Genetics in Medicine Open (2023) 1, 100820

ELSEVIER

Genetics in Medicine OPEN
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine-open

ARTICLE

Evolution of virtual gene panels over time and implications for genomic data re-analysis

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ARTICLE INFO

Article history:
Received 2 May 2023
Received in revised form
19 May 2023
Accepted 22 May 2023
Available online 30 May 2023

Keywords:
Clinical genomics
Diagnosis
Disease genes
Panelapp Australia
Rare genetic disease
Re-analysis of genomic data

ABSTRACT

Purpose: Re-analyzing genomic information from patients without a molecular diagnosis is known to improve diagnostic yields. There are different mechanisms responsible for this increase, but the discovery of new, and refinement of existing, gene-disease relationships are one of the most prominent drivers of new diagnoses. This study examines the incorporation of new knowledge into virtual diagnostic gene panels and how this affects the potential for re-analysis.
Methods: We used PanelApp Australia to explore how the gene content of 112 rare-disease panels evolved between 2019 and 2022. By dividing these panels into groups that examined Specific and Broad rare-diseases clinical testing indications, we determined the granular changes in panel composition.
Results: Characterizing how the panels present at the launch of PanelApp Australia changed, revealed that the diagnostic genes available for analysis increased in 82% of the Specific rare-disease panels and in 97% of the Broad rare-disease panels. Examining how the panels had evolved showed that different panels were changing at different rates and in different ways. The median number of diagnostic grade genes in the Specific rare-disease panel increased by 4 (0-63), whereas the median number of gene gains in the Broad rare-disease panels was 27 (0-432). Monthly snapshots demonstrated that these changes were highly variable among different panels.
Conclusion: Knowledge about gene-disease associations is changing dynamically. Using fixed time periods may not be the best strategy to guide re-analysis frequency, as a result, some conditions may benefit from an approach based on the availability of new information rather than the passage of time.

The Article Publishing Charge (APC) for this article was paid by Nicola Waddell.

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doi: https://doi.org/10.1016/j.gimo.2023.100820

Introduction

The use of genomic sequencing in clinical laboratories has significantly improved diagnostic rates in rare disease,[1,2] providing more patients with a molecular diagnosis.[3,4] This has resulted in benefits to individuals, families, and health care systems, through improved prognostication, the avoidance of unnecessary investigations and procedures, and the provision of accurate reproductive counseling and timely access to targeted therapies.[5-11] Although a significant achievement, it is essential to recognize that large numbers of rare-disease patients who undergo genomic testing do not receive a molecular diagnosis.[1,12]

The re-analysis of existing genomic data from undiagnosed patients has emerged as a key method to increase diagnostic yield and provide more individuals with a molecular diagnosis.[5,13-16] Although there are no universally accepted guidelines for re-analysis, many publications report an increase of approximately 10% in yield after a period of 2 to 3 years after the initial analysis.[13-16] This has led to many accepting this window as an appropriate period for re-analysis. However, this is not the only determinant of re-analysis frequency, as many clinicians elect to re-analyze their patient's data before this period, after the emergence of new clinical information or familial data.[5,17,18]

Routine re-analysis has the potential to provide more patients with a molecular diagnosis. However, there are still many questions and challenges that must be overcome to implement practical re-analysis solutions in a health care setting.[5,18] One question surrounds the appropriate frequency for re-analysis. A recent meta-analysis of the patients who have received a new diagnosis after re-analysis revealed that 62.5% of these diagnoses were because of the discovery of new variants and new genes associated with the relevant condition and gaining a better understanding of the connections between previously identified variants and the condition,[14] a result consistent with other similar studies.[5,16]

Many of the publications reporting a new diagnosis for patients through re-analysis were performed on heterogeneous cohorts of patients, which included a range of different conditions and clinical presentations.[13,14,16] Because our understandings of the molecular components of different conditions are likely changing at different rates, it is possible that some conditions might see different increases in yield when re-analyzed. As a result, patients with different conditions may benefit from different re-analysis intervals. However, the role that an individual's condition plays when determining when it is most appropriate to re-analyze their existing genomic data, is unknown.

Here, we examined how the content of virtual gene panels available on an open database, PanelApp Australia, changed over a period of 2 and half years. This allowed us to determine how our understandings of the molecular components of different groups of conditions have changed over time and theorize how these changes may inform re-analysis practices. To achieve this, we characterized the specific changes in panel composition from virtual panels used in rare-disease genomic analysis, determined the median amount of change in each panel as well as more granular patterns of change over time.

Materials and Methods

PanelApp Australia background

PanelApp is an open knowledge base that brings together experts to crowd source the development and refinement of virtual gene panels used in the analysis for different clinical indications.[19] Originally developed by Genomics England for the 100,000 Genomes Project, a separate instance was deployed in November 2019 by Australian Genomics. A key feature of PanelApp is the traffic light system, which is used the classify each of the genes in a panel.[19] This classification system uses a combination of case-level and experimental data to determine which genes have sufficient evidence to be analyzed for a specific clinical indication. The green rating denotes genes with significant clinical evidence, which can be used in diagnostic reporting (Diagnostic Genes). A green PanelApp rating is comparable to a "Definitive" or "Strong" rating from ClinGen or a "Confirmed" rating by G2P.[20,21] The amber rating is given to "borderline" genes that have not accumulated enough evidence to be confidently used clinically, whereas the red rating is indicative of genes that only have low levels of evidence. It is not recommended that genes with an amber or red rating are used clinically.[19]

Study design

To characterize how genes associated with different conditions have changed over time, we assessed every version of selected panels in PanelApp Australia at monthly "snapshots," ranging from the first release of PanelApp Australia (November 2019) to May 2022. This period of time is similar to the suggested re-analysis window.[13,14,16] To ensure the most comprehensive version of the panel released in each month was examined, the version present at 11:59 PM on the last day of the month was selected as the monthly representative.

Selection of panels to be included in the analysis

In addition to diagnostic panels, PanelApp Australia contains screening panels as well as large, amalgamated panels made up of multiple smaller, independent panels termed "super panels." To reduce duplication and to focus on the conditions in which the identification of a single pathogenic variant in a known disease gene can produce a new diagnosis, we removed all panels listed as "super panels," as well as panels designed to be used for screening. In addition

to this, we also restricted our analysis to panels labeled with the "rare-disease" tag. Panels that did not contain any genes, such as panels that exclusively contained copy number variants or short tandem repeat regions, were excluded. The panels collected from PanelApp Australia were then manually reviewed and classified as either "specific" or "broad" panels to distinguish panels used in the testing of individuals with very specific clinical indications, for example, Alagille syndrome, Rasopathies, and those that are used for testing individuals with nonspecific clinical presentations, such as intellectual disability. This classification was independently reviewed (A.R. and Z.S.). For the analyses that specifically examined how panels had changed over time, only panels that existed in November 2019 and May 2022 were included.

