Microdeletions detected using chromosome microarray in children with suspected genetic movement disorders: a single-centre study

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AIM Chromosome microarray (CMA) can determine copy number variants such as microdeletions or microduplications. Microdeletions of movement disorder genes including epsilon-sarcoglycan (SGCE) and thyroid transcription factor-1 (TITF1) have been described in patients with myoclonus dystonia and benign hereditary chorea respectively. We examined whether CMA is a valuable tool in the investigation of children with suspected genetic movement disorders.

METHOD A genetic movement disorder was suspected if there was a positive first-degree family history, or two or more of the following factors: normal or near-normal magnetic resonance imaging, negative history of brain injury, and negative investigations for metabolic disorders. Tic disorders were excluded. Twenty-five patients (18 males, seven females) with a mean age at movement disorder onset of 4 years 5 month (range 1mo–14y) were prospectively recruited with the following primary movement disorders: dystonia (n=10), paroxysmal kinesigenic dyskinesia (n=5), tremor (n=4), chorea (n=3), myoclonus (n=2), and paroxysmal non-kinesigenic dyskinesia (n=1). Comorbid associated features were common, particularly developmental delay or intellectual disability (19 out of 25) and attention-deficit–hyperactivity disorder (six out of 25). CMA was performed using Agilent aCGH 60K array.

RESULTS Seven out of twenty-five patients had a microdeletion determined by CMA. None of the microdeletions were considered benign variants. Four patients had a deletion of a known movement disorder gene including paroxysmal kinesigenic dyskinesia (PRRT2; n=2), SGCE (myoclonus dystonia, n=1), and TITF1 (benign hereditary chorea, n=1). Three patients had novel microdeletions of unknown but potential significance including 14q13.3 (chorea, n=1), 19p13.12 (tremor, n=1), and 19q13.12 (progressive dystonia). All seven patients had associated neurodevelopmental or behavioural problems.

INTERPRETATION Assays that determine copy number variants may be a valuable first-tier investigation in patients with suspected genetic movement disorders, particularly when associated with intellectual disability or developmental disorders. Microdeletion syndromes may help the search for new movement disorder genes.

Chromosomal microarray (CMA) is used to estimate copy number for any segment of the genome and can identify genomic microduplications or microdeletions that are beyond the resolution of standard G-banded karyotyping.1,2 Microdeletions involving epsilon-sarcoglycan (SGCE) and thyroid transcription factor-1 (TITF1) have been found in some patients with myoclonus dystonia and benign hereditary chorea respectively.3,4 As most movement disorders of genetic origin exhibit autosomal dominant inheritance,5 de novo or inherited microdeletions or duplications could be a significant cause of genetically derived movement disorders. We, therefore, assessed the use of CMA in a cohort of children with suspected genetic movement disorders.

METHOD

Patients

The study was initiated after the description of an index family with myoclonus dystonia and psychosis that had a familial 7q21.3 microdeletion involving SGCE.6 After that time, all children with a suspected genetic movement disorder had a CMA performed before direct gene sequencing.

Between January 2009 and June 2011, 120 patients with movement disorders were assessed at the Movement Disorder Clinic at the Children's Hospital at Westmead, NSW, Australia and were subgrouped as follows: (1) suspected genetic movement disorders (inclusion criteria below; n=25); (2) previously proven monogenic movement disorders including DYT1, dopa-responsive dystonia, tyrosine hydroxylase...
deficiency, or myoclonus dystonia (n=10); (3) other genetic disorders including Huntington disease, neurodegeneration with brain iron accumulation (n=8); (4) symptomatic movement disorders including inflammatory disorders, metabolic disorders, and dystonic cerebral palsy (n=31); (5) Tourette syndrome or other tic disorder (n=38); (6) psychogenic movement disorder (n=4); (7) stereotypy (n=4).

The inclusion criteria for a suspected genetic movement disorder (n=25) was a positive first-degree family history, or two or more of the following factors: normal or near-normal magnetic resonance imaging (MRI), negative history of brain injury, and negative investigations for metabolic disorders. Tic disorders were excluded from this study.

