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GENETICS

Chromosome microarray proficiency testing and analysis of quality metric data trends through an external quality assessment program for Australasian laboratories

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Summary

Chromosome microarrays are an essential tool for investigation of copy number changes in children with congenital anomalies and intellectual deficit. Attempts to standardise microarray testing have focused on establishing technical and clinical quality criteria, however external quality assessment programs are still needed. We report on a microarray proficiency testing program for Australasian laboratories. Quality metrics evaluated included analytical accuracy, result interpretation, report completeness, and laboratory performance data: sample numbers, success and abnormality rate and reporting times. Between 2009 and 2014 nine samples were dispatched with variable results for analytical accuracy (30–100%), correct interpretation (32–96%), and report completeness (30–92%). Laboratory performance data (2007–2014) showed an overall mean success rate of 99.2% and abnormality rate of 23.6%. Reporting times decreased from >90 days to <30 days for normal results and from >102 days to <35 days for abnormal results. Data trends showed a positive correlation with improvement for all these quality metrics, however only 'report completeness' and reporting times reached statistical significance. Whether the overall improvement in laboratory performance was due to participation in this program, or from accumulated laboratory experience over time, is not clear. Either way, the outcome is likely to assist referring clinicians and improve patient care.

Key words: Proficiency testing; chromosome microarray; quality improvement.

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INTRODUCTION

Chromosome microarrays (CMAs) are designed to identify chromosome and gene copy number mutation (deletions and duplications) and they have changed the nature of clinical cytogenetics profoundly. The clinical utility of CMA has been demonstrated among children with intellectual deficit and dysmorphic features, via either comparative genomic hybridisation (CGH) arrays[1–3] or single nucleotide polymorphisms (SNP) genotyping arrays.[4,5] A large meta-analysis of 33 studies confirmed the clinical utility of microarray testing, which led to the consensus that it should be the first-tier test for individuals with congenital abnormalities, intellectual deficit, development delay, and behavioural or autistic spectrum disorders.[6] Although the application of microarrays has since extended to prenatal diagnosis,[7,8] as well as cancer diagnosis and prognosis,[9–11] we limit discussion in this paper to best practice guidelines and standards for quality improvement of postnatal 'constitutional' microarrays only.

Implementation of chromosome microarrays within the diagnostic laboratory is technically challenging and necessitates quality control metrics for many aspects of laboratory practice. The prime challenge resides in the confident discrimination of (1) true copy number variants (CNVs), and (2) for SNP arrays, those loci revealing absence of heterozygosity. Both entail the minimising of false positive and negative results. Many variables affect these diverse aberration calls, including the integrity of the DNA sample, variation in the efficiencies of labelling and hybridisation, microarray design (genome-wide or targeted), effective array resolution, as well as the software used to analyse the data.[12] Consequently, extensive standards and guidelines have been published, with a view to the validation of microarray testing, within the clinical diagnostic laboratory.[13]

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Despite establishing criteria for microarray quality control and assay validation, there remained concern about, and empirical evidence of, wide variation in reporting standards among different clinical laboratories. This was partly due to the many different microarray platforms in use, with differing designs and resolutions, complicated by the challenge of interpreting CNVs of arguable clinical significance.[14,15] In an attempt to optimise microarray results and standardise reports further, Vermeesch *et al.* proposed best practice guidelines, on both technical and clinical quality criteria. It was noted that internal and external quality control programs were urgently needed to evaluate and standardise results between laboratories, although few were available at the time.[16] External quality assessment program (eQAP) and proficiency testing (PT) are frequently used interchangeably, however eQAP tends to be used preferentially in Europe and Australia, whereas PT is used in the United States. PT is widely accepted as referring to a program in which reference samples are distributed for analysis and where results are compared with other laboratories as a group. Hence, we use PT where samples are distributed to laboratories for analysis to distinguish this activity from other retrospective auditing and statistical data collection activities within our program. We use eQAP to refer to our overall program or scheme.

