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Review

Interpreting clinical microarray genomic data in 2012: What have we learnt and what challenges remain?

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ABSTRACT

Genome analysis using microarrays is increasingly used in genetic testing laboratories to detect clinically significant copy number variants (CNVs). Professional guidelines for this type of testing have been developed in several countries; in general terms, these address mainly practical issues. Although microarray testing provides significant improvements in diagnostic success compared with conventional chromosome analysis by microscopy (karyotyping) the complexities of analysing and interpreting microarray data make the writing of clear, accurate, clinically useful, evidence-based reports challenging. A major impediment is the incompleteness of the human variome, a catalogue of all variation in the genome and its associated phenotypes. Furthermore, it has become clear that CNVs can exert their pathological effects through a wide range of complex genetic and multifactorial mechanisms. The process of evidence-based interpretation is neither clearly defined nor widely understood. The conclusions of research publications in this field are often inferential rather than experimentally tested and the skills needed for thorough assessment are not widespread. Consequently, there is a clear need to incorporate much of this new knowledge into the education and training of the providers and users of microarray reports. These same challenges are also relevant to the emerging use of whole exome and whole genome sequencing which promise comprehensive sequence and CNV detection. There is therefore a need to re-evaluate interpretation and reporting issues and to address this, a forum entitled 'Microarray Reporting Best Practice Workshop' was organized by the Genetics Advisory Committee of the Royal College of Pathologists of Australasia. This commentary is the outcome of that discussion.

KEYWORDS: CNV, genetic variation, interpretation, microarray, guidelines, reporting, genome

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ABBREVIATIONS

CNV - copy number variant; LCSH – long continuous stretch of homozygosity; UPD - uniparental disomy; SNP - single nucleotide polymorphism; FISH - fluorescence *in situ* hybridization; MLPA - multiplex ligation dependent probe amplification

INTRODUCTION

Until recently, genetic analysis for clinical diagnosis has been separated by methodology into targeted detection of sequence variants at the gene/exon level and genome-wide detection of large chromosomal rearrangements which are visible by light microscopy. The introduction of microarrays now makes it possible to analyse the entire genome of a patient for submicroscopic losses and gains of DNA segments which are called copy number variants (CNVs). This transforming technology provides an improvement in analytical resolution of about two orders of magnitude enabling detection of CNVs as small as 50 kilobases and on occasion, with adequate probe coverage, down to the exon level [1-3]. Microarray analysis has therefore significantly added to the existing armamentarium of the genetic diagnostic laboratory. The relatively rapid adoption of this genomic technology has been facilitated by the commercial manufacture of high quality microarrays containing hundreds of thousands of oligonucleotide probes with tailored genome coverage for robust CNV detection. Concomitant with this has been the provision of analytical software which in the earliest forms processed raw fluorescence data into intelligible copy number and SNP genotyping information. Ongoing software development uses improved genomic normalization, automatic calling and interrogation of public CNV databases and clinical categorization of CNVs (for comprehensive review see de Leeuw *et al.* [4]).

Microarray analysis is now the recommended 'first tier' test for diagnostic evaluation of congenital malformations, developmental delay, intellectual disability and autism [5, 6]. Many studies have demonstrated significant improvements in diagnostic success compared with conventional karyotyping by microscopy (reviewed extensively by Koolen *et al.* [7] and Sagoo *et al.* [8]). This has fostered the so-called 'reverse dysmorphology' or 'genotype-to-phenotype' approach to genetic diagnosis [9-11]. Microarray analysis is now being proposed as the method of choice for genetic investigation of fetal ultrasound abnormality. In this setting, the detection rate of clinically significant CNVs (including findings of uncertain significance) above that of karyotyping has been shown to be 5.2-6.6% [12, 13]. Furthermore, microarray analysis may have utility in the diagnosis of chromosome abnormality associated with increased risk of fetal abnormality ascertained through maternal serum screening programmes. In this setting, improvements of 1.6-5.2% have been described compared with conventional karyotyping [13, 14].