Capturing the information from PanelApp Australia

We developed PanelApp Downloader, Analyzer-Web Application Navigator (PADA-WAN) to capture and characterize the information within PanelApp Australia. PADA-WAN is made up of set of Python custom scripts. PADA-WAN operates in 2 parts. The first part identifies every panel within PanelApp and sets about systematically downloading every available version of each panel before summarizing this information (Supplemental Figure 1A). The second part compares the same panel at different time points to characterize how each panel has changed (Supplemental Figure 1B). PADA-WAN was run the 1st of June 2022. To ensure PADA-WAN was operating correctly, the information from a select number of versions, from a select number of panels that had been downloaded by PADA-WAN were compared to the matched versions of these panels in the PanelApp web interface.

All scripts used in these analyses are available at: github.com/MedicalGenomicsLab/PanelApp_Pipeline.

Summarizing the information from PanelApp Australia

The distribution of panel size in the cohort of panels downloaded from PanelApp Australia was assessed with a Shapiro-Wilk test to determine the metrics that should be reported in this manuscript.

Changes to panel content over 30 months

The specific changes in the genes associated with each panel were determined by comparing the individual genes in one version of a panel with other versions of the same panel (Supplemental Figure 1, Script 3, Scripts 4). Here, this was achieved by determining the genes that were present on the last day of the month for each panel and comparing this information with the genes present in the version of the panel from the month immediately preceding it, for each month from the launch of the panel through to May 31, 2022. Analysis of this information identified the number of genes added to a panel, the number of genes removed from a panel, and the number of genes that changed diagnostic status as well as the number of updates each panel received each month.

The Kruskal-Wallis test was used to determine if the differences between groups was significant.

The panels that underwent the greatest amount of change were determined by identifying those that saw the largest number of diagnostic genes added to the panel/the largest number of genes upgraded to diagnostic status (95th percentile). To ensure that larger panels were not biased, the panels with the largest proportional increases were also identified.

Visualization

Figures were produced in R (4.1.3) using the ggplot2 (3.3.5) library.

The code used to generate figures is available from: github.com/MedicalGenomicsLab/PanelApp_Pipline/PADA-WAN/2-3_Visualisations.

Results

A summary of PanelApp Australia and an introduction to the rare disease panel portfolio

A total of 271 different panels were downloaded from PanelApp Australia on June 1, 2022. Representing the period of time from the launch of the database on November 19, 2019, to May 31, 2022, a total of 63,901 different versions of panels were downloaded (Supplemental Table 1).

At launch, PanelApp Australia contained 150 panels, and over the 2.5-year analysis period, an additional 121 panels were added to the database (Table 1). The addition of new panels to the database was not uniform, with certain periods associated with the release of larger numbers of panels that examined additional conditions (Figure 1A). The number of new panels added to PanelApp Australia has decreased over the analysis window. No new panels were added between the beginning of 2022 and May 31, 2022. This reflects the completion of activity directed at consolidating existing panels from Australian research studies, clinical groups, and diagnostic laboratories.[22,23]

To explore the potential relationship between new gene-disease associations and new diagnoses from existing data, we focused on conditions in which the identification of a single, pathogenic variant in a known disease gene can produce a new diagnosis. Therefore, we chose to examine rare-disease panels, while excluding super panels, screening panels, and the panels that only contained copy number variants and short tandem repeat regions. We also excluded a panel that examined Mendeliome. Although this panel is

Table 1 A summary of the panels within PanelApp Australia and the different rare-disease panel cohorts

Cohort	No. of Panels at Time of Analysis (May 2022)	No. of Panels at Launch (Nov 2019)	No. of Updates Made to Panels (May 2022)
Every panel in PanelApp Australia	271	150	63,901
Specific rare disease panels	131	79	9962
Broad rare disease panels	65	33	25,579

not technically a super panel, it shares many traits. After this process, 196 panels remained.

Rare-disease panels were available for a large range of clinical indications. Some panels were designed to study very specific conditions, whereas others were broader and contained genes associated with a wide range of related clinical phenotypes. This reflects clinical practice and demand.[22] Each of the 196 rare-disease panels were manually reviewed and classified as either "specific" or "broad." This review identified 131 Specific rare-disease panels and 65 Broad rare-disease panels (Table 1).

There was a large range in the number of genes present in the rare-disease panels. As of May 31, 2022, there was a significant difference in the number of genes present in each of the 131 Specific rare-disease panels and the 65 Broad rare-disease panels (P value $= 2.2 \times 10^{-16}$, Kruskal-Wallis). The number of genes in the Specific rare-disease panels ranged from 2 to 144 (Figure 1B). Certain panels, such as those used to provide testing in Hyperoxaluria, Alagille Syndrome, and Foveal Hypoplasia, all contained less than 5 genes. Larger panels, such as Congenital Disorders of Glycosylation, Vasculitis, and Ciliopathies panels contained more than 100 genes. The range in panel size was larger for the Broad rare-disease panels, with some panels having fewer than 10 genes, whereas others contained thousands (Supplemental Table 1). Analysis of the 131 Specific rare-disease panels showed that the median panel size was 25 genes with the median number of diagnostic genes in each panel was 20. In contrast, analysis of the 65 Broad rare-disease panels showed that the median number of genes was 103, whereas the median number of diagnostic genes was 86.

During the 2.5-year analysis window, 9962 updates were made to the Specific rare-disease panels, and 25,579 updates were made to the Broad rare-disease panels. The number of updates made each month were variable (Figure 1C). There was a large range in the number of updates made to the Specific rare-disease panels (6 to 480); however, the range was an order of magnitude larger in the Broad rare-disease (6 to 4807). Analysis of all 196 rare-disease panels showed a strong correlation between the number of genes in a panel and the number of updates a panel received (Pearson correlation = 0.80), with larger panels having more updates (Figure 1D).

There were multiple reasons why panels were updated, including the addition of more supporting evidence or the revision of a comment for an existing entry. Four types of update of particular relevance to re-analysis were identified (Table 2). Each of these updates represented a single change to a diagnostic gene, which altered the information available to clinical laboratories and thus have the potential to affect diagnosis. We termed these events as gene changes.

Characterizing the number of gene changes in rare disease panels over a 2.5-year period

Given the capacity of gene changes to provide undiagnosed patients with a molecular diagnosis, we characterized how the rare-disease panels had evolved between November 2019 and May 31, 2022. We determined the specific genes present on the last day of the month for every month between these 2 time points. Of the 196 rare-disease panels, 112 were present at the launch of PanelApp Australia in November 2019, and as a result, it was only possible to examine the changes over the full analysis window in 79 of the Specific rare-disease panels and 33 Broad rare-disease panels.