Twenty-five index patients (18 males, 7 females) fulfilled the criteria for a suspected genetic movement disorder during this period. The mean age at movement disorder onset was 4 years 5 months (median 4y, range 1mo–14y), and the median age at assessment was 10 years 8 months (median 11y, range 3–16y). A positive first-degree family history of movement disorder was present in five out of 25. A single movement disorder was present in 20 out of 25 patients, and a mixed movement disorder was present in five out of 25. The primary movement disorders were as follows: (1) dystonia including dystonic tremor (n=10, generalized in nine and segmental in one); (2) paroxysmal kinesigenic dyskinesia (PKD; n=5; all patients were carbamazepine responsive); (3) tremor (n=4); (4) chorea (n=3); (5) myoclonus (n=2, one with myoclonus dystonia); (6) paroxysmal non-kinesigenic dyskinesia (n=1).

The movement disorder had a static course in 20 out of 25 and a progressive course in five out of 25. Gait disturbance was present in 15 out of 25. Associated features were present in 22 out of 25, as follows: (1) Developmental delay or intellectual disability (19 out of 25). The intellectual disability was mild in most children, and 21 out of 25 patients attended normal school, some with additional support. (2) Attention-deficit–hyperactivity disorder (DSM-IV; n=6); (3) Epilepsy (n=4), including two patients with PKD with benign infantile seizures (infantile convulsions choreoathetosis syndrome); (4) Anxiety (DSM-IV; n=3); (5) less common associations including Asperger syndrome (n=2), joint hyperextensibility (n=2), dyspraxia (n=2), spasticity, neuropathy, catacar, limb deformity, sensorineural deafness, oligodontia, and cleft palate (n=1).

All 25 patients had brain MRI: 21 were normal and four showed subtle white matter changes of unknown significance that showed partial resolution on repeat imaging. The following investigations were performed, which were normal: studies of cerebrospinal fluid including glucose and neurotransmitters (n=16), metabolic studies including urine metabolic screen (urine amino acids, organic acids, and glycosaminoglycans), copper, caeruloplasmin, thyroid function, and urate (n=15). A trial of levodopa was performed in nine patients without significant clinical benefit. Specific negative investigations for patients 4, 6, and 7 who had novel copy number variant (CNV) are listed below Table I.

In patients with a positive finding, the assay was also performed in parents to determine if the microdeletion was de novo or inherited. Written consent and ethical approval were acquired from the all described individuals and the hospital.

Chromosome microarray
The clinical details of the seven patients are described below and in Table I. Arrays were handled and loaded according to the manufacturer’s specifications. Array scans were performed at 3μm resolution using an Agilent DNA Microarray Scanner, model SYS-SN-ARRAY (Agilent Technologies, Santa Clara, CA, USA). For all patients except patient 2, the SurePrint G3 Human CGH Microarray (Agilent Technologies) 8×60K non-targeted array design was used for the initial test. For patient 2, the ISCA 8×60K design, version 1, was used. Array data analysis was undertaken using Agilent CytoGenomics software (Agilent Technologies), with routine settings as follows: aberration algorithm: ADM-2; threshold: 6.7; neutralization: ON; bin size: 10; centralization threshold 6.0; fuzzy zero: ON; combine replicates (intra array): ON; genome: hg18; aberration filters: minProbes=5 AND minAvgAbsLogRatio=0.25 AND maxAberrations=30 AND percentPenetrance=0; expand non-unique probes: OFF. Some initial array results were confirmed as follows: patient 1 by fluorescence in-situ hybridization for proband; patient 4 by array testing of an affected brother and affected mother; patient 5 by the 60K array result was suggestive only (because only four probes were deleted), so the result was confirmed, for all three affected family members, by SurePrint G3 Human CGH Microarray 2×400K (e.g. 252185010124); patient 6 by array testing of affected mother.

RESULTS
Seven out of 25 patients with a suspected genetic movement disorder had a microdeletion demonstrated on CMA (28% Table I). Column 7 of Table I shows the size of the deletion (in megabases [Mb]), the serial number of the individual array chip used, and the number of array probes mapping to the deletion found. A minimum of five deleted probes was regarded as necessary for statistical significance, so the ‘probes deleted’ parameter gives an indication of the limits of sensitivity for these 60K arrays. For patient 5, note that 60K resolution was not quite sufficient to prove the 0.16Mb deletion in question. However, it did produce a suspect result, sufficient to engender a high-resolution CMA follow-up, confirming the 60K finding.

