic data between national and international diagnostic services and establish perpetual data sharing agreements. Complying with individual site governance requirements and review and authorisation of DSAs for all participating organisations was a main challenge for this project. Implementing, communicating, and enforcing timeframes for

key project milestones worked well to facilitate progression of the project. Establishing perpetual data sharing agreements has not yet been explored.

Additionally, the pilot included just six laboratories, which is a relatively small number in the scheme of ongoing QA programs and limits the generalisability of the findings.

Further, as resources such as ClinVar and Shariant continue to expand in their uptake across Australia and internationally, the ability to choose novel variants in the future will likely be a challenge. Ensuring evidence is not directly taken from those sources could be difficult.

Recommendations and Future Directions

RCPAQAP intends to progress a second pilot round and aims to use synthetic data. The longer-term aim will be to establish a sustainable QA program for a dry interpretive module. A natural progression for this project will be expanding to other clinical settings, which could include horizon scanning or monitoring of genomic tests progressing through MSAC. This would ensure QA programs remain current as the implementation of genomic testing in practice continues to grow.

The QA should also evolve to include a larger cohort of laboratories, which may include providing a broader range of data input files to facilitate inclusion of laboratories with different types of established pipelines. A key focus for future rounds should also be adhering to a realistic external QA calendar.

As the QA program matures, recurring issues and themes could prove valuable source of data to inform recommendations in reporting guidelines produced by NPAAC.

Conclusion

This project piloted the delivery of a dry interpretive module to test a laboratory's ability to correctly prioritise and interpret variants detected from broad genomic investigations.
Laboratories correctly identified and interpreted the variant in the selected case. There were differences in diagnostic reports, such as approaches to classification and clinical recommendations, highlighting the utility in such a QA program in ensuring consistency across Australia, which may become more critical for complicated cases. Participating laboratories would consider ongoing involvement in a routine QA program provided there are modifications and suggested areas for improvement which can be addressed in future iterations of the scheme. In general laboratories viewed this as a valuable experience and an important initiative in addressing a current QA gap. Suggestions for improving existing guidelines to enhance consistency in reporting practices may appear overtime. Handover to RCPAQAP is the first step to establishing a sustainable QA program.

Appendices

Appendix A. Project Protocol

Appendix B. Scoring Criteria

Appendix C. EMQN Schema

Appendix D. Detailed Marking Guide

Appendix E. Evaluation Survey

Appendix F. QUPP Final Report