Further molecular and clinical delineation of co-locating 17p13.3 microdeletions and microduplications that show distinctive phenotypes

Damien L Bruno, Britt-Marie Anderlid, Anna Lindstrand, et al.

J Med Genet 2010 47: 299-311
doi: 10.1136/jmg.2009.069906

Further molecular and clinical delineation of co-locating 17p13.3 microdeletions and microduplications that show distinctive phenotypes

Damien L Bruno,[1,2] Britt-Marie Anderlid,[3] Anna Lindstrand,[3] Conny van Ravenswaaij-Arts,[4] Devika Ganesamoorthy,[1,2] Johanna Lundin,[3] Christa Lese Martin,[5] Jessica Douglas,[6] Catherine Nowak,[6] Margaret P Adam,[5] R Frank Kooy,[7] Nathalie Van der Aa,[7] Edwin Reyniers,[7] Geert Vandeweyer,[7] Irene Stolte-Dijkstra,[4] Trijnie Dijkhuizen,[4] Alison Yeung,[1] Martin Delatycki,[1] Birgit Borgström,[8] Lena Thelin,[9] Carlos Cardoso,[10] Bregje van Bon,[11] Rolph Pfundt,[11] Bert B A de Vries,[11] Anders Wallin,[12] David J Amor,[1] Paul A James,[1] Howard R Slater,[1,2] Jacqueline Schoumans[3]

► Supplementary material are published online only. To view these files please visit the journal online (http://jmg.bmj.com).

For numbered affiliations see end of article

Correspondence to
Dr Howard R Slater, Cytogenetics Department, VCGS Pathology, MCRI, Royal Children's Hospital, Parkville, Victoria 3052, Australia; howard.slater@ghsv.org.au

DLB and B-MA contributed equally to this work.

Received 31 May 2009
Revised 15 September 2009
Accepted 16 September 2009

ABSTRACT

Background Chromosome 17p13.3 contains extensive repetitive sequences and is a recognised region of genomic instability. Haploinsufficiency of *PAFAH1B1* (encoding LIS1) causes either isolated lissencephaly sequence or Miller—Dieker syndrome, depending on the size of the deletion. More recently, both microdeletions and microduplications mapping to the Miller—Dieker syndrome telomeric critical region have been identified and associated with distinct but overlapping phenotypes.

Methods Genome-wide microarray screening was performed on 7678 patients referred with unexplained learning difficulties and/or autism, with or without other congenital abnormalities. Eight and five unrelated individuals, respectively, were identified with microdeletions and microduplications in 17p13.3.

Results Comparisons with six previously reported microdeletion cases identified a 258 kb critical region, encompassing six genes including *CRK* (encoding Crk) and *YWHAE* (encoding 14-3-3ϵ). Clinical features included growth retardation, facial dysmorphism and developmental delay. Notably, one individual with only subtle facial features and an interstitial deletion involving *CRK* but not *YWHAE* suggested that a genomic region spanning 109 kb, encompassing two genes (*TUSC5* and *YWHAE*), is responsible for the main facial dysmorphism phenotype. Only the microduplication phenotype included autism. The microduplication minimal region of overlap for the new and previously reported cases spans 72 kb encompassing a single gene, *YWHAE*. These genomic rearrangements were not associated with low-copy repeats and are probably due to diverse molecular mechanisms.

Conclusions The authors further characterise the 17p13.3 microdeletion and microduplication phenotypic spectrum and describe a smaller critical genomic region allowing identification of candidate genes for the distinctive facial dysmorphism (microdeletions) and autism (microduplications) manifestations.

INTRODUCTION

The advent of microarray analysis for the detection of chromosomal abnormalities in patients with developmental problems has heralded a new era of cytogenetic testing, which in turn is impacting significantly on clinical practice. It has been shown that pathogenic, submicroscopic copy-number alterations are detectable in 10—15% of patients with idiopathic mental retardation and/or multiple congenital abnormalities.[1–5] A significant outcome of this testing has been the identification of several 'new' microdeletion/duplication syndromes through so-called 'reverse phenotypics'—that is, using a genotype-to-phenotype approach.[6–11]

Novel co-locating microdeletions and microduplications in chromosome 17p13.3 distinct from isolated lissencephaly sequence (ILS) and Miller—Dieker syndrome (MDS) have recently been described in six and 10 unrelated individuals, respectively.[12–15] Prior to these, Cardoso *et al*[16] described two cases of derivative chromosomes involving similar deletions in 17p13.3 but with concurrent large duplications on other chromosomes. Here we report an additional eight individuals with the microdeletion and five with the microduplication. None of these microdeletions involve the *PAFAH1B1* gene, encoding LIS1, whose haploinsufficiency causes either ILS (OMIM 607432) or MDS (OMIM 247200), depending on the size of the deletion. The thirteen 17p13.3 copy-number variations (CNVs) were detected by genome-wide microarray screening of a cohort of 7678 patients referred to six independent clinical centres for genetic testing. All the patients tested had unexplained learning difficulties and/or autism, with or without other congenital abnormalities.

MATERIALS

DNA samples

Clinical samples (n=7678) were obtained from: Melbourne, Australia (n=349); Stockholm, Sweden (n=1289); Groningen, The Netherlands (n=2107); Antwerp, Belgium (n=100); Atlanta, USA (n=2000); and Nijmegen, The Netherlands (n=1833). We used 1171 parental samples as healthy controls to assess the extent of normal (non-pathogenic) CNV in this region of 17p13.3.

