

Replacing Conventional Karyotyping with 'Molecular Karyotyping' to Increase Diagnostic Success

It has become clear that the increased gap payment is proving to be a disincentive in referring patients to VCGS for Molecular Karyotyping. Hopefully Medicare will redress this problem in the relatively near future. Due to the very clear superiority of this new test, we will, for a limited period, provide 'Molecular Karyotyping' for private patient referrals at the same price as 'Conventional Karyotyping'.

The new test:

'Molecular Karyotyping' represents the most significant advance in cytogenetic testing since the introduction of G-banding in the 1970's and is now regarded as best practice as the first line test for patients with intellectual disability, multiple congenital abnormalities or developmental delay.

A 5ml EDTA blood sample is required along with a request for 'Molecular Karyotyping'. Please give as much relevant clinical information as possible on the referral form as this helps greatly with interpretation of genomic findings.

Why use Molecular Karyotyping?

'Molecular Karyotyping' using high density microarrays provides more than 10 times higher resolution than Karyotyping by microscopy. 'Conventional Karyotyping' can be thought of as very low resolution genome scanning which can detect up to 800 'bits' of information, to use a computing analogy. In comparison, 'Molecular Karyotyping' provides from 100,000 to 2,700,000 'bits' depending of the type of array used and detects submicroscopic chromosome imbalances, called Copy Number Variations (CNVs).

Our pilot study (Bruno et al. J Med Genet. 2009, see over) as well as several other European and USA studies have shown that 'Molecular Karyotyping' detects significantly more pathogenic chromosome abnormalities than 'Conventional Karyotyping'. Microscopy detects abnormalities in 2-3% of referrals whereas microarrays detect abnormalities in an additional 15%! Furthermore, pathogenic abnormalities are being found in individuals who would not normally be referred for karyotyping such as those with isolated or mild intellectual impairment, autism or epilepsy.

Interpretation of test results:

Such is the power of 'Molecular Karyotyping' that more than 20 new syndromes have recently been described in the literature. Furthermore, discovery of novel abnormalities is ongoing and the final number of previously unrecognised syndromes is likely to reach into the hundreds. Interpretation requires distinction of pathogenic chromosome changes from so-called 'benign or polymorphic CNVs'. This is a very important aspect of performing the test and VCGS Medical Scientists have specific expertise and experience in this area.

Test charges:

The reduced cost of the test will be \$452.00 of which Medicare will pay \$307.15, leaving patients an out of pocket payment of \$141.55. This is the same charge applied by VCGS for conventional Karyotyping and can be offered for a limited period.

Turnaround time: Test results should be available in 2-3 weeks after sample receipt. Urgent results can be prioritised on request.

Exclusions:

Molecular Karyotyping does not detect balanced chromosomal rearrangements such as reciprocal translocations and inversions. However, these are very unlikely to be the cause of intellectual impairment, developmental delay, multiple congenital abnormalities, autism or epilepsy.

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A new diagnostic workflow for patients with mental retardation and/or multiple congenital abnormalities: test arrays first

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High-density single-nucleotide polymorphism (SNP) genotyping technology enables extensive genotyping as well as the detection of increasingly smaller chromosomal aberrations. In this study, we assess molecular karyotyping as first-round analysis of patients with mental retardation and/or multiple congenital abnormalities (MR/MCA). We used different commercially available SNP array platforms, the Affymetrix GeneChip 262K *NspI*, the Genechip 238K *StyI*, the Illumina HumanHap 300 and HumanCNV 370 BeadChip, to detect copy number variants (CNVs) in 318 patients with unexplained MR/MCA. We found abnormalities in 22.6% of the patients, including six CNVs that overlap known microdeletion/duplication syndromes, eight CNVs that overlap recently described syndromes, 63 potentially pathogenic CNVs (in 52 patients), four large segments of homozygosity and two mosaic trisomies for an entire chromosome. This study shows that high-density SNP array analysis reveals a much higher diagnostic yield as that of conventional karyotyping. SNP arrays have the potential to detect CNVs, mosaics, uniparental disomies and loss of heterozygosity in one experiment. We, therefore, propose a novel diagnostic approach to all MR/MCA patients by first analyzing every patient with an SNP array instead of conventional karyotyping.

Journal of Medical Genetics 2009; **46**:123-131

Detection of cryptic pathogenic copy number variations and constitutional loss of heterozygosity using high resolution SNP microarray analysis in 117 patients referred for cytogenetic analysis and impact on clinical practice

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Background: Microarray genome analysis is realising its promise for improving detection of genetic abnormalities in individuals with mental retardation and congenital abnormality. Copy number variations (CNVs) are now readily detectable using a variety of platforms and a major challenge is the distinction of pathogenic from ubiquitous, benign polymorphic CNVs. The aim of this study was to investigate replacement of time consuming, locus specific testing for specific microdeletion and microduplication syndromes with microarray analysis, which theoretically should detect all known syndromes with CNV aetiologies as well as new ones.

Methods: Genome wide copy number analysis was performed on 117 patients using Affymetrix 250K microarrays. **Results:** 434 CNVs (195 losses and 239 gains) were found, including 18 pathogenic CNVs and 9 identified as "potentially pathogenic". Almost all pathogenic CNVs were larger than 500 kb, significantly larger than the median size of all CNVs detected. Segmental regions of loss of heterozygosity larger than 5 Mb were found in 5 patients. **Conclusions:** Genome microarray analysis has improved diagnostic success in this group of patients. Several examples of recently discovered "new syndromes" were found suggesting they are more common than previously suspected and collectively are likely to be a major cause of mental retardation. The findings have several implications for clinical practice. The study revealed the potential to make genetic diagnoses that were not evident in the clinical presentation, with implications for pretest counselling and the consent process. The importance of contributing novel CNVs to high quality databases for genotype-phenotype analysis and review of guidelines for selection of individuals for microarray analysis is emphasised.

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