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LETTER

Dual copy number variants involving 16p11 and 6q22 in a case of childhood apraxia of speech and pervasive developmental disorder

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In this issue, Raca *et al*[1] present two cases of childhood apraxia of speech (CAS) arising from microdeletions of chromosome 16p11.2. They propose that comprehensive phenotypic profiling may assist in the delineation and classification of such cases. To complement this study, we would like to report on a third, unrelated, child who presents with CAS and a chromosome 16p11.2 heterozygous deletion. We use genetic data from this child and his family to illustrate how comprehensive genetic profiling may also assist in the characterisation of 16p11.2 microdeletion syndrome.

A number of chromosome 16p11.2 aberrations have been reported in the recent literature, including gross rearrangements and submicroscopic (<1 Mb) deletions and duplications with incomplete penetrance and variable expressivity and in a heterozygous form.[2] In general, microdeletions appear to be more penetrant than their respective duplications.[3] The 'typical' 16p11.2 deletion encompasses 539 kb (from chromosome position 29.5–30.1 Mb, GRCh37/hg19) and 24 genes, but a smaller adjacent distal or 'atypical' deletion (between chromosome positions 28.7 and 28.95 Mb, GRCh37/hg19) has also been reported, as have novel anomalies outside of these specified regions.[2,4,5] Individuals have been described with deletions spanning both these regions[4,6] and families have been observed to carry both rearrangement types.[7] It has been suggested that proximal rearrangements may be associated with developmental impairments and distal variations correlated with altered body mass index,[2] although developmental delays and speech and language impairments appear to be a common feature of individuals with various anomalies across this chromosome band.[8–10]

Screens of clinical cohorts indicate that chromosome 16p11.2 rearrangements are observed at a frequency of 0.3–0.7% in patients with various developmental impairments including autistic disorder (eg, Weiss *et al*[11]), developmental delay (eg, Shinawi *et al*[3]), epilepsy[12] and schizophrenia (eg, McCarthy *et al*[13]). Deletions are also observed in apparently healthy individuals, at a similar frequency to clinical cohorts,[5] and while the majority of cases appear to be *de novo*, inherited imbalances of this region are not uncommon.

Recently there has been a drive to define a core clinical phenotype of the 16p11.2 microdeletion syndrome. In a retrospective screen of 9773 individuals referred for microarray testing, Rosenfeld *et al*[8] found that 77 carried chromosome 16p11.2 anomalies (45 deletions, 32 duplications, 0.78%). A detailed review of 18 patients found that the most consistent clinical manifestations in these individuals were intellectual impairment and speech and language delays.[8] These findings were supported by a similar study that included 7400 patients who had undergone array comparative genomic hybridisation (array-CGH) testing in a clinical context, 45 of whom carried 16p11.2 anomalies (27 deletions, 18 duplications, 0.6%).[3] Phenotypic characterisation of 27 individuals also found that all had speech and language delays and cognitive impairment.[3] Other predominant features of 16p11.2 syndrome include dysmorphism, macrocephaly and autistic disorders.[3,4,8,14] However, all of these features have been disputed and it is likely that ascertainment bias will affect the conclusions of many studies, particularly those that focus upon single cases. Thus, the characterisation of the relationships between genetic aberration and clinical presentation is ongoing and will require further, more refined, studies with detailed investigations of this chromosome region and consistent phenotyping of affected individuals.

The child described here was originally assessed for the presence of *FOXP2* (OMIM #605317) mutations and rearrangements, as part of an earlier screening project,[15] as disruptions of this gene have been implicated in rare cases of severe speech and language disorder.[16] Although no *FOXP2* mutations were identified in the child, we discovered a deletion of chromosome 16p11.2. The child is a second-born male child of unrelated and healthy parents of European (Caucasian) descent. His early development was normal until the age of 1.5 years, when there was evidence of a social withdrawal. He was referred following concerns regarding his speech and language development and received a diagnosis of developmental verbal dyspraxia, also known as CAS, and pervasive developmental disorder not otherwise specified (PDD-NOS) according to DSM-IV.[17] On assessment with the Vineland Adaptive Behaviour Scales,[18] he showed a remarkable impairment in language and communication. At age 14, he scored at an age equivalent of 3 years and 3 months in the communication domain, with a major impairment in the expressive subdomain. He also displayed stereotypic movements and behavioural disturbances with self-aggressive episodes. He did not have hearing or ocular problems and had normal height and weight. The patient showed macrocephaly and peculiar facial features, such as heavy eyebrows with mild synophris, down-slanting palpebral fissures, hypertelorism, short philtrum, carp shaped mouth and full lips. He had brachydactyly and single crease bilaterally. His parents were clinically normal with no family history of speech delay, autistic disorders or mental retardation.