We found that 72 of the 79 (91.1%) Specific rare-disease panels underwent at least 1 gene change during the analysis window, 65 of which contained at least 1 gene gain. All the Broad rare-disease panels contained a gene change, of which 32 of the 33 panels (96.9%) included at least 1 gene gain. The only panel not to see a gene gain was the Susceptibility to Fungal Infections panel; a small panel of 7 genes that saw 2 genes downgraded from diagnostic status (Supplemental Table 2).

Examining gene changes in both rare-disease panel groups (Figure 2), showed similar trends between the rare-disease groups with certain types of gene changes occurring more frequently than others. For example, at least 1 gene was upgraded to diagnostic status in 75.8% (n = 25/33) of Broad rare-disease panels, whereas only 41.8% (n = 33/79) of Specific rare-disease panels contained this type of gene change. It was noted that, though gene downgrades occurred in most rare-disease panels, gene removals were a rare occurrence in both groups.

The median number of gene changes from the 79 Specific rare-disease panels was 9 (0 to 83). This corresponded to a median of 4 gene gains (0 to 63) and a median of 3 gene losses (0 to 35). The median number of gene changes in the 33 Broad rare-disease panels was much larger, at 41 (1 to 919), which corresponded to 27 gene gains (0 to 432) and 12 gene losses (0 to 487). A summary of the gene changes detected in the Specific rare-disease panels and the Broad rare-disease panels are described in Table 3 and Supplemental Table 2.

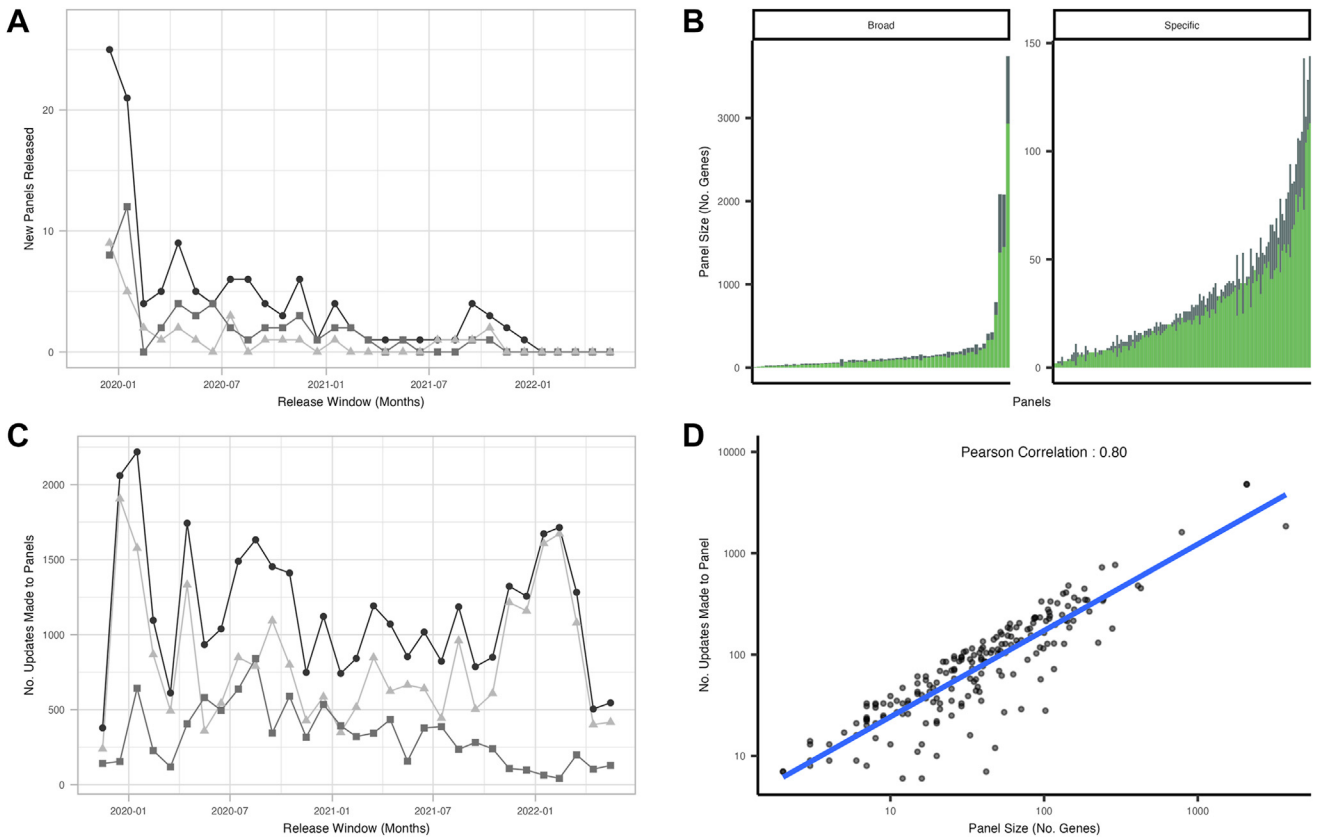

Figure 1 **A summary of the evolution of the rare-disease panels in PanelApp Australia as of May 31, 2022.** A. The number of new panels added to PanelApp Australia each month between December 2019 and May 2022. The black line shows every panel added to PanelApp Australia, over the analysis window, the gray lines show the number of rare-disease panels added each month separated into Specific rare disease panels (light-gray line with triangle markers) and Broad rare disease panels (dark-gray line with square markers). The *x*-axis represents time, shown as yyyy-mm. B. The number of genes from the 68 Broad rare-disease panels and the 128 Specific rare-disease panels from PanelApp Australia between December 2019 and May 2022 (As of May 31, 2022). Colors represent the number of genes in a panel, with the total number in gray and the diagnostic (or "Green") genes are in green. C. The number of updates made to PanelApp Australia each month between December 2019 and May 2022. The black line shows every panel added to PanelApp Australia, over the analysis window, the gray lines show the number of rare-disease panels added each month separated into Specific rare disease panels (light-gray line with triangle markers) and Broad rare disease panels (dark gray line with square markers). The *x*-axis represents time and dates are shown as yyyy-mm. D. The correlation between the number of genes in a panel and the number of updates released for the 196 rare-disease panels (Broad + Specific) as of May 2022. Both axes use a log10 scale.