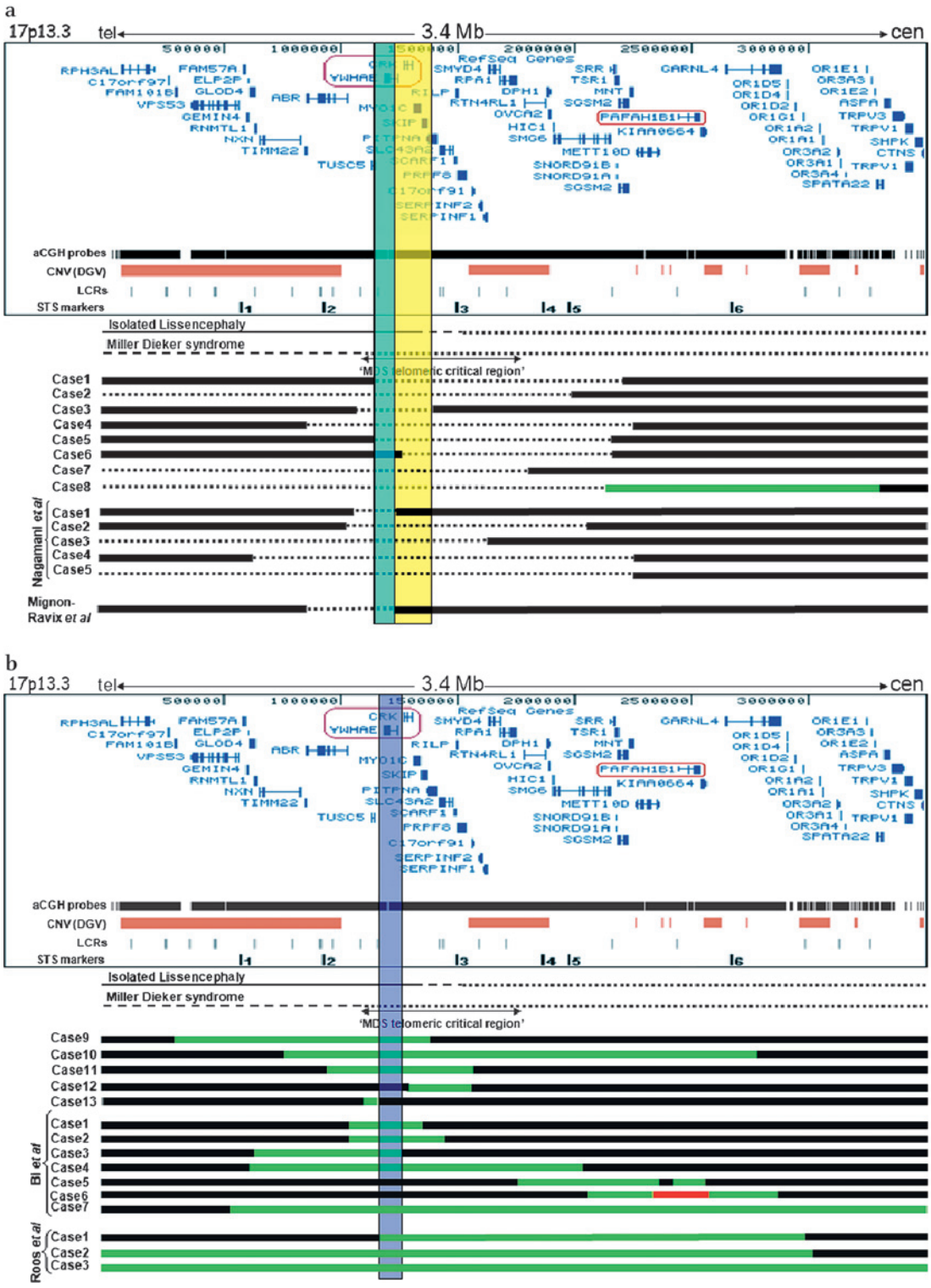

Figure 1 Schematic representation of the terminal 3.4 Mb of 17p13.3 and summary of the molecular findings in individuals with microdeletion (a) and microduplication (b). The numbers at the top represent the genomic distance (in base pairs) from the 17p telomere according to UCSC Genome Browser Build 36.1 (2006). Known genes are noted in blue and labelled according to their RefSeq name. Low-copy repeats (LCRs), copy number variation (CNVs) and the high-resolution array comparative genomic hybridisation (aCGH) probe set are depicted as grey vertical lines, reddish-brown boxes and black vertical lines, respectively. Sequence-tagged site (STS) markers used for parent-of-origin studies are shown as black vertical lines and labelled according to their number (see table 4). For each individual, the solid lines below the map represent non-deleted/duplicated DNA (two copies). The dotted lines represent deletions (one copy), whereas the green solid lines represent duplications (three copies) and the red line triplication (four copies). Dashed lines denote isolated lissencephaly sequence (ILS)/Miller—Dieker syndrome (ILS/MDS) microdeletions with variable telomeric breakpoints. The 'MDS telomeric critical region'[19 21] is depicted by horizontal arrows. The deletion (yellow shading) and duplication (blue shading) critical regions spanned 258 kb and 72 kb, respectively. The delineated microdeletion facial dysmorphism critical region (aqua) spanned 109 kb. The duplication critical region was delineated using the inherited microduplication in case 13, which was considered benign, and the proximal breakpoint of the microduplication in case 3 from Bi *et al*. The class II microduplications in case 7 (Bi *et al*[12]) and case 3 (Roos *et al*[14]) extend to 3 860 158 bp and 4 052 847 bp, respectively. tel, telomere; cen, centromere; DGV, Database of Genomic Variants.

Table 1 Molecular details for the new genomic rearrangements in the 17p13.3 region

Case	Genomic abnormality	Genomic region (bp)*	Size (Mb)	Detection method	Confirmation	Inheritance	Parental origin
1	Interstitial deletion	Chr17: 1135022–2202835	1.07	244K array	FISH	de novo (FISH)	Paternal
2	Terminal deletion	Chr17: 514–2065664	2.07	SNP6.0 array	FISH	de novo (FISH)	Paternal
3	Interstitial deletion	Chr17: 1067074–1394633	0.33	44K array	FISH	de novo (FISH)	No STS markers in segment
4	Interstitial deletion	Chr17: 842389–2257857	1.42	44K array	FISH	de novo (FISH)	NT
5	Interstitial deletion	Chr17: 1136270–2168155	1.03	HumanCytoSNP-12	Custom aCGH	de novo (MLPA)	Paternal
6	Interstitial deletion	Chr17: 1254527–2164139	0.91	250K Nsp	Custom aCGH	Maternal (250K Nsp)	NA
7	Terminal deletion	Chr17: 18901–1820903	1.80	250K Nsp	MLPA	Parental samples unavailable	NT. Parental samples unavailable
8	Terminal deletion with Concurrent duplication	Chr17: 29169–2123816 Chr17: 2124215–3321560	2.09 1.20	MLPA† 244K array	FISH MLPA	de novo (FISH) de novo (MLPA)	Maternal Maternal
9	Interstitial duplication	Chr17: 294421–1371895	1.08	244K array	MLPA	de novo (MLPA)	Maternal
10	Interstitial duplication	Chr17: 738991–2813518	2.07	BAC subtelomeric array	FISH	de novo (FISH)	Paternal
11	Interstitial duplication	Chr17: 936249–1573187	0.64	HumanCytoSNP-12	Custom aCGH	Not Maternal (MLPA)	Not Maternal. Paternal sample unavailable.
12	Interstitial duplication	Chr17: 1288474–1558459	0.27	370K array	Custom aCGH	Maternal (370K array)	NA
13	Interstitial duplication	Chr17: 1123160–1182563 (min) Chr17: 1105258–1193712 (max)	0.059 (min) 0.088 (max)	180K array	180K array	Maternal (180K array)	NA

*Breakpoints and copy number variation size determined on the basis of high-density custom oligonucleotide aCGH (cases 1, 3–6 and 8–12), multiplex ligation-dependent probe amplification (MLPA) analysis (case 2) and 180K array analysis (case 13). In case 7, the genomic coordinates are taken from the 250K Nsp array.
†Microdeletion first detected using the DiGeorge syndrome MLPA kit (MRC-Holland, http://www.mrc-holland.com/). aCGH, array comparative genomic hybridisation; FISH, fluorescent in situ hybridisation; NA, not applicable; NT, not tested.

