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REVIEW

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Reproductive genetic carrier screening and inborn errors of metabolism: The voice of the inborn errors of metabolism community needs to be heard

Edwin P. Kirk[1,2,3] | **Martin B. Delatycki**[4] | **Nigel Laing**[5,6]

[1]Centre for Clinical Genetics, Sydney Children's Hospital, Randwick, New South Wales, Australia

[2]New South Wales Health Pathology Randwick Genomics Laboratory, Randwick, New South Wales, Australia

[3]School of Women's and Children's Health, University of New South Wales, Randwick, New South Wales, Australia

[4]Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[5]Centre for Medical Research, University of Western Australia and Harry Perkins Institute of Medical Research, Nedlands, Western Australia, Australia

[6]Department of Diagnostic Genomics, PathWest Laboratory Medicine, Department of Health, Nedlands, Western Australia, Australia

Correspondence
Edwin P. Kirk, Centre for Clinical Genetics, Sydney Children's Hospital, Level 9, Bright Alliance Building, High Street, Randwick, NSW 2031, Australia.
Email: edwin.kirk@health.nsw.gov.au

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Abstract

Reproductive genetic carrier screening (RGCS) has a history spanning more than 50 years, but for most of that time has been limited to screening for one or a few conditions in targeted population groups. The advent of massively parallel sequencing has led to rapid growth in screening for panels of up to hundreds of genes. Such panels typically include numerous genes associated with inborn errors of metabolism (IEM). There are considerable potential benefits for families from screening, but there are also risks and potential pitfalls. The IEM community has a vital role to play in guiding gene selection and assisting with the complexities that arise from screening, including interpreting complex biochemical assays and counselling at-risk couples about phenotypes and treatments.

KEYWORDS

autosomal recessive, carrier screening, inborn errors, metabolism, X-linked

1 | BACKGROUND

The purpose of reproductive genetic carrier screening (RGCS) is to identify couples who are carriers of pathogenic variants in genes associated with autosomal recessive (AR) or X-linked (XL) conditions, before they have an affected child. The timing of screening is important, because the reason for identifying carrier couples is to give them access to reproductive options, including preimplantation genetic testing (PGT-M) and prenatal diagnosis. Ideally, screening would be offered and conducted prior to any pregnancy. However, the reality is that many pregnancies are unplanned, and couples who have planned pregnancies are not always aware of the availability of RGCS,

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or consider it a priority prior to the pregnancy. This means that screening often occurs early in an established pregnancy. This limits options for those who are found to be at risk of having an affected child and leads to a need for urgent counselling about predicted phenotypes and, increasingly, potential treatments. Couples may find themselves needing to make quick decisions, sometimes in the context of uncertain clinical outcomes.

Screening for inborn errors of metabolism (IEM) has been a part of RGCS almost since its beginning. The first RGCS programmes, in the 1960s and 1970s, focused on haemoglobinopathies, but screening for Tay-Sachs disease was not far behind.[1,2] For most of the history of RGCS, screening has been population-specific and targeted—screening for beta-thalassaemia in Mediterranean populations, or for Tay-Sachs disease in Ashkenazi Jewish populations were early examples. The effectiveness of screening in that setting is well established. In an Australian community genetics programme, no children with Tay-Sachs disease have been born to parents who had accessed screening in the 25+ years since the programme began.[3]

The advent of massively parallel sequencing has led to the availability of assays that screen for panels of up to hundreds of genes, which will always include multiple genes associated with IEM. Screening for a large number of genes has the advantage of increasing the likelihood of detecting carrier couples. However, it also means that if screening is conducted sequentially—typically with the female reproductive partner being screened first—there is a high chance of carrier status for at least one AR condition being identified, leading to the need to screen the other partner. This creates a need for genetic counselling and adds to the time taken for screening, which may be particularly problematic if there is already a pregnancy. Screening both partners concurrently (couple screening) has obvious advantages despite added cost, and can be considered with individual reporting of carrier status or as a couple-based analysis, with variants reported only if the couple are found to be at risk of having an affected child.