Table 2 The different types of clinically relevant changes that occur over the lifetime of panel

Type of Change	Gene Gains	Gene Losses
Inclusion change	Addition of a new diagnostic gene to a panel (ie, Gene A is added to a panel)	Removal of a diagnostic gene from a panel (rare) (ie, Gene B is removed from a panel)
Classification change	Upgrade of a gene to diagnostic status (ie, Gene C is upgraded from amber status to green status)	Downgrade of a gene from diagnostic status (ie, Gene D is downgraded from green status to red status)

One of the more active panels was the Ciliopathies panel. This Specific rare-disease panel contained 112 diagnostic genes in November 2019, and 113 diagnostic genes in May 2022; however, this panel saw the addition of 20 new diagnostic genes, 10 existing genes upgraded to diagnostic status, 3 diagnostic genes removed from the panel, and 23 diagnostic genes downgraded (Supplemental Table 2). This analysis also revealed activity in the Rasopathy panel, a clinical entity considered to be well defined. In total 9 gene changes were made; 6 gene gains (4 new, 2 upgrades) and 3 gene losses (3 downgrades).

Examining some of the changes in the Rasopathy panel in more detail showed that the first gene change was the addition of *RRAS* to the panel in January 2020 and was based on information in the literature. However, on July 3, 2020, this gene was downgraded to amber status after

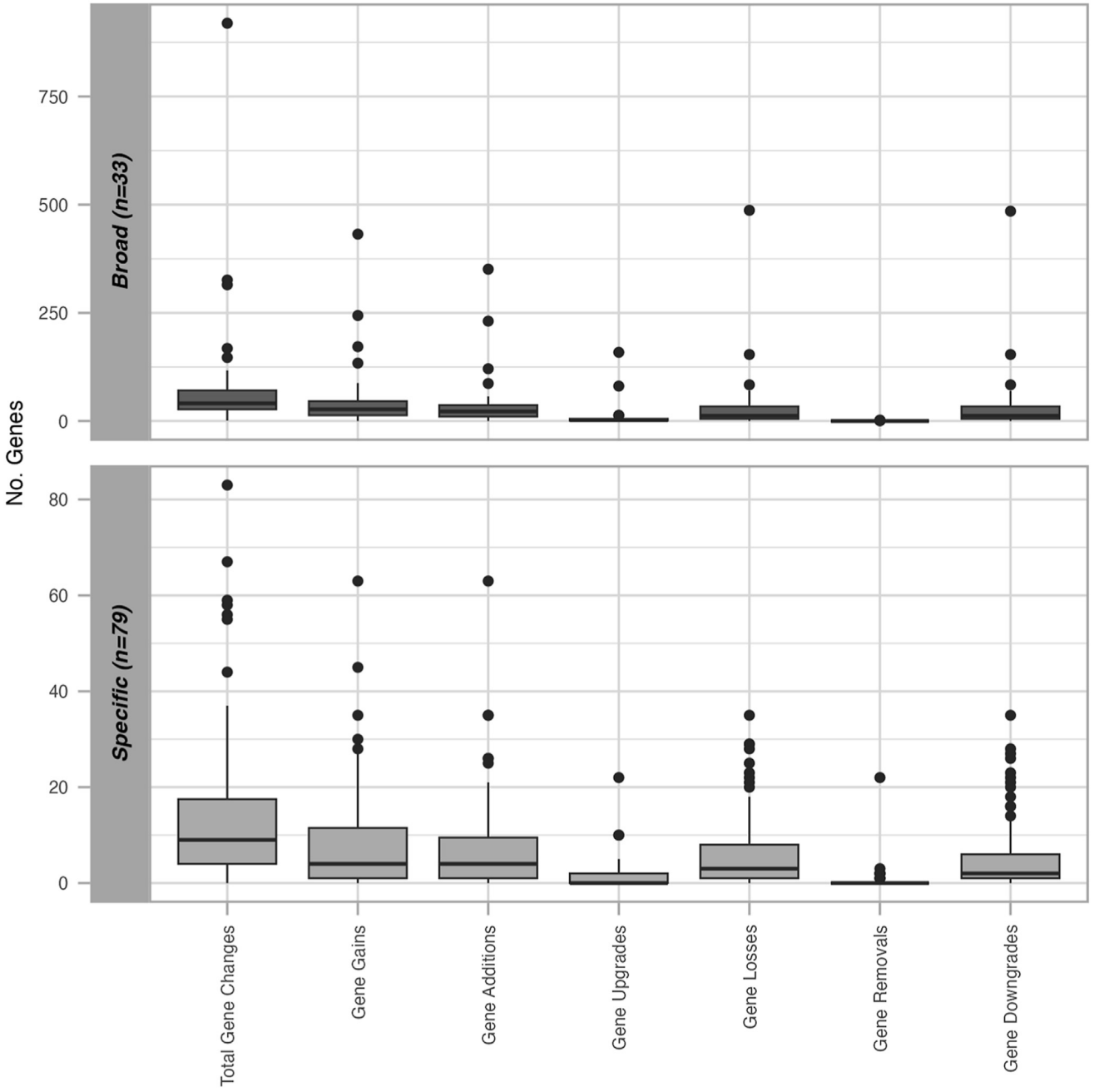

Figure 2 The number of the different types of gene changes in the rare-disease panels in PanelApp Australia between November 2019 and May 2022. The disruption of gene changes for the Specific and Broad rare-disease groups are shown as a boxplot. These plots show the median (center line), the 1st and 3rd quartile (bottom and top of "the box," respectively), and range of the nonoutlier samples (whiskers) for the classes of gene changes examined in this manuscript (*x*-axis). The number of changed genes are shown in on the *y*-axis; however, it is important to note that the Broad and Specific sub-figures have distinct *y*-axes.

the supporting information collected by PanelApp Australia was compared with the corresponding information in ClinGen.[23] As a result of this harmonization effort, *A2ML1* was downgraded to Red status because of conflicting information identified in the literature,[24] with ClinGen having classified the gene as "disputed." After alignment with ClinGen, *MRAS* was also added as a diagnostic gene on the same day. However, an updated literature search identified additional patients, resulting in green rating as opposed to the moderate rating by ClinGen.[23,25,26] This example highlights that a relatively large number of changes can be made to a panel within a short period of time because of international harmonization efforts.[23]

Table 3 Gene Changes in rare-disease panels between November 2019 and May 2022

	Panel Type	Panel Size - Nov 2019	Panel Size - May 2022	Total No. Diagnostic Gene Changes	No. Diagnostic Grade Genes Added	No. Genes Upgraded to Diagnostic Grade	No. Diagnostic Grade Gene Gains (Add + Upgrade)	No. Diagnostic Grade Genes Removed	No. Genes Downgraded from Diagnostic Grade	No. Diagnostic Grade Gene Losses (Removals + Downgrades)
Median	Specific[a]	19	21	9	4	0	4	0	2	3
	Broad[b]	64	74	41	22	2	27	0	12	12
Range - Min	Specific[a]	2	1	0	0	0	0	0	0	0
	Broad[b]	7	5	1	0	0	0	0	0	0
Range - Max	Specific[a]	115	113	83	63	22	63	22	35	35
	Broad[b]	1505	1450	919	351	159	432	2	485	487

Max, maximum; *Min*, minimum.
[a]Specific rare-disease panels, $n = 79$.
[b]Broad rare-disease panels, $n = 33$.