It is therefore surprising that these undoubted advances have been achieved against a background of incomplete knowledge of the variome [15-18], the catalogue of all genetic variation in the human genome, benign and pathogenic. Furthermore, the task of identifying the genotype-phenotype relationships of pathogenic copy number changes is in its infancy and there is a burgeoning knowledge of the relevance of rare genetic variants and complex genetic/multifactorial mechanisms to neurocognitive, neurodevelopmental and congenital disorders [19]. The inevitable consequence has been inconsistency in how different genetic testing centres process, analyse, interpret and report microarray genomic data for use by clinicians.

Best practice guidelines for constitutional microarray genomic analysis have already been developed in several countries [20-27]. In general terms, these guidelines cover the major practical issues associated with microarray testing service provision. However, there is a clear need to reevaluate current policies for analysis, interpretation and reporting of genomic data. Accordingly, a forum was organised by the Genetics Advisory Committee of the Royal College of Pathologists of Australasia to address the key unresolved issues that remain. This was attended by clinical scientists, genetic pathologists, clinical geneticists and specialist physicians all of whom are experienced in the provision and use of diagnostic genetic microarray testing. This commentary is the outcome of that discussion.

Terminology: How do we categorize CNVs?

Unfortunately, there is no uniform set of descriptive terms used to describe the different CNV interpretation categories. Even the term CNV is not universally used, some preferring the term 'Copy Number Change' to avoid confusion regarding the term variant. It would seem prudent therefore to agree on a clearly defined and universally accepted terminology for reporting that reduces risks of misunderstanding or of unintended confusion [28]. Irrespective of how CNVs are classified, there is a substantial number of CNVs of 'unknown significance' for which there is no relevant, peer-reviewed literature or database evidence to assist interpretation, i.e. there is no evidence base on which to determine clinical significance. These are usually CNVs that have not previously been described or that are identified at very low frequencies (e.g. <0.1%) in clinical and control populations combined. Reporting these does not assist meeting the immediate clinical diagnostic challenge that prompted the test request.

Classification of CNVs into the widely used categories 'pathogenic', 'uncertain' and 'benign', requires an evidence base. We suggest that the 'unknown' category as defined above cannot therefore be included within this spectrum. Unfortunately, CNVs of 'unknown significance' are sometimes described as of 'uncertain significance' [22] or as a subclassification of 'uncertain significance' [20]. In the interests of facilitating genetic counseling, there needs to be a clear distinction drawn between CNVs of 'unknown' and those of 'uncertain' significance. Consensus guidelines need to be provided by the relevant national professional bodies. The term 'pathogenic' should be reserved for CNVs where clinical association is very well established with multiple peer-reviewed literature reports. Classification of a CNV as pathogenic should not be expected to require revision. The term 'benign' should be reserved for CNVs that occur commonly in general populations without enrichment in clinical cohorts, or identified in local databases with frequencies exceeding a pre-set level. Between these relatively easily defined categories are CNVs of 'uncertain significance'; for which the supporting evidence base is variable and inadequate. Despite widespread adoption, use of the term 'uncertain significance' remains ill-defined and there is ongoing discussion about the weighting associated with different levels of supporting evidence [20, 22].

So called 'susceptibility CNVs' [4, 20], which are often categorized as 'pathogenic' (although some clinical laboratories prefer to report them as 'uncertain'), have been shown from association studies to be more common in patients with neurodevelopmental disorders (autism, epilepsy, schizophrenia, and cognitive impairment) than in control groups. Collectively, these are found in approximately 2-4% of individuals referred for investigation of developmental delay, intellectual disability, autism spectrum, disorder and congenital abnormalities [29]. However, it may be better to report these as 'susceptibility factors' in keeping with recognition that they are important, emerging, contributory factors but are not sole determinants of the phenotypic abnormality under investigation [19].

Assessing the available evidence: Do we emphasize the evidence base?