Table 2 Phenotypic features of individuals with 17p13.3 class I microduplications

	Case 9	Case 11	Case 12	Case 1*	Case 2*	Case 3*	Case 4*
Age (years)	2	14	3	6	8	5	15
Gender	Male	Male	Female	Male	Female	Male	Male
Gestational age (weeks)	Full term	Full term	Full term	Full term	Full term	NI	36
Birth weight (g)	3400 (M)	3487 (M)	3320 (M)	3900 (M)	4597 (+2.5 SD)	NI	3750 (+2 SD)
Birth length (cm)	51 (M)	N	48 (−1 SD)	NI	57 (+3 SD)	NI	55.9 (+3 SD)
Birth head circumference (cm)	NI	50th centile	-	NI	NI	NI	NI
Postnatal growth retardation	−	−	−	−	−	−	−
Overgrowth	−	+ (90–97th)	(+) (75–90th)	+ (90th)	+ (+3 SD)	−	+ (97th)
Feeding difficulties	−	+	−	−	−	−	−
Muscle hypotonia	−	+	−	+	+	−	−
Delay in motor function	**Delay in fine motor skills**	+	+	+	+	+	**Delay in fine motor skills**
Cognitive development	Normal at 2 years	Mild delay	Global delay	Mild MR	Global delay	Mild delay	Normal
Speech delay	+	+	**Pronounced**	++	++	++	−
Neurobehavioural symptoms	**Autism**, hyperactivity	**Autistic traits**, facial tic	**Autism**	**Suspected autism**, aggressive tendencies, disobedience	Preoccupation with food	**Autistic behaviour**	ADHD
Repeated infections	−	−	−	−	−	+	+
Facial features:							
Face	Triangular	N	N	N	N	N	Long
Forehead	Broad	N	Narrow	N	Broad	N	N
Eyes	N	Broad, sparse eyebrows	N	Thick eyebrows, synophrys	Synophrys, upslanting palpebral fissures	Prominent eyebrows	Synophrys, hypotelorism, upslanting palpebral fissures
Nose	N	Squared, upturned tip	Broad	Squared tip	Squared tip, broad base	Broad base	High bridge
Ears	Prominent	Large, fleshy	N	Large	Large	N	N
Mouth	**Prominent cupid bow**	**Prominent cupid bow**	Prominent cupid bow, high palate	Thin upper lip	Thin upper lip	N	Thin upper lip
Mandible	**Pointed chin**	**Pointed chin**	Pointed chin	N	**Prominent chin**	**Mild prognatia**	**Pointed chin**
Hand/foot abnormality	Bilateral groove between toe one and two	Hallux valgus, sandal gap, abnormal toe nails	Fetal finger pads	Large hands, small distal phalanges	Large hands, long middle finger	−	Prominent proximal interphalangeal joints
Malformation	−	Genu valgum	−	−	−	Increase in kidney size	Aortic root dilatation, mitral valve prolapse
MRI	NP	NP	Normal	NP	Normal	NP	NP

Bold type indicates common features among individuals with 17p13.3 class I microduplications. Case 12 was considered non-pathogenic.
*Bi *et al*.[12]
−, not present; +, present; ADHD, attention deficit hyperactivity disorder; M, mean; MR, mental retardation; N, normal; NI, no information; NP, not performed.

Table 3 Phenotypic features of individuals with 17p13.3 class II microduplications

	Case 10	Case 5*	Case 6*	Case 7*	Case 1†	Case 2†	Case 3†
Age	6.5 y	2 y 8 m	17 y 4 m	10 y 5 m	14 y	2 y 4 m	1 y 10 m
Gender	Male	Male	Male	Female	Male	Female	Male
Gestational age (weeks)	36+2	42	NI	38	38+4	40	38+1
Birth weight (g)	2670 (−0.5 SD)	2970 (−2 SD)	2753	3060 (−1 SD)	3350 (M)	4200 (+1 SD)	3380 (M)
Birth length (cm)	NI	NI	NI	53 (+1 SD)	53 (+1 SD)	NI	50 (M)
Birth head circumference (cm)	NI	NI	NI	31 (−2 SD)	NI	NI	N
Postnatal growth retardation	−	++ (height−3.5 SD) Microcephaly	++ (height−6 SD) Microcephaly	− (+1 SD)			
Overgrowth	−	−	−	−	Height +3.5 SD (not corrected for tall parental heights) weight +1 SD	−	Height increased from M to +2 SD at 22 months
Feeding difficulties	+	+	+	NI	−	+	−
Muscle hypotonia	**Axial hypotonia**	NI	**Profound**	−	**Severe**	+	+
Delay in motor function	+	NI	NI	+	+	**+ (mild)**	**+ (severe)**
Cognitive development	**Normal**	Developmental delay	Mental retardation	Moderate developmental delay	+ (no reading)	+	+
Speech delay	−	NI	NI	+	+	+	+
Neurobehavioural symptoms	**Autism, hand flapping**	NI	**ADHD, OCD**	**Autism**, insensitivity to pain, unprovoked screaming, self-abusive behaviours	NI	NI	NI
Recurrent infections	+	NI	+	−	+	+	+
Facial features:							
Face	Triangular, malar flatness	Triangular, sagittal craniosynosthosis	N	N	Plagiocephaly	Broad face, midface retrusion	Broad midface
Forehead	N	Prominent	N	N	Frontal bossing	Frontal bossing	Frontal bossing
Eyes	N	N	N	Strabism	Hypertelorism	Hypertelorism, down-slanting palpebral fissures	Hypertelorism, down-slanting palpebral fissures
Nose	Full tip	N	N	N	Broad nasal bridge	Broad nasal bridge	Broad nasal bridge
Ears	N	N	N	N	Low set	Low set	N
Mouth	**Prominent cupid bow**	Thin upper lip	High arched palate	N	N	Small	Small, high palate
Mandible	**Pointed chin**	**Pointed chin**, micrognathia	**N**	N	N	**Triangular chin**	**Triangular chin**
Hand/foot abnormality	Sandal gap	−	−	−	−	5th finger clinodactyly	Simian crease (R), equino valgus (R)
Malformation	−		Scoliosis, dislocated hips, varus leg deformity		Muscle biopsy: increased type one cells	PFO	Pectus excavatum, micropenis, hernia inguinalis, hip luxation
MRI	NP	NI	Corpus callosum dysgenesis	Corpus callosum hypoplasia, cerebellar volume loss	N	NP	Corpus callosum agenesis, dilated lateral ventricles, abnormal signal intensities of white matter

Bold type indicates common features among individuals with 17p13.3 Class II microduplications.
*Bi *et al*[12];
†Roos *et al*.[14]
−, not present; +, present. ADHD, attention deficit hyperactivity disorder; m, month; M, mean; MR, mental retardation; N, normal; NI, no information; NP, not performed; OCD, obsessive compulsive disorder; PFO, persisting foramen ovale; R, right.