Table 4 Proportional gene changes in rare-disease panels between November 2019 and May 2022

	Panel Type	Panel Size - Nov 2019	Panel Size - May 2022	Total No. Diagnostic Gene Changes	No. Diagnostic Grade Genes Added	No. Genes Upgraded to Diagnostic Grade	No. Diagnostic Grade Gene Gains (Add + Upgrade)	No. Diagnostic Grade Genes Removed	No. Genes Downgraded from Diagnostic Grade	No. Diagnostic Grade Gene Losses (Removals + Downgrades)
Median	Specific[a]	19	21	47.4%	18.2%	0.0%	25.0%	0.0%	13.6%	15.4%
	Broad[b]	59	73	63.6%	37.9%	3.4%	44.9%	0.0%	19.5%	19.5%
Range - min	Specific[a]	2	1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Broad[b]	7	5	10.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Range - max	Specific[a]	115	113	360.9%	273.9%	100.0%	273.9%	31.0%	127.3%	127.3%
	Broad[b]	1505	1450	509.4%	380.0%	248.4%	406.7%	3.4%	240.6%	240.6%

[a]Specific rare-disease panels, $n = 79$.
[b]Broad rare-disease panels, $n = 33$.

Characterizing the proportional gene changes in rare-disease panels over a 2.5-year period

To ensure our analysis captured significant changes in smaller panels, we also examined the proportional gene changes over this 2.5-year period. Using the version of a panel from November 2019 as a base and comparing this with the version from May 2022, the proportional gene changes were determined for the 79 Specific rare-disease panels and 33 Broad rare-disease panels that existed at the launch of PanelApp Australia. When examining gene changes, the median proportional increase from the Specific rare-disease panels was 47.4% (range = 0 to 361%) of the panel size at launch (Table 4). The median proportional gene changes in the Broad rare-disease panels was 63.6% (range = 10.0% to 509.4%) of the size of the panel at launch (Table 4).

Although the characterization of 79 panels that examine specific conditions, and 33 panels designed to probe more Broad clinical indications, provide some insight into the ways our understanding of these diseases have changed over a 2.5-year window, this sample size is too small to provide definitive answers. However, the findings presented here may provide some insight into the typical amount of change seen in comparable panels in the standard 24 to 36 month analysis window.

Differences in panels with the greatest amount of change

To better understand the individual changes that may lead to new diagnoses, we focused on the panels that had the largest gene gains because this metric captures the number of additional diagnostic genes available for curation. Within the 79 specific rare-disease panels that existed over the 2.5-year analysis window, we identified 4 panels in the 95th percentile for the number of gene gains and 4 panels in the 95th percentile for the proportional increase (Table 5).

The panels in the 95th percentile were Craniosynostosis, Cerebellar and Pontocerebellar Hypoplasia, Congenital Disorders of Glycosylation, and Ciliopathies panels, and they

Table 5 The four Specific rare-disease panels with the greatest gene gains (95th percentile)

Panels With the Greatest Number of Gene Gains				Panels With the Greatest Proportional Increase in Gene Gains			
Panel	Gained Diagnostic Genes	Diagnostic Genes: Nov 19 –	Diagnostic Genes: May 22	Panel	Proportion of Gained Diagnostic Genes	Diagnostic Genes: Nov 19 –	Diagnostic Genes: May 22
Craniosynostosis (226 updates)	+63[a] (−20)[b]	23	66	Craniosynostosis (226 updates)	293.9% (+63/−20)	23	66
Cerebellar and pontocerebellar hypoplasia (277 updates)	+45 (−22)	49	72	Congenital diaphragmatic hernia (103 updates)	136.8% (+26/−0)	19	45
Congenital disorders of glycosylation (399 updates)	+35 (−23)	98	110	Corneal dystrophy (85 updates)	127.3% (+28/−28)	22	22
Ciliopathies (479 updates)	+30 (−29)	112	113	Ichthyosis (126 updates)	109.1% (+24/−3)	22	43

[a]No. of diagnostic genes gained by the panel between November 2019 and May 2022.
[b]No. of diagnostic genes lost by the panel between November 2019 and May 2022.

Table 6 The 2 panels Broad rare-disease panels with the greatest gene gains (95th percentile)

Panels With the Greatest Number of Gene Gains				Panels With the Greatest Proportional Increase in Gene Gains			
Panel	Gained Diagnostic Genes	Diagnostic Genes: Nov 19 –	Diagnostic Genes: May 22	Panel	Proportion of Gained Diagnostic Genes	Diagnostic Genes: Nov 19 –	Diagnostic Genes: May 22
Intellectual disability syndromic and nonsyndromic (4708 updates)	+432[a] (−487)[b]	1505	1450	Cerebral palsy (213 updates)	406.7% (+61/−3)	15	73
Genetic epilepsy (1596 updates)	+244 (−71)	463	636	Early-onset dementia (154 updates)	268.8% (+172/−154)	64	82

[a]No. of diagnostic genes gained by the panel between November 2019 and May 2022.
[b]No. of diagnostic genes lost by the panel between November 2019 and May 2022.

contained between 30 to 63 gene gains (Table 5). The panels that saw the greatest proportional increase in the number of diagnostic genes (Table 5) were also identified. The panels with the largest increase saw gene gains between 109% to 293% and included the panels used to characterize Craniosynostosis, Congenital Diaphragmatic Hernia, Corneal Dystrophy, and Ichthyosis (Table 5). These panels were also considerably higher than the median proportional gene gains (25%).

The same approach was applied to the 33 Broad rare-disease panels. Determining the panels in the 95th percentile of gene gains from this smaller cohort identified 2 panels in both the numerical and proportional analyses. The Intellectual Disability Syndromic and Nonsyndromic and Genetic Epilepsy panels were found to have the largest number of gene gains, and the Cerebral Palsy and Early-onset Dementia panels had the largest proportional gene gains (Table 6).

Although it is expected that the panels in the 95th percentile of gene gains to be more active than the majority of the cohort, when considering that the median number of gene gains was 4 for the Specific rare-disease panels and 22 for the Broad rare-disease panels (Table 2), the presence of a Specific rare-disease panel with 63 gene gains or a Broad rare-disease panel with 432 gene gains suggests that there could be some benefit in re-analyzing undiagnosed patients who have been previously examined by the panels with significant amounts of change, more frequently than 24 to 36 months.

Examining gene gains in monthly snapshots reveals different modes of panel evolution

To understand the precise ways each of the panels that contained the greatest amount of gene gains had evolved over the analysis window, the number of monthly gene gains in each of these panels were examined (Figure 3). To contextualize these results, we compared the monthly gene gains in each panel to the median number of gene gains seen over the analysis window (Specific = 4 and Broad = 27) and the median proportional gene gain (Specific = 25.0% and Broad = 44.9%). Although far from a perfect approach, these metrics provide some perspective surrounding the number of genes typically gained by a panel in this period.