PT is an essential component of a laboratory's quality management system and a good program not only evaluates analytical performance but also pre-analytical and post-analytical processes. In general, this can include aspects of sample registration and data entry, method performance, *in vitro* diagnostic devices in use in the laboratory, reporting practices, and staff education/training. PT should provide clinically relevant challenges that mimic patient samples and check the entire examination process. For maximum benefit, laboratories should process samples and report results as they would for routine patient samples. These programs may also serve as a component of the regulatory requirements for licensing and/or accreditation of laboratories. Furthermore, if PT is to be effective, then satisfactory versus unsatisfactory performance should be defined, within the program itself.[17] Such programs are provided across many disciplines of pathology with the ultimate aim being improved patient care.[17,18] Unfortunately, there are few providers of PT for genetic pathology,[19] and these tend to be for specific single-gene disorders, rather than for the typically multi-gene copy number changes commonly detected in array testing.

Accordingly, there is scarce literature on PT for microarray. In Europe, the current Cytogenetics External Quality Assessment Service (CEQAS) in the United Kingdom operates a program in collaboration with the European Molecular Genetic Quality Network (EQMN). However, we are unaware of any published data from this program. In the United States, a small pilot microarray program commenced in 2007, which was jointly operated by the College of American Pathologists (CAP) and the American College of Medical Geneticists (ACMG). Their aim was to evaluate the reproducibility and concordance of 'reported' abnormalities, and their significance. Tsuchiya *et al.* reported on the pilot program and found considerable variation in interpreting clinically significant CNVs, which involved the results of 13 single copy number variants being distributed to 11 different laboratories. None were reported with 100% concordance in respect of the clinical significance of the CNV. From 2010, submissions were graded if at least 80% of laboratories reached a 'consensus', or agreement, on the reportable findings. Unfortunately, they did not discuss whether there was overall improvement in quality of reported results between laboratories over this period.[20] The primary concern here relates to patient care, and whether the copy number change identified is interpreted and reported correctly (i.e., as clinically relevant, benign, or of unknown significance), or not reported at all. In this context, a need for internal and external quality assessment programs is self-evident.

While North American and European groups were formulating PT or eQAP, there was also a call for such a program from Australasian laboratories. Up until 2007, only five laboratories in Australia were performing microarray testing, however the number had doubled by 2009. Furthermore, medical diagnostic testing is regulated in Australia under Federal law, mandating that medical testing laboratories be accredited according to the standard ISO-15189, which requires participation in external quality assessment activities.

The Australasian Society of Diagnostic Genomics (ASDG, formerly known as the Australasian Society of Cytogeneticists, ASoC) Quality Assessment Program, operated in conjunction with the Human Genetics Society of Australasia (HGSA), has been providing an eQAP in Clinical Cytogenetics to over 40 laboratories within the region since 1997; i.e., in Australia, New Zealand, Malaysia, Singapore, Indonesia and Hong Kong. In 2009, we designed and released a small proficiency testing pilot program for chromosome microarray, which was limited to the detection of postnatal constitutional CNVs. This program aimed to evaluate analytical performance, including pre-analytical (data entry and sample accessioning) and post-analytical (interpretation and reporting) practices. In 2012, the ASDG QAP was accredited (microarray PT inclusive) according to the international standard ISO/IEC17043: 'Conformity assessment – general requirements for proficiency testing'.

Here, we report six years experience of microarray proficiency testing, and present data trends covering this same period. The aspects of laboratory performance monitored included analytical accuracy, result interpretation, and completeness of written reports. Basic laboratory performance metrics also reviewed included test success rates, reporting times, and abnormality detection rates. As far as we are aware, this is the first report of a proficiency testing program including inter-laboratory comparison of this range of metrics, within the context of clinical microarray services.

MATERIALS AND METHODS

Proficiency testing samples

Between 2009 and 2014 the program provided known (reference) DNA samples with pathogenic copy number changes, plus two 'normal' samples, to participating laboratories. The de-identified samples (designated 'PT') were representative of routine patient samples and each comprised ~2 μg DNA. Each was dispatched along with clinical notes indicating age, sex and clinical phenotype, or indication for testing. Single PT samples were dispatched for the initial pilot survey in 2009, and 2010 and 2011 surveys. Between 2012 and 2014, we dispatched two PT samples per year. A summary of all samples is provided in Table 1.

Prior to dispatch, each PT sample was assessed to ensure the integrity and purity of DNA, by gel electrophoresis and optical density ratios (260 nm/280 nm and 260 nm/230 nm). Each PT sample was then array-tested using two independent microarray platforms, which preferably involved both oligonucleotide CGH and SNP microarray platforms. Alternatively, either