METHODS

Microarray analysis

The control samples were tested using the 105K Agilent array (n=631), the 250K Nsp (n=240) or the 6.0 Affymetrix array (n=300). Detection of CNVs, pathogenic and non-pathogenic, in the patient samples used the following array platforms: 250K Nsp (n=1650) and 6.0 (n=81) array (Affymetrix, Santa Clara, California, USA); 370K (n=100) and HumanCytoSNP-12 300K (n=65) array (Illumina, San Diego, California, USA); 33K (n=30) and 38K (n=79) BAC array (Swegene, Lund, Sweden)[17]; 32K (n=386) BAC array (Microarray Facility, Nijmegen, the Netherlands), 244K catalogue oligonucleotide array (n=858), 180K catalogue oligonucleotide array (n=322) and 105K custom-designed oligonucleotide array (n=1292) (design ID 019015; Agilent Technologies, Santa Clara, California, USA); subtelomere BAC array containing 500 clones[18] (n=345), 1Mb BAC array containing 6465 BAC clones (including two BACs covering *YWHAE* and one BAC for *CRK*)[19] (n=470), and a custom-designed 44K oligonucleotide array[20] (n=2000).

CNV confirmation

Independent confirmation of deletions/duplications in the 17p13.3 region was performed by multiplex ligation-dependent probe amplification (MLPA), fluorescence in situ hybridisation (FISH) and/or array comparative genomic hybridisation (aCGH) according to previously described methods. Rearrangements of 17p13.3 were further analysed with the use of a custom oligonucleotide array (see High-resolution breakpoint mapping below).

Parental studies

Parental analyses were performed to investigate whether the finding (gain/loss) was de novo or inherited. This was performed by FISH, MLPA or microarray analysis. Six sequence-tagged site (STS) markers spanning the maximum deleted/duplicated segment of 17p13.3 were typed to determine the parental origin of de novo rearrangements (where possible). Primer details are available upon request. Amplification was carried out with a 5′-FAM(carboxyfluorescein)-labelled primer, and PCR products were separated by capillary electrophoresis using a MegaBACE sequencer (GE Healthcare, Milwaukee, Wisconsin, USA). The results were analysed with Fragment Profiler V1.2 (GE Healthcare).

High-resolution breakpoint mapping

The breakpoint regions were further refined in 11 of the 12 cases, in 10 cases using a custom high-density CGH array (ie, CGH 2105K format (Agilent Technologies) (cases 1, 3–6 and 8–12), and in one case (case 2) by MLPA analysis (data not shown). The custom array consisted of 24 469 60-mer catalogue probes spanning the distal 3.4 Mb of 17pter and a further 23 235 tiling oligonucleotide probes targeting the 'apparent' breakpoint regions. The effective resolution was 60–1000 bp across this 3.4 Mb region. The custom array was designed using Agilent's web portal eArray (https://earray.chem.agilent.com/earray/) with a very high density of probes in the first 3 352 600 bp of 17 p. Firstly, we created a probe set with probes selected from the HD CGH Database within eArray (total of 24 469 probes). We then designed custom-made probes to tile across the previously defined breakpoint regions filtering out repeat-masked regions (23 235 probes). The two probe sets (47 704 probes) were combined into an array with an average density of one probe every 43 bp in the breakpoint regions and one probe every 130 bp in the intervening regions. Genome background coverage was achieved by adding the entire Agilent 44 K Whole Human Genome CGH probe set. Hybridisations were performed as previously described[21] with minor modifications. Briefly, patient DNA and sex-matched controls (Promega, Madison, Wisconsin, USA) were double-digested with *RsaI* and *AluI* (Promega). One microgram of each digested sample was labelled by random priming (Enzo Life Sciences, New York, USA) with Cy3-dUTP or Cy5-dUTP, and labelled products were column purified with the Qiaquick PCR purification kit (Qiagen AB, Solna, Sweden). After probe denaturation and pre-annealing with 50 μg Cot-1 DNA (Invitrogen, Carlsbad, California, USA), hybridisation was performed at 65°C with rotation for 40 h. The array was analysed with the Agilent scanner and Feature Extraction software (V10.2). A graphical overview was obtained using the DNA analytics software (V4.0). Genomic start and stop positions of the deletions and duplications were determined by visual inspection of the numerical normalised $\log_2$ ratio values in the table view of the DNA analytics software package. The genomic architecture at the breakpoints was assessed by referring to the Human Genome Build 36.1 reference sequence (University of California Santa Cruz (UCSC) genome browser), and shared sequences between the telomeric and centromeric breakpoints were identified using BLAST2 (http://www.ncbi.nlm.nih.gov/BLAST/).

Junction fragment analysis

Long-range PCR primers were designed from the high-resolution custom-aCGH coordinates of CNV breakpoints for cases 1, 9 and 12. PCR products spanning the junctions were obtained and sequenced using the ET SEQ Mix (Applied Biosystems, Scoresby, Victoria, Australia) on a MegaBACE 1000 (GE Healthcare). Sequences were aligned using Mutation Surveyor (GE Healthcare). Junction sequences were further analysed using BLAST2 and the Human Genome Build 36.1 reference sequence in order to identify shared sequences between the telomeric and centromeric breakpoint sequences.

RESULTS

Molecular results

In total, 13 co-locating CNVs were detected in chromosome 17 band p13.3. This included seven simple microdeletions

Table 4 Parent-of-origin studies

STS marker		Case 1	Case 2	Case 5	Case 8	Case 9	Case 10	Case 11
1	D17S1308	Outside del	**Paternal (del)**	Outside del	**Maternal (del)**	**Maternal (dup)**	Outside dup	Outside dup
2	D17S1840	Outside del	**Paternal (del)**	Outside del	**Maternal (del)**	Not Informative (dup)	**Paternal (dup)**	Outside dup
3	D17S1533	**Paternal (del)**	**Paternal (del)**	Not Informative (del)	**Maternal (del)**	Outside dup	**Paternal (dup)**	**Not maternal***
4	D17S831	**Paternal (del)**	**Paternal (del)**	Outside del	Not Informative (del)	Outside dup	Not Informative (dup)	Outside dup
5	D17S1528	**Paternal (del)**	**Paternal (del)**	**Paternal (del)**	Not Informative (del)	Outside dup	Not Informative (dup)	Outside dup
6	D17S1583	Outside del	Outside del	Outside del	**Maternal (dup)**	Outside dup	Not Informative (dup)	Outside dup

Boldface indicates informative markers.
*Paternal sample unavailable for testing.
del, deletion; dup, duplication; STS, sequence-tagged site.