Peripheral blood samples were collected from the proband and his parents, and DNA extracted according to standard procedures.[19] To identify genomic imbalances, DNA samples were hybridised to Agilent 244K and Agilent 4 × 44K arrays (Agilent Technologies Inc., Santa Clara, CA, USA) for the proband and his parents, respectively. The array-CGH was completed as part of an assessment of 36 children with specific language impairment, PDD-NOS and autism spectrum disorders (ASD). Image data were extracted using Agilent Feature Extraction software version 8.5 (Agilent Technologies Inc.) and analysed using Agilent CGH Analytics software version 3.4 (*z*-score method setting) (Agilent Technologies Inc.). The reference genomic DNA samples used throughout the study were from the same consented individuals, one male and one female. We estimate that the mean resolution of the Agilent 244 K arrays is ~40 kb.

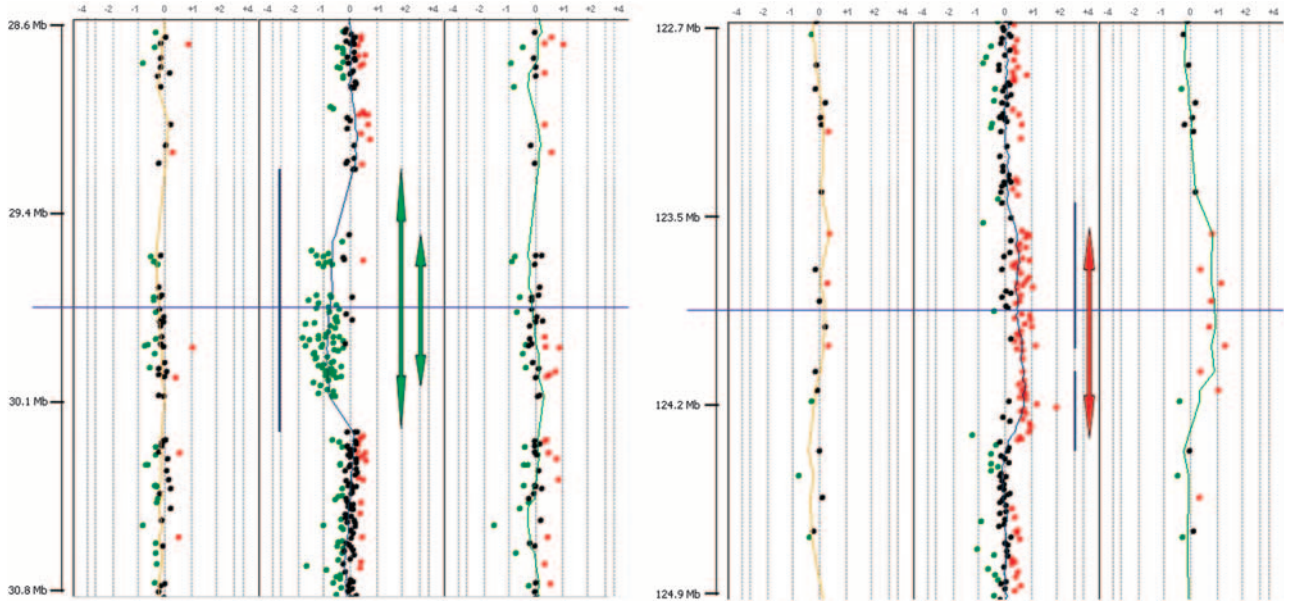

Figure 1 Chromosome 16p11.2 deletion and chromosome 6q22 duplication found in proband with CAS. The chromosome 16 deletion is shown in the left panel and the chromosome 6 duplication in the right panel (the minimally deleted and duplicated regions are indicated by the green and red double-ended arrows, respectively. The proband's DNA was examined using an Agilent 244 K array and the parents on the lower density 44K arrays.