PAROXYSMAL KINESIGENIC DYSKINESIA
Two out of five patients with PKD (patients 1 and 2) tested had a microdeletion in 16p11.2, the region incriminated in

What this paper adds
• We have shown that a significant proportion of children with suspected genetic movement disorders have chromosomal microdeletions.
• Some of these microdeletions involve known movement disorder genes or known loci.
• Some of these microdeletions are of unknown significance.
• CMA should be considered in children with suspected genetic movement disorders, particularly if there are associated neurodevelopmental problems.
• Microdeletions may be useful at defining new movement disorder genes.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at MD onset (y)</th>
<th>Mode of inheritance</th>
<th>Suspected clinical syndrome</th>
<th>Associated features</th>
<th>Chromosome band location</th>
<th>Microdeletion size (array serial number; probes deleted)</th>
<th>Chromosomal coordinates (hg18)</th>
<th>Candidate or OMIM disease-associated gene deleted, or susceptibility locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>De novo</td>
<td>PKD</td>
<td>Speech delay, mild</td>
<td>16p11.2</td>
<td>0.43 Mb (25192414173; 25)</td>
<td>29,581,455–30,013,488</td>
<td>PRRT2 16p11.2 locus</td>
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<tr>
<td>2</td>
<td>10</td>
<td>De novo</td>
<td>PKD/ICCA</td>
<td>Benign infantile seizures, mild ID</td>
<td>16p11.2</td>
<td>0.60 Mb (25227831011; 8)</td>
<td>29,500,284–30,098,069</td>
<td>PRRT2 16p11.2 locus</td>
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<tr>
<td>3</td>
<td>1.5</td>
<td>De novo</td>
<td>Benign hereditary chorea</td>
<td>DD, mild ID, ligamentous laxity, oligodontia, gait disturbance</td>
<td>14q13.2–q21.1</td>
<td>3.27 Mb (25219241358; 51)</td>
<td>35,327,739–38,602,335</td>
<td>TITF1, PAX9, MIPOL1, SEC23A</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>Maternal</td>
<td>Benign hereditary chorea</td>
<td>DD, gait disturbance</td>
<td>14q13.3</td>
<td>0.16 Mb (25219241347; 6)</td>
<td>35,730,648–35,885,742</td>
<td>Unknown (TITF1 is 0.18Mb outside deletion)</td>
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<tr>
<td>5</td>
<td>3</td>
<td>Paternal</td>
<td>Myoclonus dystonia</td>
<td>Mild ID, anxiety, gait disturbance</td>
<td>7q21.3</td>
<td>0.16 Mb (252192410062; 4)</td>
<td>93,943,241–94,100,725</td>
<td>SGCE</td>
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<tr>
<td>6</td>
<td>5</td>
<td>Maternal</td>
<td>Tremor</td>
<td>DD, mild ID, ADHD, mild dysmorphism</td>
<td>19p13.12</td>
<td>0.41 Mb (252192411961; 11)</td>
<td>14,069,071–14,482,692</td>
<td>19p13.12 locus</td>
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<tr>
<td>7</td>
<td>4</td>
<td>De novo</td>
<td>Progressive dystonia</td>
<td>Mild ID, gait disturbance</td>
<td>19q13.12</td>
<td>0.63 Mb (252192414203; 28)</td>
<td>40,300,506–40,925,348</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*See Results section: ‘Chromosome microarray’ for validation of this result.*

Mode of inheritance: de novo, both parents tested and negative using same CGH microarray. Additional normal investigations in patients with unknown microdeletions; patient 4, brain scan by magnetic resonance imaging, cerebrospinal fluid neurotransmitters and glucose, urine metabolic screen, copper, caeruloplasmin; patient 6, brain scan by magnetic resonance imaging, thyroid function, urine metabolic screen, copper, caeruloplasmin; patient 7; brain scan by magnetic resonance imaging, cerebrospinal fluid neurotransmitters and glucose, urine metabolic screen, DYT1, levodopa trial. MD, movement disorder; PKD, paroxysmal kinesigenic dyskinesia; ICCA, infantile convulsions, choreoathetosis syndrome. DD, developmental delay; ID, intellectual disability; ADHD, attention-deficit-hyperactivity disorder.
PKD. One of these patients had been previously reported. The microdeletions in these unrelated individuals with PKD were very similar and both delete PRRT2, the recently defined PKD gene (Table I). Testing of parents confirmed that the mutations were de novo in both patients.

**Chorea**

One of the three patients with chorea had a de novo microdeletion involving TITF1, the gene incriminated in benign hereditary chorea (patient 3, Table I). This patient had a mixed movement disorder with chorea plus additional myoclonus and dystonic posturing. The patient did not have lung disease but did have biochemical hypothyroidism, hyperextensible joints, and hypodontia, the last probably related to deletion of the contiguous gene PAX-9. A further patient with chorea (patient 4) had a microdeletion adjacent to but not involving TITF1 (Table I). Patient 4’s biological mother and brother were similarly affected and carried the same microdeletion. The significance of this microdeletion is unknown, although it is possible that this microdeletion contains a gene with distal regulatory effects on TITF1.