The evaluation of a gene or region affected by genomic imbalance in an evidence-based manner is paramount for proper interpretation of clinical significance. Few would disagree that the peer-reviewed literature should be regarded as the gold standard for the primary evidence required to assess a particular genomic region/gene [30]. When evaluating literature, the quality of the publication needs to be taken into account. *Critical review of literature cited in reports is a requisite competency skill for any laboratory geneticist*. This requires expertise gained through training and experience and includes consideration of ascertainment bias and the statistical significance requirements in case/control and comprehensive family studies. In general, clinical interpretations should not be based solely on the predicted gene function or on functional data obtained in model organisms (e.g. mouse knock-outs, zebra fish). Decisions based on *in vitro* studies are even less satisfactory. Inferences based on these categories of information, rather than well characterized data from human subjects, should remain purely speculative.

Large-scale case-control series are particularly valuable. However, care should be taken to avoid over-interpreting early reports of CNV enrichment in 'clinical' versus 'control' populations, particularly those that have not been verified in replication studies. When using publicly available population frequency data, account should be taken of sample sizes, ascertainment bias, availability of phenotypic data (preferably gender-specific), ethnic differences and the confidence associated with individual CNV calls. The latter is influenced by the type of microarray, analysis algorithms and study design. The nature of the 'control' population also deserves close scrutiny.

There are approximately 1800 genes with variants that have been associated with Mendelian diseases [31]. The clinical significance classification of these is variable with some soundly evidence based and others not (see ISCA Dosage Sensitivity Map on NCBI; http://www.ncbi.nlm.nih.gov/projects/dbvar/ISCA/index.shtml). These variants can be useful in the interpretation of CNV significance. For example, a novel, heterozygous deletion involving a gene for which there are well-established, pathogenic dominant, loss of function sequence variants could be classified as 'likely pathogenic', especially if supported by genotype-phenotype correlation. As the issue being considered is the potential clinical consequence of dosage imbalance, the evidence base for sequence variants has to be carefully scrutinized.

Analytical considerations: The need for quality clinical information on microarray request forms

It has long been recognized that the role of the laboratory geneticist in a medical team is to facilitate accurate clinical diagnosis; this increasingly offers improved management options for patients and wider family members. However, the laboratory geneticist's ability to effectively address the clinical issue that prompted the test referral is often dependent on the quality of clinical details provided by the referring doctor. Astute clinical assessment and laboratory-clinician liaison can focus analysis on specific genomic regions, which sometimes enables diagnosis of single gene dominant and recessive Mendelian disorders [3, 32].

Unfortunately, the clinical details written on test request forms are often insufficient. Efforts to address this would be highly beneficial [33]. This might be promoted through wider use of phenotype checklists attached to the test request form. Such checklists could be similar to those developed by the ISCA Consortium [33] and DECIPHER [34], the main repositories for cataloguing clinically significant CNVs. A role for the laboratory-based genetic counsellor in this effort has also recently been expressed [33]. Again, strengthening laboratory liaison with requesting practitioners, particularly those in clinical genetic services, would be beneficial.

Should analysis be extended to the regions flanking CNVs?

To some extent this is determined by the probe coverage. Early arrays used low coverage BAC (Bacterial Artificial Chromosome) clones containing human genome inserts and 'calls' were made on as few as two to three neighboring clones with the same copy number change. The spacing between the loci covered by these arrays was so large that defining the boundaries of a CNV was imprecise and it was prudent to consider the regions flanking the 'called' boundaries to avoid missing relevant genes. Most laboratories now use high density arrays containing tens to hundreds of thousands of oligonucleotide probes giving much more precise breakpoint definition. Microarray analysis has progressed to the stage where it is evident that optimal array design includes adequate genome coverage, which is primarily gene focused and specifically targeted to clinically significant genomic regions. These issues are extensively reviewed in Kearney *et al.* [23], Vermeesch *et al.* [20] and Riggs *et al.* [30]. Many laboratories do not assess neighbouring sequences but focus on the array coordinates marking the maximum deletion/duplication boundaries. Some, however, review sequences/genes up to 1Mb upstream and downstream of CNV breakpoints. This may be fruitful where there is an established genotype-phenotype relationship for a gene within the neighbouring region or where clinically-directed, targeted analysis is being carried out.

Should analysis consider agenic regions?