2 | SELECTION OF GENES FOR SCREENING

Gene selection for RGCS is challenging and various approaches have been advocated.[4–6] While there is general agreement that the intent of screening is not to identify couples with an increased chance of having a mild condition, there is no consensus definition of 'mild' and different groups have made varying judgements about whether conditions are severe enough to include in screening. In any case, many genes are associated with a wide spectrum of severity; a gene may be included in a panel because of a phenotype that is lethal in infancy, but variants in that gene may be identified in carrier screening that are predicted to be associated with attenuated or late onset phenotypes, or with incomplete penetrance. For example, Gaucher disease has a spectrum of severity ranging from type 2 disease, with death typically before the age of 5, through to people who survive to advanced age with no or only mild clinical features.[7] Similar variability is seen in Krabbe disease[8] and in other conditions. Confident predictions of phenotype from genotype are not always possible. This can lead to reporting challenges for laboratories, counselling challenges for clinicians and genetic counsellors and fraught decision-making for couples.

The recent Practice Resource on carrier screening of the American College of Medical Genetics and Genomics (ACMG)[6] recommends restricting routine carrier screening to a list of 113 genes (97 autosomal and 16 X-linked), chosen on the basis of severity of the condition and frequency of pathogenic or likely pathogenic variants. In principle, these are reasonable grounds for choosing such a gene list, but the resulting list omits many genes associated with conditions that are very severe and are not very rare. A possible reason for this is that the frequency data used were derived from the gnomAD population database, which may not yet be representative enough of population variation to accurately capture carrier frequencies for rare genetic conditions. This may be because the gnomAD dataset is not yet sufficiently large to capture the majority of rare pathogenic variants, particularly for conditions in which no one variant represents a substantial proportion of all pathogenic variants. There may be population-specific considerations as well, either because some populations are not represented at all, or because they make up a small proportion of the individuals represented in the database. The Practice Resources recommends against routine carrier screening for genes not included among the 113; this includes many that are associated with IEM that are certainly severe and not (in the experience of readers of this Journal) exceptionally rare. Examples of genes that are excluded include *GALC* (Krabbe disease), *GLDC* (glycine encephalopathy) *HPRT1* (Lesch–Nyhan syndrome), *IDS* (Hunter syndrome), *PEX1* and *PEX6* (peroxisomal biogenesis disorders) and *SGSH* and *NAGLU* (Sanfilippo syndrome).

If these are sins of omission, selection of gene lists for carrier screening is also subject to sins of commission—the inclusion of genes that do not belong. Ideally, given the importance of IEM as a group of conditions, those who compile gene lists for carrier screening would always seek advice from practitioners familiar with the area. That they do not always do this is evidenced by the frequent appearance of genes for which evidence for gene-phenotype relationship is limited, or for which there may

be a phenotype in some but penetrance is low. Examples include *ACADS* (short chain acyl-CoA dehydrogenase deficiency),[9] *ACSF3* (combined malonic and methylmalonic aciduria)[10] and *MCCC1* and *MCCC2* (3-methylcrotonyl-CoA carboxylase deficiency),[11] all of which appear on some current commercial panels (*MCCC2* is on the ACMG Practice Resource list, and *ACSF3* was initially included in the list used by the Australian Reproductive Genetic Carrier Screening Project,[5] but was subsequently removed). If there is any benefit in telling a couple that there is a 'risk' of having a child affected by one of these conditions, it is surely greatly outweighed by the potential harms.

While it is difficult to see an argument against screening for a condition as severe as Zellweger syndrome, or in favour of screening for a condition that may only represent a biochemical variant with no associated clinical phenotype (such as combined malonic and methylmalonic acid) there are other IEM that pose considerable challenges in thinking about the purpose and potential value of RGCS. An exemplar of this issue is *ACADM*. Since the advent of newborn screening (NBS) by tandem mass spectrometry, clinical outcomes in babies affected by medium chain acyl-CoA dehydrogenase deficiency (MCAD deficiency) have generally been excellent, with treatment that is usually not overly burdensome to affected children and their families.[12] However, there remains a small percentage of babies who present with severe complications of MCAD deficiency, before the results of NBS become available. There are still rare deaths from MCAD deficiency among children born in countries with effective NBS for the condition.[13] Moreover, some parents of affected children choose to take steps, such as PGT-M, to avoid having another affected child. Should *ACADM* be included in RGCS? The gene represents an edge case—some would advocate for, and others against, its inclusion.