We identified a *de novo* chromosome 16p11.2 deletion in the proband's sample (see Figure 1). The minimal region affected by this deletion spans from chromosome position 29 652 999 to 30 199 351 (GRCh37/hg19) and encompasses 28 Refseq genes, thus coinciding with the 'proximal' type reported in the literature.[2] The presence of speech and language abnormalities, macrocephaly and PDD in this child coincides with previously reported core phenotypes of chromosome 16p deletions.[1,3,4,8,14] The CAS diagnosis of this case provides further support to the findings of Raca *et al*[1] and their theory as to the importance of proximal chromosome 16p11.2 abnormalities in CAS. It would thus be of interest to fully assess the CAS in this child using the Madison Speech Assessment Protocol and other relevant speech batteries suggested by Raca *et al*.[20,21]

Using the array-CGH data, we catalogued all observed imbalances that spanned four or more consecutive oligonucleotide probes with values outside the $\log_{10}$ Cy-dye threshold ratios for the proband. We excluded any region that had been observed repeatedly either in control data deposited in the Database of Genomic Variants (DGV)[22] or within our own sample sets, and small imbalances that mapped to regions without noted reference genes or mRNAs. This approach allowed us to identify an additional novel duplication of chromosome 6q22.31, which occurred both in the proband and in his clinically normal mother. This duplication has a minimal region from chromosome position 123 527 545 to 124 311 813 (GRCh37/hg19) and does not overlap significantly with any known CNVs in the DGV. The duplication covers two genes: the entire coding region of *TRDN* (OMIM #603283) (triadin), a ryanodine-sensitive calcium channel expressed in cardiac and skeletal muscle,[23] and the first exon of *NKAIN2* (OMIM #609758) (sodium/potassium-transporting ATPase subunit beta-1-interacting protein 2 isoform 2), a transmembrane protein. Truncation of *NKAIN2* has been described in patients with developmental delay[24] and complex neurological impairment.[25] All other events found in the patient overlapped with those reported in the DGV. A full list of events can be found in Supplementary Table 1.

Thus we hypothesise that the inherited chromosome 6q22.31 duplication may compound the presence of the *de novo* 16p11.2 deletion, leading to the observed clinical phenotype in this patient. As most researchers focus solely on the chromosome 16p abnormality, or choose to exclude inherited CNVs, most cases of 'dual CNV disorder' such as this will have been missed in the literature. In an attempt to identify similar cases, we performed a PubMed search for '16p11.2' that matched 130 articles (August 1990–February 2012). Fifty of these manuscripts described the characterisation of 16p11.2 anomalies, of which only nine explicitly reported information regarding concurrent CNVs.[7,10,12,14,26–30] When limiting our search to cases with typical proximal (29.5–30.1 Mb) 16p11.2 anomalies, we were unable to identify any 16p11.2 cases reported to co-occur with *NKAIN2* CNVs. Across the nine studies available, the only regions that were consistently reported across multiple studies as secondary CNVs in 16p11.2 patients were 15q11.2 (Prader–Willi syndrome region, found in 2 of 31 16p11.2 patients studied in Bachmann-Gagescu *et al*[10] and 1 of 427 autistic individuals studied in Marshall *et al*[28]), 15q13.2 (found in 2 of 138 16p11.2 duplication carriers studied in Jacquemont *et al*[27] and 1 of 427 autistic individuals studied in Marshall *et al*[28]) and 22q11.2 (DiGeorge syndrome critical region, found in 1 of 31 16p11.2 patients studied inBachmann-Gagescu *et al*[10] and 1 of 36 autistic individuals studied in Davis *et al*[30]). Interestingly, these recurrent secondary CNVs align with regions known to be involved in autism and developmental delays.[31–33]

When we widened our search to include novel chromosome 16p11.2 CNVs outside of the typical region (28.0–31.4 Mb), we did find one study that documented cases with co-occurring chromosome 16p11.2 and 6q22.31 abnormalities.[29] This study, by Sanders *et al*,[29] investigated 1124 individuals with autism and their unaffected family members (2248 parents and 872 sibs) and identified several recurrent copy number events associated with autism, including rearrangements of 16p11.2, both within and outside the 'typical' region. As part of their Supplementary data, the authors published full lists of all high-confidence CNVs found in samples passing quality control. These