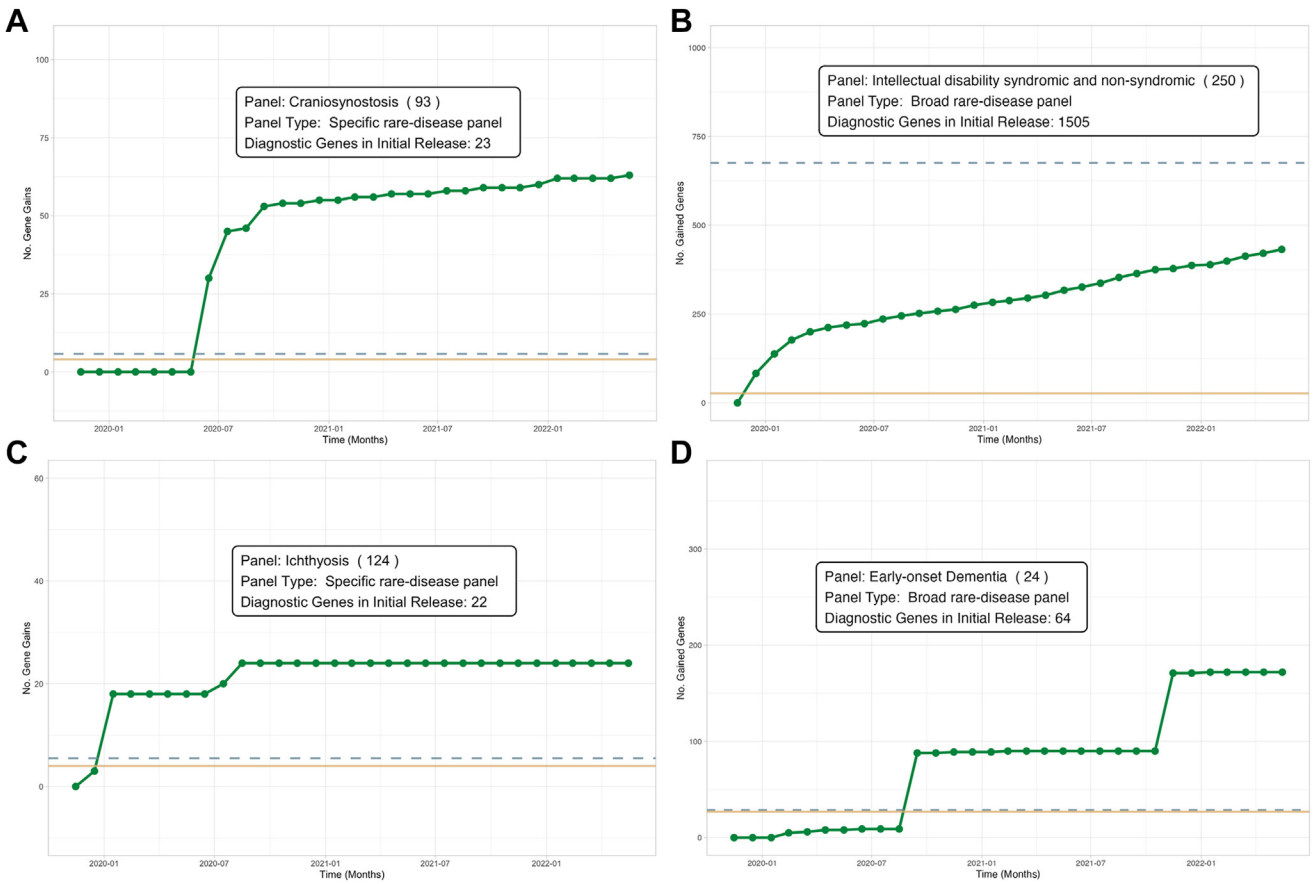

Figure 3 The number of new diagnostic genes gained by panels (gene gains) over a period of 30 months. A. Gene gains in the Craniosynostosis panel. The number of gene gains (green) in the panel increased rapidly between May and June 2020. B. The intellectual disability—syndromic and nonsyndromic—the number of gene gains in the panel increased at more steady rate. C. The Ichthyosis panel saw a "spike" in gene gains and after a smaller period of activity remained completely static. Panel (D). The early-onset dementia panel contained 2 "spikes," and 3 periods of reduced activity. For each panel, the median number of diagnostic genes added to each panel in PanelApp Australia is shown in brown, whereas the proportional median (calculated for each panel individually) is shown by a gray dashed line.

The number of monthly gene gains in these panels did not always increase at a steady rate. Panels, such as the Craniosynostosis panel, contained periods that saw rapid increases in the number of diagnostic genes available for curation (Figure 3A). However, there were also panels in which the number of gene gains increased at a more steady rate (Figure 3B), such as the Intellectual Disability – syndromic and nonsyndromic panel, which, despite seeing 432 gene gains, did not pass the proportional median that had been determined for this panel.

Some panels displayed multiple behaviors over the 2.5-year analysis window. For example, the Ichthyosis panel saw a spike in the number of diagnostic genes between December 2019 and January, passing both the median milestones within 3 months of its release (Figure 3C). However, this panel has been dormant since August 2020. Similar periods of activity and dormancy were also seen in the Early Onset Dementia panel (Figure 3D).

To further explore how new knowledge may impact the re-analysis of existing clinical genomic data, we expanded the scope of our analyses and examined gene gains in all of the 128 Specific and 68 Broad rare-disease panels (Supplemental Table 3). Although this analysis included panels that did not exist for the entire analysis window, we identified additional panels with spikes, panels that did not see any gene gains, and panels with a steady increase in the number of diagnostic genes (Supplemental Table 3).