Regions that contain no genes, so called 'agenic regions', present an ongoing challenge for the

microarray analyst. It is now known that some functional elements (e.g. cis-regulatory elements) may be located a considerable distance upstream or downstream of genes that they control [35]. Consequently, there is recognition of an expanding number of developmental disorders caused by disruption of flanking functional elements [36-40]. At present, individual laboratory policies about evaluating and reporting agenic deletions and duplications vary considerably. It should be noted also that the interpretation of agenic CNVs may in the future need to consider microRNAs, particularly in view of a recent report of a putative association with a congenital genetic syndrome [41], and also as a result of enhanced understanding of the organization and regulation of our genes and genome [35].

Interpretational considerations: What are the ongoing interpretational challenges?

Novel and rare CNVs pose the greatest interpretational challenge owing largely to the continued lack of high-quality, large-scale control data. Additionally, there is often a lack of information on novel and rare variants for evidence-based assessment [30]. Efforts to catalogue genetic variation (including CNVs) in well-phenotyped control populations is beginning to emerge [15, 17, 18, 42] and will progressively reduce this problem, although the extent of the task is not to be underestimated.

Currently, the clinical interpretation of CNVs is heavily influenced by case-control frequency data (where available), CNV size and gene content. Uniqueness (novelty) or rarity are key discriminators, with most laboratories dismissing variants as insignificant if there are several reports in external databases showing comparable frequency in 'control' and 'clinical' populations. Furthermore, variants may be dismissed when frequencies exceed 1% within suitably-sized, in-house data sets. In doing so, consideration should be given to the copy number state (e.g. a homozygous deletion versus a heterozygous deletion that is known to be benign) and the size and position of the variant compared with the recognised polymorphism. Note also has to be taken of the emerging knowledge of the extent of misannotation rates in clinical databases of genetic variants [43].

Grounds for considering a CNV as pathogenic include overlap with a known disease-associated deletion/duplication, and involvement of a gene(s) with a well established dosage effect. A CNV involving a gene associated with a recessive Mendelian disorder may be pathogenic if acting in concert with a second allelic variant (sequence or copy number). Depending on the phenotypic information provided, this aetiology needs to be considered. Analysts should also be aware that a partial gene duplication or insertional rearrangement may disrupt gene function [3] and have consequences functionally equivalent to a partial gene deletion [44].

Genomic load/burden

It is becoming evident that in many patients with a neurodevelopmental disorder, the phenotype(s) results from the cumulative (oligogenic) effects of multiple clinically significant alleles. As such, consideration should be given to the possibility that the initial assessment of the underlying cause of the clinical problem might be incomplete. Specifically, there may be additional undetected, significant variants [19, 45]. Accordingly, the microarray result should not be over-interpreted, especially where a variant of uncertain significance or 'susceptibility' CNV is concerned. Constant awareness of the limitation of our knowledge of the variome is required, and the challenge this brings to the task of interpreting CNV findings.

Extended tracts of homozygosity

Microarray platforms designed for genome-wide SNP detection allow detection of extended tracts of homozygosity (i.e. 5-10 megabases), which are often referred to as long continuous stretches of homozygosity (LCSH) [46-49]. LCSH may indicate chromosomal segments that are identical (i.e. homozygous) by descent (IBD) [50], or rarely may be segments of isodisomy associated with a uniparental disomy (UPD). Several recent publications describe LCSH findings of relevance to the investigation of a neurodevelopmental disorder(s) and/or congenital abnormality [3, 32, 46, 49, 51, 52].

Detection of LCSH may be used to identify relevant recessive disease genes, or prioritise a list of candidate genes that may assist with

clinical diagnosis. The ubiquity of LCSH, however, even in outbred populations [53, 54], poses significant challenges in the setting of routine diagnostic testing. Nevertheless, there are now several reports of a microarray (LCSH)-assisted diagnosis of an autosomal recessive disorder [3, 32]. Often these successes have foreshortened the time taken to reach the correct diagnosis with obvious benefits for patient management, family counselling and reduced investigational costs. Importantly, this approach does not necessarily require a family history of consanguinity. It should be emphasised that astute clinical assessment and laboratory-clinician liaison is important for this strategy to be successful.