Table 1 Probands with Concurent 16p11.2 and 6q22.31 CNVs from the Sanders *et al*[29] Study (a) Chromosome 16p11.2 anomalies and (b) Concurrent chromosome 6q22.31 anomalies

Individual	*16p11.2 start (hg18)*	*16p11.2 end (hg18)*	*16p11.2 size*	*16p11.2 State*	*16p11.2 Inheritance*	*16p11.2 Genes*	*Intronic/Exonic?*	*DGV frequency range*[a]	*Average DGV frequency*[b]
(a)									
Our patient	29 560 500	30 106 852	546 352	Deletion	*De novo*	*SPN* to *CORO1A* (inclusive)	Exonic, 30 genes	0.00–0.00	0.00
11009.p1	28 521 466	28 528 253	6787	Duplication	Paternal	*SULT1A1*	Exons 1-7 (of 8)	0.03–0.30	0.17
11087.p1	28 522 302	28 528 253	5951	Duplication	Paternal	*SULT1A1*	Exons 1-7 (of 8)	0.03–0.30	0.17
11096.p1	28 521 466	28 528 253	6787	Deletion	Maternal	*SULT1A1*	Exons 1-7 (of 8)	0.03–0.30	0.30
11229.p1	31 386 212	31 396 534	10 322	Duplication	Maternal	*TGFB1I1*	Exons 1-11 (of 11)	0.00–0.00	0.00
11246.p1	30 497 961	30 502 245	4284	Deletion	Paternal	*ZNF785*	Exon 3 (of 3)	0.00–0.00	0.00
11996.p1	28 522 302	28 528 253	5509	Duplication	Unsure	*SULT1A1*	Exons 1-7 (of 8)	0.03–0.30	0.17
12961.p1	28 522 744	28 528 253	5951	Duplication	Paternal	*SULT1A1*	Exons 1-7 (of 8)	0.03–0.30	0.17
(b)									
Individual	*6q22.31 start (hg18)*	*6q22.31 end (hg18)*	*6q22.31 size*	*6q22.31 State*	*6q22.31 Inheritance*	*6q22.31 Genes*	*Intronic/Exonic?*	*DGV frequency range*[a]	*Average DGV frequency*[b]
Our patient	123 581 324	124 201 824	620 500	Duplication	Maternal	*TRDN, NKAIN2*	Exons 1-41 (of 41), Exon 1 (of 6)	0.00–0.00	0.00
11009.p1	124 477 640	124 510 591	32 951	Duplication	Maternal	*NKAIN2*	Exon 2 of BC035062 mRNA	0.001–0.029	0.01
11087.p1	124 477 640	124 510 591	32 951	Duplication	Maternal	*NKAIN2*	Exon 2 of BC035062 mRNA	0.001–0.029	0.01
11096.p1	124 479 205	124 510 591	31 386	Duplication	Paternal	*NKAIN2*	Exon 2 of BC035062 mRNA	0.001–0.029	0.01
11229.p1	124 480 321	124 510 591	30 270	Duplication	Maternal	*NKAIN2*	Exon 2 of BC035062 mRNA	0.001–0.029	0.01
11246.p1	124 477 640	124 510 591	32 951	Duplication	Paternal	*NKAIN2*	Exon 2 of BC035062 mRNA	0.001–0.029	0.01
11996.p1	124 959 283	124 961 396	2113	Deletion	Unsure	*NKAIN2*	Intronic	0.00–0.00	0.00
12961.p1	124 477 640	124 510 591	32 951	Duplication	Paternal	*NKAIN2*	Exon 2 of BC035062 mRNA	0.001–0.029	0.01

Abbreviation: DGV, database of genomic variants.
[a]DGV range frequency gives the frequency range of deletions or duplications (as appropriate) in all DGV studies that included at least 30 individuals and the CNV is reported (NB: this includes populations other than European).
[b]Frequency DGV gives the average frequency of deletions or duplications (as appropriate) reported in studies including at least 30 European individuals in the DGV.