Finally, we investigated gene losses because these events may also affect the need to re-analyze individuals. We examined the monthly gene losses from the 79 Specific rare-disease panels and the 33 Broad rare-disease panels that existed over the entire 2.5-year analysis window. This analysis revealed panels that contained rapid decreases in the number of genes (Figure 4A), panels that lost genes at a more uniform rate (Figure 4B), and stable panels. Some panels displayed multiple behaviors over the analysis window (Figure 4C). The gene losses present in a panel were independent of the gene gains. For example, the Craniosynostosis panel saw a large gene gain spike in mid-2020 (Figure 3A); however, when examining gene losses,

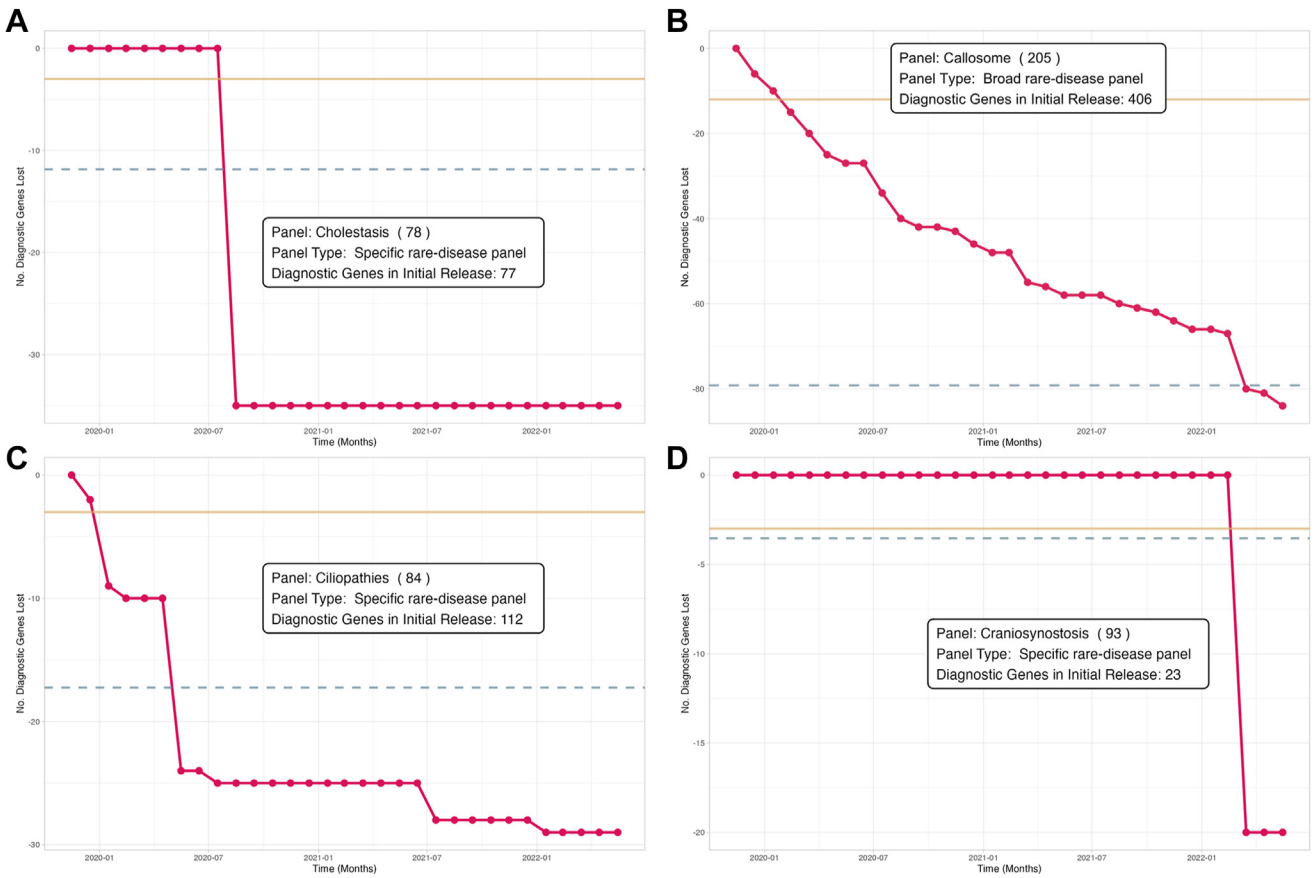

Figure 4 The number of diagnostic genes lost by panels (gene losses) over a period of 30 months. A. Gene loses in the Cholestasis panel. The number of gene losses (red) in the panel decreased by more than 30 genes in the space of 1 month. B. Gene losses in the Callosome Panel. Genes were steadily lost from the panel. C. Gene losses in the Ciliopathies panel. This panel contained multiple periods of gene loss and multiple periods of stability. D. Gene losses in the Craniosynostosis panel. The gene losses seen in a panel are independent of the gene gains (Figure 3A). For each panel, the median number of new gene losses is shown in brown, whereas the proportional median (calculated for each panel individually) is shown by a gray dashed line.

this panel was static until February 2022, in which it saw a rapid decrease in the number of diagnostic genes (Figure 4D).

Discussion

PanelApp Australia has consolidated and harmonized virtual gene panels in use by Australian diagnostic laboratories and research groups.[23] Many of the panels hosted by PanelApp Australia were originally created to support early translational studies that assessed the utility of genomic testing in specific rare diseases, such as those by Melbourne Genomics, Queensland Genomics and Australian Genomics.[22,27] Other panels were created by diagnostic laboratories through a process of literature review, expert review, and consultation. Cases in which more than 1 panel was developed for the same clinical indication by multiple laboratories (eg, intellectual disability), these have been consolidated with discrepancies that are critically reviewed and resolved in the process by a panel of expert gene curators.[28] Assessment of evidence predominantly considers published sources. Occasionally, unpublished sources, such as variants deposited by other laboratories in ClinVar,[29] as well as those identified internally by Australian laboratories or by Genomics England, are considered,[19,23] particularly in cases in which detailed information is available about patient phenotypes and the robustness of the variant assessments.

Internationally, PanelApp Australia is involved in systematic efforts to improve the evidence base for gene-disease associations in collaboration with Genomics England[23] and also as part of the Gene Curation Coalition.[28] In addition to these harmonization efforts, the PanelApp Australia team updates virtual panels on a monthly basis following literature reviews. These changes are captured by an audit trail, which records every change as an updated version.[19,23] As a result, PanelApp Australia represents an open resource that contains up-to-date, evidence-based associations between specific conditions/clinical indications and specific groups of genes.

Here, we used the information captured by PanelApp Australia to observe how the knowledge of genes associated

with common clinical indications for testing in rare disease have changed over a period of 2 and a half years. Because the current recommendations for re-analysis typically suggest that re-examination occurs approximately 24 to 36 months after the initial analysis,[13,14,16] and because this analysis occurred over a period of 30 months, this study provides insights into the changes seen in 79 Specific and 33 Broad rare-disease panels during this period of time.

The information captured by PanelApp Australia demonstrated that, over this 2.5 year period, our understanding of the genes associated with different rare-diseases testing indications changed by different amounts, in different ways, and at different rates. Some panels, such as the Ichthyosis panel, saw the number of diagnostic genes almost double (22 to 43) during this period, whereas the genes associated with other conditions, such as Spondylocostal Dysostosis, remained static. Some panels received hundreds of updates and others received less than 10. Some panels contained large numbers of gene gains, some saw large numbers of gene loses, and some saw both types of gene changes occur during the analysis window. Together, these findings highlight how dynamically different panels can evolve over the standard re-analysis period and suggest that undiagnosed patients could benefit from re-analysis strategies that incorporate gene change data.