**Myoclonus**

The patient with myoclonus dystonia had a microdeletion involving SGCE, which had been previously described (patient 5). Her symptomatic sibling and father had the same microdeletion.

**Tremor**

One patient (patient 6) with tremor, attention-deficit–hyperactivity disorder, and mild intellectual disability had a microdeletion in 19p13.12. There is no known benign variant in this region. This 19p13.12 microdeletion has been reported to cause learning difficulties, attentional problems, and stereotypical movements. The mother, who had intellectual disability but no tremor, carried the same microdeletion.

**Dystonia**

One patient with progressive generalized dystonia including bulbar involvement had a microdeletion in 19q13.12 (patient 7). There is no known benign variant in this region.

**DISCUSSION**

**Microarray testing considerations**

CMA testing may measure DNA copy number only, or it can be coupled with single nucleotide polymorphism genotyping. The former (described as comparative genomic hybridization by array or aCGH) can also be combined with single nucleotide polymorphism analysis, in a single ‘hybrid’ array platform. All such genome-wide screening tests can detect microdeletions or -duplications in approximately 15 to 20% of patients referred for intellectual disability and autism, a significantly higher diagnostic yield than karyotyping (approximately 3%).

Routinely, we used an ‘Agilent aCGH’ 60K targeted array (60-mer oligo probes) with a mean effective resolution of 0.25Mb, based on our standard requirement that at least five consecutive probes must be deleted or duplicated to report a positive finding. We note that all microarrays analyses have their limitations, and they are unable to detect the smaller microdeletions or duplications, including most intragenic changes in copy number. Indeed, the number of positive findings is related to the resolving power of the array platform used, a parameter dependent on the array’s designed probe density. However, there is a trade-off in this regard: very high-density arrays achieve more positive findings, but lack specificity and tend to detect the many very small and innocuous CNVs that abound in the normal population. Hence, intermediate levels of resolution are usually preferred. Accordingly, the American College of Medical Genetics (ACMG) has recently recommended a minimum resolution of 0.4Mb, genome-wide, as desirable for postnatal testing arrays. The CMA used in the present study achieved a resolution of 0.25Mb (i.e. greater than the 0.4Mb recommended by the ACMG), and even higher density arrays were available to us if needed such as the ‘400K’ arrays with mean effective resolution, in our hands, of 0.06Mb. Indeed, 400K arrays were used to confirm the findings for our patient 5, and family, where the 60K platform was suggestive only.

We did not use single nucleotide polymorphism arrays here, as this study aimed to discover changes in gene copy number as potential causes of movement disorder detected on routine referral for array testing. The ACMG statement concurs, noting that ‘[single nucleotide polymorphism] analysis is not a requirement at present for clinically appropriate genomic microarrays, as the primary goal of these analyses is to reliably detect copy number alterations’. The 60K arrays proved to be generally adequate for our purpose although, as we note above, higher-resolution arrays may be required for confirmatory testing. We would have found additional positives if the 400K arrays had been used throughout. However, as noted, many ‘false’ positives would be among these additional findings, and in any case, the increased costs would have been prohibitive for routine testing in clinical practice at this time.

The decision as to whether any novel CNV is pathogenic, or otherwise, is not straightforward. We follow others in regarding as innocuous any CNV that is well known among ‘controls’, as per the literature, or databases such as the Database of Genomic variants (http://projects.tcag.ca/cgi-bin/variation/gbrowse hg18/) or the Copy Number Variation Project of the Children’s Hospital of Philadelphia (http://cnv.chop.edu). This presumption may be corroborated by observation in unaffected relatives. Conversely, a familial CNV not found in such databases, and limited to the affected family members, is regarded as likely to be pathogenic (e.g. patients 4, 5, and 6 of this study). Furthermore de novo (non-familial) CNVs, unknown to the literature, are also regarded as likely to be pathogenic. Concerning de novo CNVs, we are informed by arguments such as that of Mefford et al., who discuss ‘... the structural-variant disease hypothesis’, whereby it is proposed that ‘[large (>100kb) structural genetic changes] arise often in the germ line but are typically ‘under strong selective constraint’. Hence, they argue that ‘de novo events will have a significant impact...’ on phenotype." In other
words, we assume that de novo deletions of greater than 100kb are good candidates as causal for the disease in question. Patients 1 to 3, and 7, are examples here of this principle. Of course, these classification principles are not foolproof. There is also the matter of incomplete penetrance to consider, when considering ‘normal’ carriers of those CNVs not well described in the literature.