Technical issues may also impact a decision regarding inclusion of a gene. Most RGCS panels rely on massively parallel sequencing (MPS), with separate assays for triplet repeat expansion in *FMR1* and exon 7 deletions in *SMN1*. Some genes present challenges for MPS, either because of variant profiles that include copy number variants as a common type of pathogenic variant requiring robust CNV analysis, or because of limitations on variant calling imposed by the presence of pseudogenes. In the IEM field, the most prominent example of the latter issue is *GBA*. While, as discussed above, there can be difficulties in variant interpretation, and counselling related to attenuated forms of Gaucher Disease may not be straightforward, there is no doubt that Type 2 Gaucher Disease—with neurologic involvement leading to death in early childhood—meets clinical criteria for inclusion of the associated gene in a carrier screening panel. However, there is a pseudogene that has 96% exonic sequence homology with *GBA*, greatly limiting capacity to detect pathogenic variants by standard MPS methods.[14] Unless a specific method for analysis of this gene can be implemented in the laboratory,[15] it is our view that it is preferable not to include the gene in panels for RGCS, as this may lead either to couples being erroneously identified as carriers or falsely reassured regarding carrier status for this gene.

3 | THE CHALLENGES OF VARIANT CLASSIFICATION AND REPORTING

Another area of controversy is the reporting of variants of uncertain significance (VUS). There is general agreement that variants classified as Likely Pathogenic (LP) or Pathogenic (P) should be reported, with the possible exception of variants only associated with very mild phenotypes. However, there are differing views regarding reporting of VUS, with some taking the view that this is inappropriate whereas others routinely report VUS, creating additional challenges for couples and their healthcare providers. Although there is consensus that variants classified as Likely Benign or Benign should not be reported, some laboratories do report variants such as the GALT p.-Asn314Asp (Duarte) variant, despite conclusive evidence that it is benign.[16] Reporting this type of information will often cause confusion and anxiety for individuals undergoing screening, as well as creating a requirement for genetic counselling, without any potential benefit.

Reflecting this controversy, the ACMG Practice Resource[6] has a bet each way, recommending that only LP and P variants should routinely be reported, but also suggesting that reporting of VUS should be considered in partners of previously identified carriers. This approach is problematic. This is arguably the setting in which it is most important *not* to report VUS, because it leads to couples effectively being asked to make reproductive decisions on the basis of uncertain information (that by definition would not usually be considered medically actionable), and because it may mean that variants are reported differently depending on which partner is tested first—a challenging prospect for the reporting laboratory.

Variant classification and counselling of carrier couples regarding expected phenotypes, in the absence of an affected individual in the family, may sometimes be straightforward, but can be extremely difficult. The scenario in which both partners carry well known loss of function variants, with the variant combination predictably associated with a severe phenotype, does not require specific high level clinical genetic or IEM expertise to manage. However, in common with other areas of genetics, there are variants associated with IEM that have conflicting functional, clinical and population evidence.

Classification of a variant as likely pathogenic may be difficult and require expert interpretation of functional data; consideration of likely phenotypes similarly requires experience with the condition and its spectrum. For example, there are variants in *GALC*, such as NM_000153.3: c.956A>G, p.Tyr319Cys (also known as p.Tyr303Cys), that have been reported in individuals with varied clinical phenotypes but are relatively common in at least one subpopulation in the gnomAD database. Interpretation of pathogenicity of these variants requires expert assessment of the phenotypes of reported patients as well as of enzymology and in vitro functional assays.[17,18] Similarly, interpretation of variants in *ALDOB*, such as NM_000035.4: c.911G>A, p.Arg304Gln, may rely heavily on assessment of clinical phenotype and enzymology.[19] For some genes, the existence of pseudodeficiency variants[20] means that enzymatic analyses cannot always be taken at face value, an issue unlikely to be familiar to most outside the field. A common theme here is that variant interpretation, particularly for missense variants, relies heavily on the prior probability that a variant identified in a particular gene will be pathogenic. The expertise of metabolic physicians and diagnostic laboratory scientists in integrating clinical, biochemical and other data in order to accurately assess this prior probability is indispensable.