For example, undiagnosed patients previously tested with a panel that is rapidly gaining additional diagnostic genes may benefit from frequent intervals of re-analysis. Conversely, undiagnosed patients tested with more static panels may benefit from a re-analysis strategy that relies on discovery and reclassification of variants rather than new gene-disease associations. The low level of gene discovery in some disease groups and their corresponding panels may be suggestive that these disorders are either well understood or that they may benefit from more research.

Although we have predominantly focused on gene gains, the significance of gene losses must not be overlooked. A considerable number of panels contained genes that were downgraded from a diagnostic status, and though panels that saw diagnostic genes removed were much rarer, these events are likely to still be of clinical significance. Reclassification events, which have seen pathogenic or likely pathogenic variants downgraded,[30-32] have prompted a suggestion that re-analysis should not be limited to patients awaiting a diagnosis.[5] We show that similar proportions of panels saw gene gains and gene losses and that the median number of gene loses for the specific rare-disease cohort was 3—a similar number to the median gene gains ($n = 4$), suggesting that the clinical benefits of post-diagnosis re-analysis should be explored. Moreover, given the exponential amount of work this approach would place on clinicians, this finding highlights the need to explore the development of automated re-analysis solutions.

Examination of the number of new genes added per month to each panel, showed that the accumulation and integration of new knowledge for certain conditions did not occur at a linear rate. Some conditions saw multiple "spikes" or sharp increases in the number of diagnostic genes available for curation, whereas others remained more stable. The presence of spikes in activity in PanelApp Australia commonly reflects harmonization efforts between different Australian groups and with international efforts such as with Genomics England,[23] ClinGen,[33] and the Gene Curation Coalition.[28] Other spikes of activity represent monthly updates as a result of literature reviews directed at identifying newly published gene-disease associations.[23] Although the large amount of clinically significant change seen in some panels suggests that these panels would benefit from more frequent re-analysis intervals, these spikes in the number of available diagnostic genes also suggest that there may be some benefit to triggering re-analysis, after instances in which a panel undergoes a large change, irrespective of when the initial test was undertaken.

It is important to recognize that the adoption of a system that utilizes different frequencies for re-analyzing an individual's existing genetic information may place an additional workload on the health care system. Although re-analyzing genomic data, with the additional context provided by new knowledge, may represent an ideal space to develop and test approaches that incorporate elements of automation, we must acknowledge that traditional automated approaches to recuration will do little to address the challenges associated with reinitiation, recontacting patients, and providing care to the individual patients who require recounseling.[5]

These results from this analysis PanelApp Australia could also be interpreted as supporting a longer re-analysis period for those who have been tested with a more stable panel; however, this approach completely overlooks the role of new variant-level information in providing new diagnoses and highlights the need for future work to incorporate both gene- and variant-level information.

Interestingly, our analysis showed that some of the panels used to study conditions that might be considered specific, well defined, and stable were associated with a sizable number of gene gains. For example, in our examination of the Rasopathy panel, we detected 9 gene changes, of which 6 were gene gains (4 novel diagnostic genes + 2 gene upgrades) and 3 downgrades of genes from diagnostic status. Because the Rasopathy panel contained 22 diagnostic genes at launch, it is our opinion that these 9 gene changes represents a large proportional change. A comparison with the corresponding ClinGen RASopathy curation of 17 genes that has not been updated since 2018[34] further highlights the clinical importance of the more dynamic PanelApp Australia approach.

Analysis of the number of new panels released each month in PanelApp Australia revealed that the number of new panels being added to the database was declining. No new specific rare-disease panels had been released after November 2021, and no new panels had been added to PanelApp Australia since January 2022 in our analysis

window. These results are indicative of PanelApp Australia reaching a state of maturity and moving to a model that encourages consolidation of activity into existing panels. Activity was not affected by the COVID-19 pandemic as evidenced by the volume of updates during that period: the online, crowdsourced nature of the platform being ideally suited to asynchronous activity from many contributors "working from home."

There are several caveats to the work presented here. Sample size was limited to the rare-disease panels and the classification of which was not ideal. Furthermore, we have made a number of assumptions. We have assumed that each new gene added to a panel will provide an equal number of patients with a diagnosis, we assumed that the range conditions examined by PanelApp Australia is reflective of the cohorts of undiagnosed patients characterized by the re-analysis literature, and we assumed that the median amount of gene gains in the rare-disease cohorts are representative of the amount of change that produces an increase of diagnostic yield of approximately 10%. Although our approach was not ideal, it does provide some indication of the amount of change seen across 79 Specific and 33 Broad rare-disease panels and represents the first time the information in PanelApp Australia has been used to study how our understanding of different conditions is changing.

Future studies should aim to better understand when it is most beneficial to re-analyze an individual's existing genomic data. Additional work should be done to combine the diagnostic genes associated with a panel and the pathogenic variants reported, at specific time points to examine how the diagnostic resources available for curation have changed over time. Future work should also explore the impact of associating multiple disease entities with single genes over time, especially in cases the mode of inheritance is different. Moreover, there may be some benefit to suggest different re-analysis intervals for common indications for genomic testing but such guidance will need to be informed by evidence from re-analysis studies across a range of disorders.

Conclusion

Analyzing the information captured by PanelApp Australia has revealed that the evidence base for gene-disease associations is changing at different rates. This has important implications for re-analysis of genomic data from unsolved rare disease patients, suggesting that a dynamic rather than a fixed time period approach may be more appropriate.

Data Availability

All data analyzed here can be downloaded from PanelApp Australia. Results from the analysis of the data for this study are available from the corresponding author on request.

Funding

A.R. is supported by the DHCRC "bringing digital excellence to clinical excellence" PhD scholarship (DHCRC:0083), which is co-funded by Queensland Health, The University of Queensland and Digital Health CRC Limited. Digital Health CRC Limited is funded under the Australian Commonwealth Government's Cooperative Research Centres Program. N.W. is supported by an Australian National Health and Medical Research Council (NHMRC) Senior Research Fellowship (1139071) and Investigator Grant (2018244).

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Conceptualization: A.J.R., N.W.; Formal Analysis: A.J.R.; Methodology: A.J.R.; Software: A.J.R., K.T.; Investigation: A.J.R., Z.S., N.W.; Visualization: A.J.R.; Funding Acquisition: A.J.R.; Writing-Original Draft: A.J.R., N.W.; Writing-Review: K.T.; Writing-Review and Editing: C.P., C.S., Z.S.; Supervision: C.S., Z.S.; Resources: N.W.; Project Administration: N.W.

Ethics Declaration

The information retrieved from PanelApp Australia manuscript does not contain any patient data and as a result it does not require ethics approval.

Conflict of Interest

A.J.R. is the founder and CEO of ClearSKY Genomics Pty Ltd. N.W. is a founder and board member of genomiQa Pty Ltd. All other authors declare no conflicts of interest.

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