included lists of rare CNVs that did not overlap more than 50% with a CNV present at >1% frequency in the DGV[29] (Supplementary Table S8). Using these Supplementary data, we were able to identify seven autistic probands who carried concurrent 16p11.2 (five duplications and two deletions) and *NKAIN2* (six duplications and one deletion) anomalies, all inherited from healthy parents (Table 1). However, all of these events were small (<50 kb) and none overlapped with those observed in our patient. No 16p11.2 events were found to be concurrent with *TRDN* CNVs. Furthermore, the chromosome 16p11.2 anomalies identified by Sanders *et al*[29] in these concurrent cases were all outside of the typical region and, on review of the DGV, we noted that those involving the *SULT1A1* (OMIM #171150) gene overlapped significantly with regions of common variation (Table 1). Similarly, the *NKAIN2* anomalies were intronic to the RefSeq *NKAIN2* consensus sequence, or overlapped with common CNVs in the DGV (Table 1). However, it is worth noting that we observed a mRNA, BC035062, that is annotated to include an exon contained within the common 'intronic' duplication, suggesting these events may affect a splice variant.

The patient we describe in this letter carries the typical 16p11.2 loss co-occurring with a further 6q22.31 duplication, both of which are distinct from those described above. The latter does not overlap significantly with noted DGV variants. However, further mining of the Sanders *et al*[29] Supplementary data identified three healthy individuals carrying apparently identical 6q22.31 duplications (a father and son and another father) (Supplementary Table S8). Thus the 6q22.31 duplication in our case may represent a very rare CNV with little independent effect, but we cannot rule out a modifying role in combination with the 16p11.2 loss, particularly in view of the gene content. The 6q22.31 duplicated region in our patient encompasses all of the coding regions of *TRDN* and the first exon of *NKAIN2. TRDN* codes for a muscle-specific protein, deletion of which leads to cardiac arrhythmia.[34] Although primarily expressed in cardiac tissue, this gene is also expressed in skeletal muscle, where it is involved in the regulation of resting calcium levels.[35] *NKAIN2* is a transmembrane protein with four homologues (*NKAIN1–4*), all of which are highly conserved and have brain-specific expression.[36] Interestingly, it is the only gene in common with the smaller 6q22.31 CNV regions described above. The cellular functions of the NKAIN proteins are unknown but they have been shown to localise and interact with the plasma membrane protein ATP1B1. *Drosophila dNKAIN* mutants show decreased co-ordination and temperature-sensitive paralysis.[36] Microdeletions in *NKAIN2* have previously been reported as rare events contributing to the risk of schizophrenia[37] and Attention

Deficit/ Hyperactivity Disorder (ADHD),[38] and variants within this gene have been associated with neuroticism.[39]