Table 5 Phenotypic features of individuals with microdeletions in 17p13.3

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6a	Case 6b	Case 6c	Case 7
Age (years)	21	13	2	9	17	4.5	2.5	38	50
Gender	Male	Female	Male	Female	Female	Male	Male	Female	Female
Gestational age (weeks)	35	Full term	36+4	42	42	38+6	39+1	36	Premature
BW (g)	2490 (−0.5 SD)	3390 (M)	2270 (−2 SD)	3120 (−1.5 SD)	2875 (−2 SD)	2825 (−1 SD)	3000 (M)	2000 (−1.5 SD)	IUGR
BL (cm)	44 (−1.5 SD)	51 (M)	46 (−1.5 SD)	48 (−1 SD)	NI	NI	NI	NI	NI
BHC (cm)	33 (M)	34 (M)	N	NI	36 (+1 SD)	NI	NI	NI	NI
Postnatal growth retardation	++	++	++	++	−	++	++	+	++
Feeding difficulties	−	−	Neonatal	Neonatal	−	−	−	−	−
Muscle hypotonia	−	−	+	−	−	−	+	−	−
Developmental disorder	**Mild MR**	**Moderate MR**	**DD**	**Mild MR**, speech delay	**Mild learning difficulties**, problems with concentration, poor coordination	**DD**	**DD**	Speech delay	**Mild MR**
Behavioural problems	−	−	−	Echolalia	−	−	−	−	−
Recurrent infections	−	−	−	−	−	+	−	+	−
Facial features:									
Face	N	N	Broad	Midface retrusion	Round	N	N	N	N
Forehead	Bitemporal narrowing, high anterior hairline	Frontal bossing	N	Broad, high anterior hairline	N	N	N	N	N
Eyes	Short and down-slanting palpebral fissures, ptosis, **Laterally extended eyebrows**	**Infraorbital folds, laterally extended eyebrows**	Down-slanting palpebral fissures, **laterally extended eyebrows**	Narrow palpebral fissures, **laterally extended eyebrows**	N	**Infraorbital fold**	N	**Infraorbital fold**	N
Nose	**Broad nasal tip**	**Broad nasal tip** anteverted nares	**Broad nasal tip** and base	Low insertion of columella	Broad	**Broad nasal tip**	**Broad nasal tip**	**Broad nasal tip**	N
Ears	Small with overfolded helix	N	Low set, cupped	Darwinian tubercles	N	Addition al crus of antihelix	N	N	Low set
Maxilla	**Maxillary Prominence**	**Maxillary Prominence**	N	**Maxillary Prominence**	N	N	**Maxillary Prominence**	N	N
Mandible	**Micro-retrognathia**	**Retrognathia**	**Micrognathia**	N	N	N	N	N	N
Mouth	Thick upper lip vermillion, Thick and everted lower lip vermillion	Prominent upper lip with thin vermillion	Thick and everted upper lip vermillion	−	−	Wide mouth with thick and everted lower lip vermillion	−	Wide mouth	Thick upper lip vermillion, thick and everted lower lip vermillion
Hand−foot abnormality	−	5th finger clinodactyly, broad distal phalanges, narrow fingernails	−	−	Mild shortening of 2nd and 5th fingers, medially deviated great toes	5th finger clinodactyly	5th finger clinodactyly	−	Brachydactyly, 5th finger clinodactyly
Malformation	Cryptorchidism, hypospadia	Submucous CP, PDA	Iris coloboma	−	−	−	−	−	−
MRI	**No LIS. Wide perivascular spaces, white matter abnormalities**, low cerebellar tonsils	**No LIS. White matter abnormalities**, low cerebellar tonsils	NP	**No LIS. Wide perivascular spaces, white matter hyperintensities**	NP	N	**Signal abnormalities in white matter**	NP	NP

Bold type indicates common features among individuals with novel 17p13.3 microdeletions.
*Sreenath Nagamani *et al*.15
−, not present; +, ++, present; ACC, agenesis of the corpus callosum; BHC, birth head circumference; BL, birth length; BW, birth weight; CP, cleft palate; DD, developmental delay; ILS, isolated lissencephaly sequence; LIS, lissencephaly; M, mean; MDS, Miller−Dieker syndrome; MR, mental retardation; N, normal; NI, no information; NP, not performed; PDA, persistent ductus arteriosus; PFO, persistent foramen ovale; VUR, vesico−urethral reflux.

Case 8	Case 1*	Case 2*	Case 3*	Case 4*	Case 5*	Mignon-Ravix	ILS	MDS
3	13	5.5	10.5	8	17	3.5		
Female	Male	Female	Female	Male	Female	Male		
39	NI	NI	NI	NI	NI	39		
2350 (−2 SD)	3180 (−1.3 SD)	2630 (−2.1 SD)	2560 (−2.0 SD)	2540 (−2.2 SD)	NI	3260 (25th centile)		Low
44 (−3 SD)	NI	47.5 (−1.8 SD)	45.0 (−3.0 SD)	45.7 (−2.8 SD)	NI	46.5 (−1 SD)		
34 (M)	NI	NI	NI	NI	NI	38 (+2−3 SD)		
++	− (−1.3 SD)	**++ (−4.4 SD)**	**++ (−3.3 SD)**	**++ (−4.1 SD)**	**++ (−3.5 SD)**	NI height macrocephaly (+3 SD)	Microcephaly	++ Microcephaly
++	NI	NI	NI	NI	NI	NI	−	Failure to thrive
−	Mild	−	Mild	−	−	NI	+	+
Moderate MR	**Mild − moderate MR**	**Mild DD**	**Mild DD**	**Mild DD**	**Mild DD**	**Motor delay, NI on cognition**	+	++
−	NI	NI	NI	NI	NI	NI	−	−
+	NI	NI	NI	NI	NI	NI	−	−
Triangular							N	Mid-facial hypoplasia
Broad, frontal bossing	Prominent	Prominent	NI	Prominent	Prominent	High, bitemporal narrowing	Bitemporal narrowing	High, bitemporal narrowing
Large eyes, eyelid coloboma	Epicanthus, down-slanting palpebral fissures	N	Down-slanting palpebral fissures	Epicanthus inversus	N	Epicanthus, hypertelorism, down-slanting palpebral fissures	N	Hypertelorism, upward slanted palpebral fissures
Broad base	N	Broad nasal root	Broad nasal root	Broad nasal root	Broad nasal root	Anteverted nares	Broad base	Short with anteverted nares, broad base
N	Low set, large auricles	N	Low set	N	N	Low set, posteriorly rotated thick and irregular helix	N	Low set, posteriorly rotated
Maxillary Prominence	NI	NI	NI	NI	NI	Marked cupid bow	N	Prominent upper lip
Micrognathia	**Retrognathia**	N	**Mild retrognathia**	**Retrusion**	N	NI	Small jaw	Micrognathia
Prominent upper lip with thin vermillion	Ni	NI	NI	NI	NI	N		
5th finger clinodactyly, long slender fingers	Arthrogryposis of upper limbs	NI	5th finger clinodactyly, short 2nd phalanges	NI	NI	N	−	Polydactyly, 5th finger clinodactyly, camptodactyly
Submucous CP, PDA, embryotoxon	Anteriorly placed anus, VUR, hydronephrosis	PFO, PDA, coloboma of iris	−	Bifid tongue, pectus excavatum, cryptorchidism	Submucosal cleft palate, PDA	−	−	CP, occasional malformation of heart and kidney
No LIS. Wide perivascular spaces, white matter abnormalities, Hypoplastic adenohypophysis and olfactory tracts.	Thinning of corpus callosum and frontal cortex	Chiari I malformation, **signal abnormalities in subcortical white matter**	N	Chiari I malformation, **wide perivascular spaces**	**Wide perivascular spaces, signal abnormalities in subcortical white matter**	Hypoplasia of corpus callosum with posterior agenesis, nodular periventricular and subcortical heteroplasias, cortical abnormalities	LIS	LIS,ACC