Regarding uniparental disomy, confirmatory parental genotyping studies are required to confirm a suspicion prompted by detection of LCSH. It should be noted that uniparental disomy may go undetected in the absence of LCSH and it is important that the requesting doctor is aware of this limitation. Although UPD of most chromosomes (i.e. other than chromosomes 6, 7, 11p, 14, 15) has in itself no known clinical significance, consideration should be given to the possibility of unmasking of a variant associated with a recessive Mendelian disorder. There is also the possibility of residual trisomy arising from trisomic rescue, but in practice this is very difficult to establish.

Utility of assessing CNV inheritance

Several recent insights into how CNVs exert phenotypic effects have raised questions about the practice of routinely assessing parental samples to determine whether a CNV is inherited or *de novo* [55-58]. This includes recognition of the following: most novel and rare CNVs smaller than 1Mb, particularly gains, are inherited [24]; the *de novo* rate of CNV formation across the genome is appreciable [59-61]; the clinical uncertainties associated with CNVs that have incomplete penetrance and variable expressivity.

The spectrum of genetic mechanisms that can contribute to the phenotypic variation associated with the same CNV, even among close relatives, includes imprinting effects, unmasked recessive mutations, gene-gene (e.g. oligogenic) and gene-environment (e.g. epigenetic) interactions as well as the inevitable chance effects inherent in pre-and postnatal development. It is important also that there is general awareness of susceptibility CNVs (e.g. 22q11.2, 15q13.3, 16p12.1, 16p13.11). As many are inherited without phenotypic correlation in the carrier parent, these aspects should not be over-interpreted, particularly as the effect sizes are presently unknown and clinical utility is questionable.

It is therefore clear that the generalization whereby an inherited CNV is seen as likely to be benign, and a *de novo* CNV is seen as likely to be pathogenic, does not always hold true.

Test reporting: Which categories of copy number changes should be reported?

This issue has ethical and legal aspects as well as resource implications. There are markedly divergent views amongst both providers and users of array tests on how this important issue should be addressed. At one extreme, some wish all CNVs to be listed and categorized in reports, notwithstanding the potentially large number of these if a high resolution array has been used. At the other, some hold the view that only those with clinical utility should be reported. The latter view implies discretionary reporting of variants of ‘unknown’ and ‘uncertain’ significance. Ultimately, the requesting doctor’s preference should direct which approach is taken. Irrespective of individual laboratory reporting policies, reports should contain a clear statement about, which, if any, CNV categories are not included in reports. It is imperative that any published data used to support or refute clinical significance (e.g. case reports, case-control frequencies, penetrance information, etc) are cited in the microarray report.

A consistent approach to reporting array findings should be aimed for, particularly for families dispersed across state or national boundaries. Inconsistencies in reports of identical familial CNVs from different laboratories are likely to cause confusion. Unfortunately, it is not uncommon to encounter variation in the description of genomic coordinates in reports of the same CNV from different laboratories. This primarily results from differences in array probe content and analytical software, but may also arise from the use of different and sometimes unspecified human

reference genome builds. It is fundamentally important that laboratories include sufficient information in reports to allow direct comparisons of assigned genomic locations of CNVs and the effective resolution of the array platform used. Differences in interpretive conclusions are of particular concern, critically so when they may influence medical management and reproductive choices.

It is important to be aware of the potential for information and interpretations in reports of a microarray test on a child or adult to have implications for prenatal testing in a subsequent pregnancy. Use of the same CNV classifications in both pre- and postnatal tests would avoid some obvious sources of confusion. The need to use sound, evidence-based, interpretation is even more critical in prenatal testing, especially with CNVs of uncertain significance. The challenge here is to meet the difficulty in communicating complex genetic information to allow parents to make informed decisions under the pressures of emotional stress and in a limited timeframe.

It should be noted that these same issues will impact at an even greater scale in the emerging clinical use of whole genome/exome sequencing. This has the potential to detect virtually all sequence variants and CNVs and other structural variants which have hitherto not been detectable genome-wide. The associated challenge of interpreting genomic variation on this scale will be much more demanding than that being experienced with microarrays. National professional bodies responsible for setting implementation guidelines in diagnostic laboratories, are currently grappling with these issues.