It should be noted that many variants remain VUS even after all available evidence is considered. This represents much more of a constraint on the sensitivity of RGCS than technical limitations on detection of variants; it is important that those undergoing screening are aware that false negative results may occur.

Counselling patients about outcomes of rare genetic conditions requires an understanding of the associated phenotypes and natural history. Increasingly, in the field of IEM, it also requires knowledge of existing and imminent therapeutic options, including their limitations. Examples include enzyme replacement therapy (ERT) for conditions such as Pompe disease[21] and the imminent prospect of gene therapy for many different conditions.[22] Importantly, there are emerging complexities associated with some new therapies. For example, children with infantile-onset Pompe disease (IOPD) treated with ERT are now being found to have variable neuromuscular outcomes and increasingly, are recognised to have multisystem phenotypes including hearing loss, gastrointestinal dysfunction and neurocognitive deficits with associated white matter disease.[23] Reproductive decision-making, particularly in the context of an ongoing pregnancy, may be profoundly affected by the knowledge that there is a treatment available that could affect the course of the condition in an affected child. That information is best conveyed by a clinician familiar with *all* aspects of the new therapies, including potential complications and the development of additional phenotypes.

The complexities of variant classification and of predicting phenotypes mean that RGCS is not without risks to couples. In the event of a false negative result, couples may be falsely reassured. On the other hand, misclassification of a benign variant as pathogenic could lead to a fetus being wrongly identified at prenatal diagnosis as affected by a severe condition, and subsequent termination of a healthy pregnancy. To mitigate these risks, it is essential that variant classification should be as rigorous as possible and that counselling of couples identified as carriers should be done by those best placed to do so, or at least by genetics professionals who have consulted with the relevant specialists.

4 | CONCLUSIONS

Despite all of these complexities, the potential benefits of RGCS are considerable. For those who have had the experience of sitting with the parents of a child recently diagnosed with infantile metachromatic leukodystrophy (MLD), describing the expected course of the condition and the lack of any curative options, the idea that there may be an alternative to having to break such news is a powerful one. Not every couple would choose to take action to avoid having a child affected by MLD, or any of the hundreds of other IEM with severe impacts on quality and/or duration of life; but given the chance, most do. The promise of RGCS is that it can provide couples with that chance, before they have an affected child.

In order for RGCS to fulfil its promise and deliver the most good, with the least harm, the readers of this Journal have an essential role to play. Selection of genes for screening, assistance with variant interpretation, clinical counselling of carrier couples and advice about follow-up assays (such as enzymatic prenatal diagnosis as an adjunct to molecular testing) all require the expertise of the IEM community, including clinicians, diagnostic laboratory staff and researchers.

One final point: a couple who have a child with an IEM are not protected against having a child with a different genetic condition. While this is particularly true for consanguineous couples, families with two or more children affected by different genetic conditions are by no means restricted to any particular ethnic or cultural group. Counselling about recurrence risks for the IEM in question, as well as reproductive options (or referring for genetic counselling) is already routine. We think that a discussion about RGCS should also be part of routine clinical care.

AUTHOR CONTRIBUTIONS

Edwin Kirk drafted the manuscript following discussions with Martin Delatycki and Nigel Laing, who then both critically reviewed and edited the text. All authors approve the content of the paper.

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CONFLICT OF INTEREST

Edwin Kirk is an employee of New South Wales Health Pathology, a public pathology service that offers reproductive genetic carrier screening on a fee for service basis.

DATA AVAILABILITY STATEMENT

The manuscript has no associated data.

ETHICS STATEMENT

Ethics approval was not required.

PATIENT CONSENT

Not applicable.

ORCID

Edwin P. Kirk https://orcid.org/0000-0002-4662-0024

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