Taking all of this information into consideration, we believe that the clinical presentation of chromosome 16p11.2 deletion cases may be modulated by the presence of additional genomic imbalances, such as the inherited duplication of chromosome 6q22.31 observed in our case. Researchers of developmental disorders have proposed a dual CNV model at other loci,[33,40–42] as well as compound heterozygotes with a CNV-mediated deletion of one allele and non-synonymous mutation of the other (mixed genomic disorders).[43,44] The genetic background, of course, extends beyond CNVs and, as genetic technologies advance, we predict that a whole-genome view will allow the elucidation of many combinatorial factors. For example, a recent study extended the dual CNV model to incorporate rare point mutations across common functional pathways, where an ASD proband was identified with both a *de novo* mutation of *FOXP1* (OMIM #605515) and an inherited mutation of *CNTNAP2* (OMIM #604569).[45] The validity of this model and the significance of concurrent CNVs can only be tested by the consistent and detailed description of CNV cohorts in a whole-genome context. This is especially true for studies such as ours, which involve only a single patient. We would therefore urge researchers characterising chromosome abnormalities to consider, and to explicitly report, the anomalies in the context of whole genome copy number variation and genomic cataloguing. Advances in genetic technology mean that there is no longer a need to consider genomic imbalances in isolation, particularly in case reports. We suggest that the capture of complete genomic contexts, alongside detailed phenotypic profiling, will allow us to develop a better understanding of the variability of the chromosome 16p11.2 phenotype and may assist in the delineation of a core clinical phenotype.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Raca G, Baas BS, Kirmani S *et al*: Childhood apraxia of speech (CAS) in two patients with 16p11.2 microdeletion syndrome *Eur J Hum Genet* 2012; e-pub ahead of print 22 August 2012; doi:10.1038/ejhg.2012.165.
2 Barge-Schaapveld DQ, Maas SM, Polstra A, Knegt LC, Hennekam RC: The atypical 16p11.2 deletion: a not so atypical microdeletion syndrome? *Am J Med Genet A* 2011; **155A**: 1066–1072.
3 Shinawi M, Liu P, Kang SH *et al*: Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet* 2010; **47**: 332–341.
4 Hanson E, Nasir RH, Fong A *et al*: Cognitive and behavioral characterization of 16p11.2 deletion syndrome. *J Dev Behav Pediatr* 2010; **31**: 649–657.
5 Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH *et al*: Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. *Eur J Med Genet* 2009; **52**: 77–87.
6 Ballif BC, Hornor SA, Jenkins E *et al*: Discovery of a previously unrecognized microdeletion syndrome of 16p11.2-p12.2. *Nat Genet* 2007; **39**: 1071–1073.
7 Tabet AC, Pilorge M, Delorme R *et al*: Autism multiplex family with 16p11.2p12.2 microduplication syndrome in monozygotic twins and distal 16p11.2 deletion in their brother. *Eur J Hum Genet* 2012; **20**: 540–546.
8 Rosenfeld JA, Coppinger J, Bejjani BA *et al*: Speech delays and behavioral problems are the predominant features in individuals with developmental delays and 16p11.2 microdeletions and microduplications. *J Neurodev Disord* 2010; **2**: 26–38.
9 Walters RG, Jacquemont S, Valsesia A *et al*: A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* 2010; **463**: 671–675.
10 Bachmann-Gagescu R, Mefford HC, Cowan C *et al*: Recurrent 200-kb deletions of 16p11.2 that include the SH2B1 gene are associated with developmental delay and obesity. *Genet Med* 2010; **12**: 641–647.
11 Weiss LA, Shen Y, Korn JM *et al*: Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008; **358**: 667–675.
12 Mefford HC, Yendle SC, Hsu C *et al*: Rare copy number variants are an important cause of epileptic encephalopathies. *Ann Neurol* 2011; **70**: 974–985.
13 McCarthy SE, Makarov V, Kirov G *et al*: Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* 2009; **41**: 1223–1227.
14 Hempel M, Rivera Brugues N, Wagenstaller J *et al*: Microdeletion syndrome 16p11.2-p12.2: clinical and molecular characterization. *Am J Med Genet A* 2009; **149A**: 2106–2112.
15 MacDermot KD, Bonora E, Sykes N *et al*: Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. *Am J Hum Genet* 2005; **76**: 1074–1080.
16 Fisher SE, Scharff C: FOXP2 as a molecular window into speech and language. *Trends Genet* 2009; **25**: 166–177.
17 American-Psychiatric-Association: *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn (DSM-IV). American Psychiatric Association: Washington DC, 1994.
18 Sparrow SS, Cicchetti DV, Balla DA: *Vineland Adaptive Behavior Scales, Second Edition (Vineland-II). A measure of adaptive behavior from birth to adulthood, 2nd edition manual*. NCS Pearson Inc: Minneapolis: MN, 2005.
19 Sambrook J, Fritsch E, Maniatis T: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory Press: Cold Spring Harbour, 1989.
20 Shriberg LD, Fourakis M, Hall SD *et al*: Extensions to the Speech Disorders Classification System (SDCS). *Clin Linguist Phon* 2010; **24**: 795–824.
21 Shriberg LD, Fourakis M, Hall SD *et al*: Perceptual and acoustic reliability estimates for the Speech Disorders Classification System (SDCS). *Clin Linguist Phon* 2010; **24**: 825–846.
22 Iafrate AJ, Feuk L, Rivera MN *et al*: Detection of large-scale variation in the human genome. *Nat Genet* 2004; **36**: 949–951.
23 Taske NL, Eyre HJ, O'Brien RO, Sutherland GR, Denborough MA, Foster PS: Molecular cloning of the cDNA encoding human skeletal muscle triadin and its localisation to chromosome 6q22-6q23. *Eur J Biochem* 1995; **233**: 258–265.
24 Yue Y, Stout K, Grossmann B *et al*: Disruption of TCBA1 associated with a *de novo* t(1;6)(q32.2;q22.3) presenting in a child with developmental delay and recurrent infections. *J Med Genet* 2006; **43**: 143–147.
25 Bocciardi R, Giorda R, Marigo V *et al*: Molecular characterization of a t(2;6) balanced translocation that is associated with a complex phenotype and leads to truncation of the TCBA1 gene. *Hum Mutat* 2005; **26**: 426–436.
26 Schaaf CP, Goin-Kochel RP, Nowell KP *et al*: Expanding the clinical spectrum of the 16p11.2 chromosomal rearrangements: three patients with syringomyelia. *Eur J Hum Genet* 2011; **19**: 152–156.