Unsolicited, incidental findings

Genomic microarray analysis will inevitably detect, albeit in a small proportion of cases, clinically significant CNVs unrelated to the clinical features that prompted testing. Experience already demonstrates that some unsolicited findings yield benefits to the patient and family, especially where effective treatment or management is available (for an extensive discussion see Boone *et al.* [62]). Examples are deletions of *STS* (causing steroid sulphatase deficiency) or deletions and duplications of *PMP22* (causing Hereditary Neuropathy with liability to Pressure Palsies and Charcot-Marie Tooth Disease Type 1A, respectively). CNVs which predict later onset morbidities in presymptomatic infants or children, such as deletions of genes such as *DMD* (causing dystrophinopathy) or *PROS1* (causing protein S deficiency), create challenging counselling issues. In assigning pathogenicity in this situation, it is important to remember the critical role played by ascertainment bias in establishing genotype-phenotype correlations in the literature. Applying these correlations where there is no such ascertainment should be done with careful consideration and caution.

Several recent studies have also shed light on issues related to incidental detection of CNVs involving genes associated with predisposition to dominant, adult-onset cancer [62-64].

These examples serve to emphasise the importance of providing pre-test information that includes explaining that microarray testing will occasionally uncover unsolicited findings, some of which may be of clinical relevance to the individual tested and some also to other family members.

It is already clear that decisions on what to include on the report should be based on clinical validity and actionability. Finally, in certain circumstances, reporting of unsolicited findings to the requesting doctor may be best managed through liaison with a specialist clinical geneticist.

Consanguinity

As the proportion of the genome that is identical (i.e. homozygous) by descent (IBD) is contingent upon the degree of parental relatedness [65], SNP microarray analysis may incidentally reveal consanguinity and incest [32, 46, 49, 66]. It is important to be aware that such findings do not provide definitive proof. In light of the legal, ethical, and medical issues associated with reporting of such results, appropriate consultation between laboratory and clinician is imperative. Several recent studies [32, 47, 49, 66] have called upon professional societies, such as the American College of Medical Genetics, the American Society of Human Genetics, and the European Society of Human Genetics to develop guidelines that address issues of consent and reporting of incidental detection of suspected consanguinity

and incest. Notwithstanding these, it is recommended that each institution has a policy whereby results indicating a possible incestuous parental relationship are discussed directly with the referring clinician before issue of the formal result, with the aim of ensuring that this information is dealt with sensitively, confidentially, and with a clear understanding of the possible interpretation of the result by non-geneticists. In addition, parents should be informed of the possibility of uncovering hidden parental relatedness before giving consent for SNP based molecular karyotyping. There is also the overlay of different local jurisdiction laws to be considered.

Carrier status for autosomal recessive disorders

A finding of an autosomal recessive allele in a healthy subject, or at least free of symptoms arising from the recessive condition, offers information about familial risk. As the level of risk is determined by the local mutation carrier frequency rate, interpretation must take this into account. Discovery of a recessive allele is arguably more significant for recessive disorders that are more frequent in particular communities (for example α thalassaemia in south-east Asian populations, or involvement of the *HEXA* gene in a child of Ashkenazi Jewish heritage). Recent guidelines such as those of the American College of Medical Genetics [22] suggest that comprehensive reporting of recessive alleles (i.e. carrier status) goes beyond the intended use of these tests. Decisions about reporting recessive alleles must take account of the clinical indication for the test, the likely clinical and familial significance of chance findings, and any agreement between patients and referring clinicians about disclosure of variants of significance. Ideally, inclusion of any disclosure policy regarding recessive carrier status on test reports would avoid any confusion amongst referring clinicians, patients and, in some instances, their families.

Follow-up testing

Assessing the significance of variants may be aided by examining genotype-phenotype relationships in relatives. This may be done by genome-wide microarray testing or by locus specific methods such as Multiplex Ligation-dependent Probe Amplification (MLPA), quantitative-PCR or Fluoroscence *in situ* Hybridization (FISH). FISH testing uniquely provides additional positional information, particularly the genomic location of duplications.