(cases 1–7), which are genomically distinct from the known microdeletions that cause ILS and MDS, five interstitial microduplications (cases 9–13) and one case of a complex rearrangement involving a concurrent deletion–duplication (case 8).

The smallest deletion (case 3) was 328 kb in size, containing only six genes (*TUSC5*, *YWHAE*, *CRK*, *MYO1C*, *SKIP* and exons 1–4 of *PITPNA*) (figure 1a). All the deletions, except in case 6, involved the *YWHAE* gene. The complex rearrangement (case 8)

showed a terminal deletion extending to *SMG6* followed by a tandem duplication extending 777 kb beyond *PAFAH1B1*. The breakpoints were scattered across the distal 3.4 Mb of 17p13.3 and all were unique (figure 1 and table 1). With the exception of two cases (cases 8 and 10), the *PAFAH1B1* gene was not involved. The smallest duplication (case 13) was 59–88 kb in size. It contained the entire *TUSC5* gene, but did not involve *YWHAE* or *CRK*.

Parental studies using FISH, MLPA or microarray analysis showed that eight of the genomic imbalances (cases 1–5 and 8–10) were de novo and three were inherited (cases 6, 12 and 13). In one case, the parents were deceased and no material was available for testing (case 7), and in another case the father was unavailable for testing (case 11). The deletion in case 6a was present in a similarly affected brother (case 6b) and their less severely affected mother (case 6c). Analysis of grandparental samples revealed that the deletion had occurred de novo in the mother. Both brothers had mild developmental delay and pronounced postnatal growth retardation, while their mother had growth retardation but normal cognition. Notably, these individuals (cases 6a–c) showed only discrete facial dysmorphism. The patient in case 12 was more severely affected than the other duplication cases; she had a global delay with severe mental retardation and autism (tables 2 and 3). The duplication in case 12 was inherited from her healthy mother and was also present in her healthy sister. Therefore this duplication may represent an incidental finding that is either not causal or only partly contributes to her phenotype. Similarly, the patient in case 13 was more severely affected than the other duplication cases. This duplication involved a single gene, *TUSC5*, and was inherited from the patient's healthy mother. Parent-of-origin analysis using six polymorphic STS markers, located in the terminal 3 Mb of 17p13.3, was performed for seven of the cases (table 4). Of these, four were of paternal origin (cases 1, 2, 5 and 10) and two of maternal origin (cases 8 and 9). The genotyping results for case 11, for which a paternal sample was unavailable, showed that the duplication was not maternal in origin.

Clinical results

The patient's phenotypes are described in detail in online supplementary material and summarised in tables 2, 3 and 5.

DISCUSSION

We are able to review and refine the molecular and clinical features of the recently described novel microdeletions[13 15] and microduplications[12 14] in chromosome 17p13.3 by describing eight and six new cases (including one sib pair), respectively. Comparing all 14 deletions, the delineated critical region spans approximately 258 kb (chr17: 1 136 270–1 394 633) and includes six genes (exons 2-3 of *TUSC5*, *YWHAE*, *CRK*, *MYO1C*, *SKIP*