27 Jacquemont S, Reymond A, Zufferey F *et al*: Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* 2011; **478**: 97–102.
28 Marshall CR, Noor A, Vincent JB *et al*: Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008; **82**: 477–488.
29 Sanders SJ, Ercan-Sencicek AG, Hus V *et al*: Multiple recurrent *de novo* CNVs, including duplications of the 7q11.23 williams syndrome region, are strongly associated with autism. *Neuron* 2011; **70**: 863–885.
30 Davis LK, Meyer KJ, Rudd DS *et al*: Novel copy number variants in children with autism and additional developmental anomalies. *J Neurodev Disord* 2009; **1**: 292–301.
31 Burnside RD, Pasion R, Mikhail FM *et al*: Microdeletion/microduplication of proximal 15q11.2 between BP1 and BP2: a susceptibility region for neurological dysfunction including developmental and language delay. *Hum Genet* 2011; **130**: 517–528.
32 Pinto D, Pagnamenta AT, Klei L *et al*: Functional impact of global rare copy number variation in autism spectrum disorders. *Nature* 2010; **466**: 368–372.
33 Wincent J, Bruno DL, van Bon BW *et al*: Sixteen New Cases Contributing to the Characterization of Patients with Distal 22q11.2 Microduplications. *Mol Syndromol* 2010; **1**: 246–254.
34 Roux-Buisson N, Cacheux M, Fourest-Lieuvin A *et al*: Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. *Hum Mol Genet* 2012; **21**: 2759–2767.
35 Eltit JM, Feng W, Lopez JR *et al*: Ablation of skeletal muscle triadin impairs FKBP12/RyR1 channel interactions essential for maintaining resting cytoplasmic Ca2+. *J Biol Chem* 2010; **285**: 38453–38462.
36 Gorokhova S, Bibert S, Geering K, Heintz N: A novel family of transmembrane proteins interacting with beta subunits of the Na,K-ATPase. *Hum Mol Genet* 2007; **16**: 2394–2410.
37 Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M: Strong association of *de novo* copy number mutations with sporadic schizophrenia. *Nat Genet* 2008; **40**: 880–885.
38 Elia J, Gai X, Xie HM *et al*: Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. *Mol Psychiatry* 2010; **15**: 637–646.
39 Calboli FC, Tozzi F, Galwey NW *et al*: A genome-wide association study of neuroticism in a population-based sample. *PLoS One* 2010; **5**: e11504.
40 Leblond CS, Jutta H, Delorme R *et al*: Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. *PLoS Genet* 2012; **8**: e1002521.
41 Girirajan S, Rosenfeld JA, Cooper GM *et al*: A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet* 2010; **42**: 203–209.
42 Veltman JA, Brunner HG: Understanding variable expressivity in microdeletion syndromes. *Nat Genet* 2010; **42**: 192–193.
43 Shiow LR, Paris K, Akana MC, Cyster JG, Sorensen RU, Puck JM: Severe combined immunodeficiency (SCID) and attention deficit hyperactivity disorder (ADHD) associated with a Coronin-1 A mutation and a chromosome 16p11.2 deletion. *Clin Immunol* 2009; **131**: 24–30.
44 Vorstman JA, van Daalen E, Jalali GR *et al*: A double hit implicates DIAPH3 as an autism risk gene. *Mol Psychiatry* 2011; **16**: 442–451.
45 O'Roak BJ, Deriziotis P, Lee C *et al*: Exome sequencing in sporadic autism spectrum disorders identifies severe *de novo* mutations. *Nat Genet* 2011; **43**: 585–589.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)