A particularly challenging issue is the need to exclude the possibility that a clinically relevant CNV might be an unbalanced derivative from a parental balanced reciprocal rearrangement, which if present, is of paramount importance for future pregnancies. However, ruling out this possibility for all such CNVs has major cost implications. Reciprocal translocation interchanges involving the ends of two chromosomes are relatively common and it would be prudent to pursue parental investigation of any pathogenic or 'likely pathogenic' CNV located at a chromosome end to exclude this possibility. FISH testing is useful in this regard. The case for following up interstitial CNVs, which are by far the majority of array findings, also needs to be considered despite the lower likelihood that they are derived from a parental balanced insertional translocation [67, 68]. The important point is that recurrence risk needs to be assessed before or at least early in any subsequent pregnancy to provide the option of prenatal diagnosis.

Systematic review of CNV interpretation

The clinical significance status of CNVs is open to reassessment in the light of new information. The American College of Medical Genetics [22] recommends that reports of CNVs of uncertain significance include a recommendation for continued surveillance of the medical literature for new information that may resolve the uncertainty. This view is also held by the European Society of Human Genetics (ESHG) [20], but goes one step further by commenting that "*it is the duty of the clinician/clinical geneticist to ask a laboratory to reanalyze the data and/or recall patients if a previously reported "benign" CNV is later found to be associated with a pathogenic disorder*." These recommendations assume that the doctor is aware of the patient's comprehensive list of CNVs. The practicality of this is highly questionable. For variants identified by massively parallel sequencing it is hard to imagine how this could be managed. The ESHG guidelines also state that '*CNV results*

must be retained by the laboratory, if possible in the patient's file and in a CNV database (local, national, or international)'. These recommendations demand that laboratories actively maintain their capability to access and reanalyze historic array data and, where required, to issue updated reports. These recommendations have resource implications, particularly for professional time.

Inter-laboratory sharing of CNV data

Sharing of CNV data amongst laboratories, especially newly-found rare variants, would progressively improve the overall quality of microarray testing services. In this regard, active involvement in international cataloguing initiatives such as the ISCA Consortium and DECIPHER is of particular value. Experience to date has shown that sharing of CNV information is manageable. However, the equivalent task for variants detected by massively parallel sequencing will be on a scale that is orders of magnitude higher in volume and complexity.

CONCLUSION

What has been learned and what challenges remain?

We have learned that, as is the case with testing for sequence variants, 'normal' copy number variation is far from completely documented and this is a major impediment to accurately distinguishing benign CNVs from pathogenic CNVs. What is clearly needed are comprehensive catalogues of benign and pathogenic CNVs. Studies such as the 1000 Genomes Project and Personal Genome Project and repositories such as DECIPHER and ISCA will make significant contributions to this aim. We have also learned that sound clinical interpretation of CNVs requires awareness of the likelihood that in many instances, the disorder under investigation has a multifactorial cause and that phenotypic expression cannot be simply explained by a dosage change in a single gene. The advances in our knowledge of genotype-phenotype relationships have shown us that interpretations made for previous tests may need to change. In the interests of reaching the correct interpretation, it is important that the evidence used to form opinions about the likely clinical significance of CNVs is clearly documented in the reports issued to referring clinicians and that systems are put in place for re-interpretations in the event of new evidence coming to light. The process of evidence-based interpretation is neither clearly defined nor widely understood. The conclusions of research publications in this field are often inferential rather than experimentally tested, and the skills needed for thorough assessment are not widespread. Specific training for microarray testing needs to be incorporated into existing professional development programmes for the laboratory geneticists who write reports. Of equal importance is the need to ensure that clinicians and genetic counsellors who use these reports have the necessary up-skilling and support to optimise interpretation of genomic test results for patient care. This should happen hand in hand with training for interpretation of next generation sequencing data as many of the issues and skills required are the same. Microarray testing has undoubtedly delivered significant improvements in the detection of clinical disorders with clear benefits to patients and their families. As the potential to confuse and mislead is real, decision making as always should be underpinned by the guiding ethical principle '*primum non nocere*'.

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