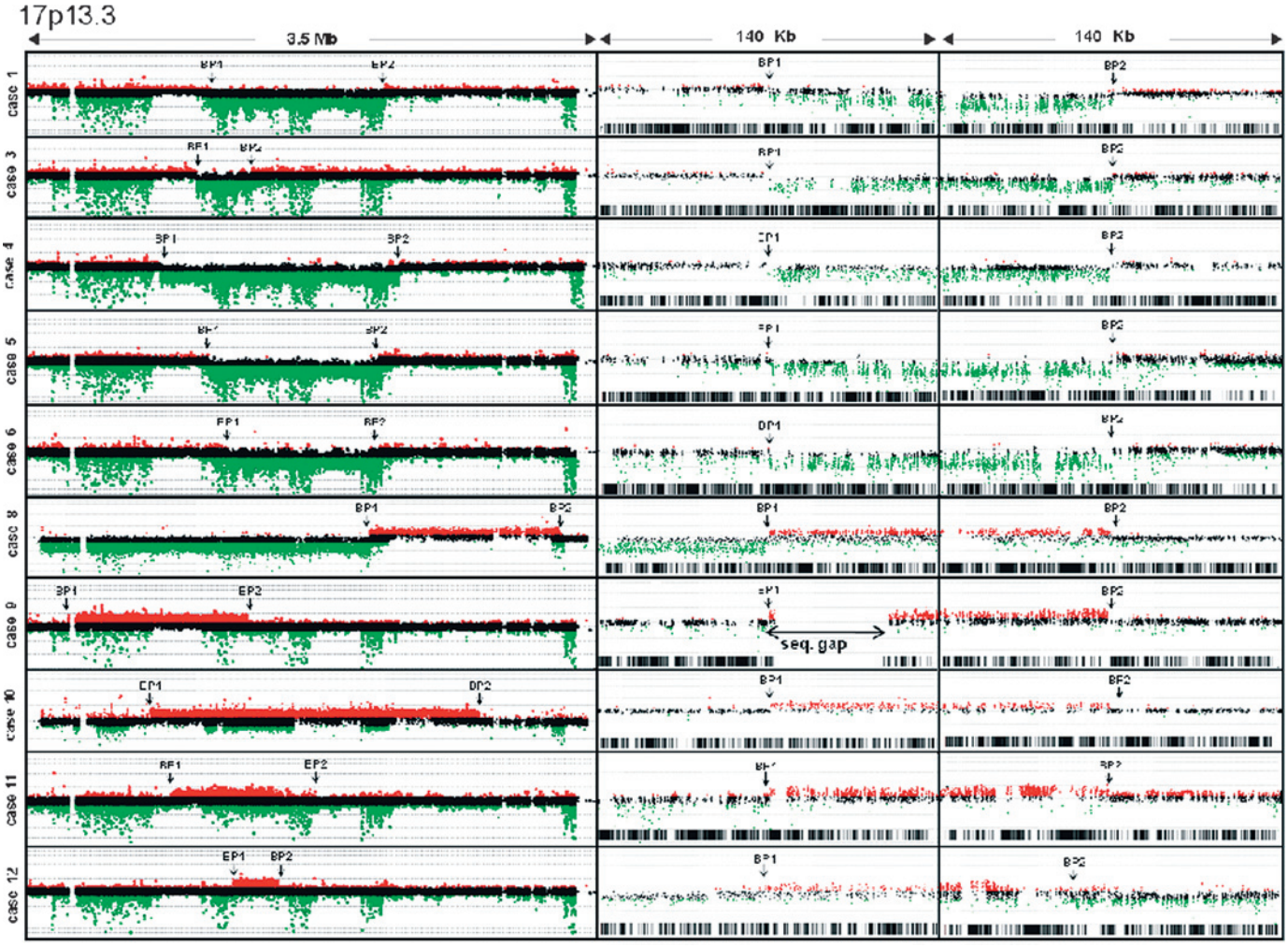

Figure 2 High-density oligonucleotide array mapping of novel 17p13.3 microdeletions/duplications (chr17: 29 169–3 352 600). Results of custom Agilent 105K array comparative genomic hybridisation on DNA samples from cases 1, 3–6 and 8–12 showed that none of the breakpoints are shared, and that they map to regions of the genome containing low-copy repeats. Black bars display repeats in each breakpoint region.

and exons 1–4 of *PITPNA*) (figure 1a). Owing to its function in the central nervous system,[12 22] *YWHAE* is very likely to play a role in the phenotypes of the deletion patients. *CRK* is the likely candidate for growth restriction; notably, case 1 described by Sreenath Nagamani *et al*[15] and the single case described by Mignon-Ravix *et al*[13] showed neither growth restriction nor deletion of *CRK* (figure 1a and tables 2, 3 and 5). Benign CNVs within the deletion minimal overlapping region (MRO) have not been reported in a series of 891 healthy individuals (see Methods) nor in the Database of Genomic Variants (http://projects.tcag.ca/variation/) or the CHOP CNV Database (http://cnv.chop.edu/).

We describe a recognisable but variable phenotype in eight individuals with microdeletions (table 5 and figure 3a) in 17p13.3 consistent with that recently described by Sreenath Nagamani *et al.*[15] All individuals with 17p13.3 microdeletions had developmental delay of varying degree, except the patient in case 5, who had only mild learning difficulties, and the mother (6c) in cases 6a and 6b who, however, experienced transient speech problems as a young child. None displayed behavioural problems. Postnatal growth retardation varied from mild to pronounced and was present in all cases except case 5. Interestingly, two of the individuals (cases 1 and 4) were treated with growth hormone, and both showed a good to excellent catch-up response. Some facial features were shared in individuals with 17p13.3 microdeletion, albeit very discrete in family 6 (cases 6a–c) (figure 3). The most common facial features were laterally extended eyebrows, infraorbital folds, broad nasal tip, maxillary prominence and prominent upper and/or lower lip. The features lacking in family 6 were high/prominent forehead, maxillary prominence and micro/retrognathia, suggesting this aspect of the phenotype maps to the region (chr17: 1 136 270–1 245 560) spanning 109 kb.

This genomic segment includes the two genes *TUSC5* and *YWHAE* (figure 1a, aqua box), further stressing the predominant role of *YWHAE* in the phenotype. Brain MRI performed in five individuals (table 5 and figure 3) showed no evidence of lissencephaly, but rather identified mild structural anomalies in the white matter (wide perivascular spaces, white matter hyper intensities), in four of them. In one of these (case 2), the frontal and upper parietal lobes were predominantly affected. Subtle hand/foot abnormalities were observed in six individuals (this study) with a microdeletion (table 5). Hence, the main characteristics of the microdeletion syndrome are significant postnatal growth retardation, mild to moderate mental retardation and facial dysmorphic manifestations.

We suggest that there are two classes of co-locating microduplications in 17p13.3. Class I duplications (six cases) involve *YWHAE* (encoding 14-3-3ϵ), but notably not *PAFAH1B1*.[12] Class II duplications (seven cases) always involve *PAFAH1B1* and may also include the genomic region encompassing the *CRK* and *YWHAE* genes.[12 14] Class I show autistic manifestations and other behavioural symptoms, speech and motor delay, subtle dysmorphic facial features, subtle hand/foot malformations, and a tendency to postnatal overgrowth (table 2). Class II microduplications have recently been shown to be associated with moderate to mild developmental and psychomotor delay and hypotonia. Some dysmorphic features, such as prominent forehead and pointed chin, are shared with the class I duplications, while overgrowth, behavioural problems and hand/foot abnormalities are less often noted (table 3). Notably, individuals with duplication of *PAFAH1B1* but not *YWHAE* or *CRK* (cases 5 and 6 from Bi *et al*[12]) show microcephaly and severe growth restriction, but are not particularly dysmorphic.

Collating the data for all class I microduplications, including cases 12 and 13, which were considered non-pathogenic, we identify a minimal region of overlap spanning 72 kb (chr17: 1 182 563–1 255 000). This region contains a single gene, *YWHAE* (figure 1b). All class II microduplications described include *PAFAH1B*, and five of the seven cases also include the class I minimal region of overlap.

All three cases with a 'pathogenic' duplication (tables 2 and 3) displayed different neurobehavioural symptoms. Two patients had autism with normal cognitive development, whereas the third patient had mild developmental delay. All had motor delay, while two patients also showed speech delay. Prenatal and postnatal growth was normal in two (cases 9 and 10), while the patient in case 11 had overgrowth. Dysmorphic manifestations were subtle and included a full tip of the nose, prominent cupid bow and pointed chin. Finally, subtle foot abnormalities were present in all three individuals. The phenotypes of individuals with 17p13.3 microduplications, including those recently reported by Bi *et al*[12] and Roos *et al*,[14] are summarised in tables 2 and 3. In conclusion, the main phenotypic characteristics of the patients with 17p13.3 microduplication are autistic manifestations, behavioural symptoms, speech delay, subtle dysmorphic facial manifestations, and subtle hand/foot malformations.