On routine diagnostic testing with 60K arrays, we classify around 30% of patient genomes as potentially abnormal, in respect of either micro-duplication or -deletion. In follow-up of apparently unaffected relatives, half of this 30% are shown to be familial, and thereby presumed innocuous. In patient 4 of this study, such a CNV was found (not described), and was assumed benign on grounds that (1) a more likely candidate CNV was also present, in both affected siblings and mother, and (2) the CNV did not co-segregate with disease among the family members tested.

**Clinical aspects**

This study suggests that CMA or other assays that measure copy number variation may be a useful first-tier investigation in children with suspected genetic movement disorders. Twenty-eight per cent of patients had an abnormal microdeletion, representing a yield that is comparable to the findings in unexplained developmental delay, intellectual disability, and autism. The relatively high yield from this method is in contrast to that of the MRI, which although important in excluding structural problems, otherwise provided no diagnostic information. We are now using CMA as a first-tier investigation in these children, and before targeted genetic investigation. Although the patients were all referred because of their movement disorder, there was a high incidence of associated features, particularly developmental delay, or intellectual disability, or both. Indeed, all seven children with an abnormal CMA had problems with learning, although these issues were mild, and all patients except one were at mainstream school. This study would have been improved if detailed neuropsychological analysis had been performed to better define the cognitive manifestations. Only one of the patients had overt dysmorphism (patient 6), although another patient had hyperextensible joints and hypodontia (patient 3).

Four of the patients had a microdeletion involving known movement disorder genes. Two unrelated patients with PKD had almost identical de novo microdeletions in 16p11.2 (Table I). The 16p11–q21 region has been implicated in PKD for some time, and recent study incriminates the haplo-insufficient gene PRRT2 in this regard. Both patients with PKD in this study had deleted one PRRT2 allele. The present finding suggests that at least a small proportion of patients with PKD have a microdeletion syndrome involving 16p11.2 that involves PRRT2. It is increasingly apparent that 16p11.2 microdeletions can cause a broad neurological phenotype including autism, developmental delay, speech delay, intellectual disability, epilepsy, and paroxysmal dyskinesia. One patient with chorea plus associated myoclonus and dystonic posturing had a microdeletion involving the whole of TITF1, supporting a diagnosis of benign hereditary chorea. A further patient with myoclonus dystonia had a microdeletion involving 90% of the epsilon sarcoglycan. This was inherited from her father who had psychosis but no myoclonus dystonia.

Three further patients had microdeletions that have not been previously reported in the context of movement disorders. Although it is possible these are incidental findings, none of these microdeletions is considered to be a benign variant. We should emphasize that any finding of a novel CNV of unknown significance should not preclude the clinician from investigating further for other diagnoses. Patient 4 had a microdeletion in 14q13.3 (Table I) that was immediately adjacent to, but not involving, TITF1; the significance of this is unknown although this region may include a gene with a distal regulatory effect on TITF1. Patient 6 had a maternally inherited microdeletion in 19p13.12, whose clinical phenotype was tremor plus associated mild intellectual disability, attention-deficit–hyperactivity disorder, and mild dysmorphism. Almost identical 19p13.12 microdeletions have been reported by Bonaglia et al. in three children with dysmorphism, intellectual disability, attentional problems, and stereotyped movements. Bonaglia described six deleted genes in the overlapping region of all three patients, and these six genes were deleted in our study (LPHN1, CD97, DDX39, PKN1, PTGER1, GIPC1). The 19p13.12 deletion we describe is the smallest yet reported, and matches very closely the ‘shortest region of overlap’, as described in the context of previously reported cases. Our finding would, therefore, suggest that one or more of the genes in this 19p13.12 region have a role in the control of movements, and may represent a new gene for tremor.

One patient with progressive dystonia had a de novo microdeletion of 19q13.12. This region contains many genes that have important neuronal function, several of which are potential candidates that require investigation.

In conclusion, although small, this cohort was representative of children with suspected genetic movement disorders seen in clinical practice. The rate of positive findings from CMA in this group is, relative to other means of investigation, high and the technique is becoming increasingly less expensive. Clearly, larger studies are needed but this study raises the possibility that CMA will provide useful diagnostic information in some patients. The technique may also help to narrow the search for new movement disorder genes.

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