All thirteen 17p13.3 microdeletions/duplications were non-recurrent, with all of the breakpoints distinct from each other. Six of the 11 rearrangements that were characterised by high-resolution aCGH/MLPA (cases 3, 5, 6, 8, 10 and 12) had one of the breakpoints that mapped to known *Alu* elements, while the other was located within a unique sequence with no repetitive elements (figure 2 and table 6). *Alu* elements, part of the short interspersed nucleotide elements family of transposable elements, have a well-established role in both benign and pathogenic CNV formation.[3 23–27] The centromeric breakpoint in case 2, with a terminal deletion, was also located within a unique sequence. In three cases (cases 1, 4 and 9), the centromeric and telomeric breakpoints were located within repetitive elements, and comparison of the breakpoint sequences revealed significant homology with the primary sequences of overlap mapping to *Alu* elements that showed identical genomic orientations (table 6). These data suggest that non-allelic homologous recombination is the likely mechanism responsible for the microdeletions (cases 1 and 4) and microduplication (case 9) in these individuals. Within the limits of high-density oligonucleotide aCGH analysis, no homology was observed between the breakpoints in cases 2, 3, 5, 6, 8, 10, 11 and 12, which is consistent with non-homologous end joining or Fork Stalling and Template Switching.[25]

Thus, high-density aCGH/MLPA analysis precisely located the breakpoint regions to either *Alu* elements (n=12) or unique sequences (n=11). Sequence analysis of the breakpoint junctions was carried out for three cases (cases 1, 9 and 12). Junction fragments were obtained for all three (data not shown). However, sequencing of deletion/duplication junctions was possible for only one of these (case 1). The inability to sequence the other junction fragments is most likely due to the complexity of the genomic sequence at these breakpoints (table 6). Interestingly, rearrangements within 17p13.3, such as those causing the co-locating duplication syndromes, ILS and MDS, have breakpoints that are not associated with the typical paired low-copy repeats.[12 28] Instead, these non-recurrent rearrangements, including the 11 characterised here, appear to result from diverse molecular mechanisms (ie, non-allelic homologous recombination, non-homologous end joining or Fork Stalling and Template Switching).

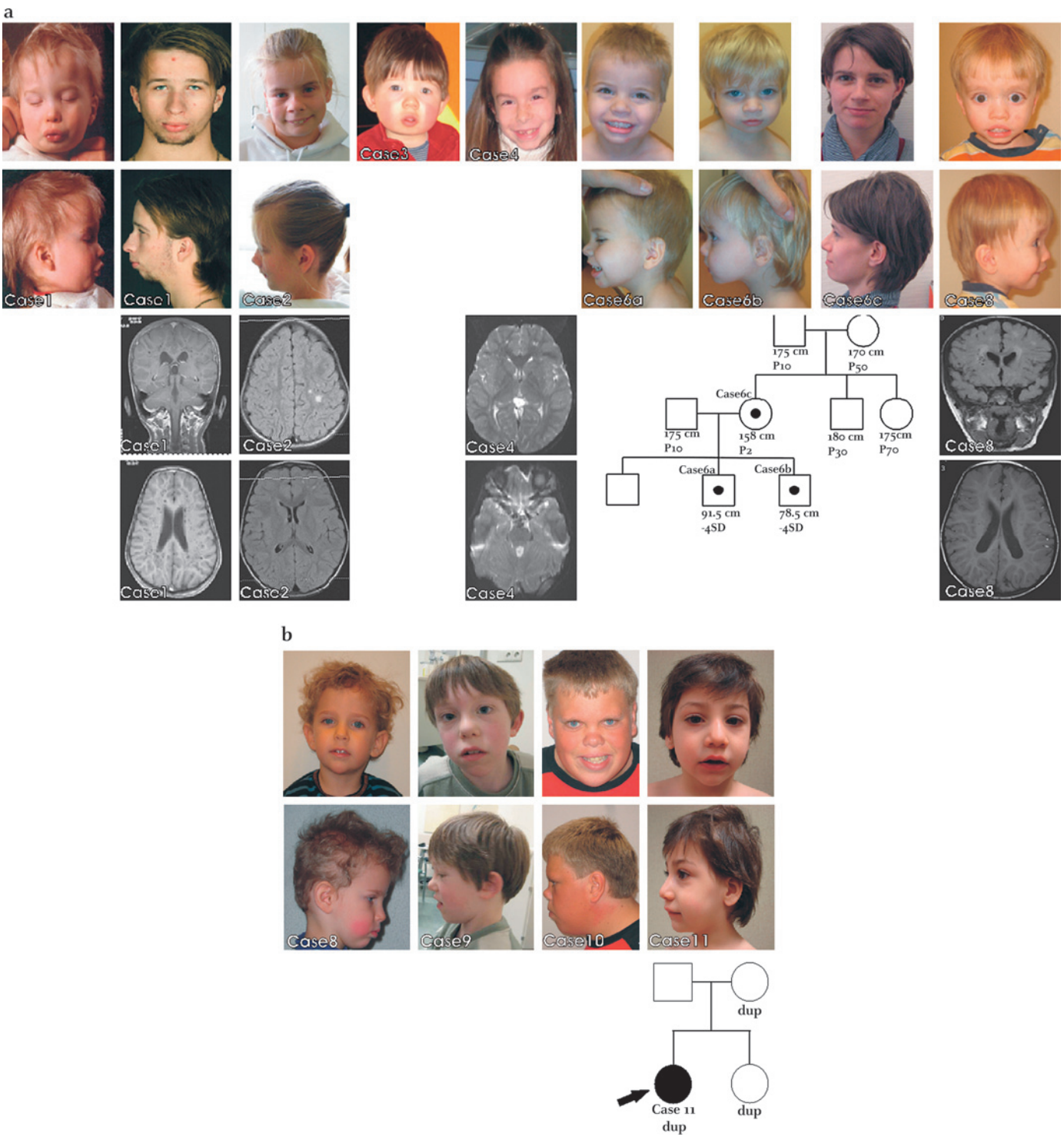

Figure 3 (a) Photographs showing facial features of individuals with microdeletion (case 1 at 2.5 and 21 years of age, cases 2—4, family 6 (6a, 6b, 6c)) and microdeletion and concomitant microduplication (case 8). The MRI scans for cases 1, 2, 4 and 8 showing mild structural brain abnormalities, are also included. Maxillary prominence, overbite, micrognathia, broad nasal tip and laterally extended eyebrows were often seen in individuals with microdeletion. All individuals or their parents gave consent to publish the photographs in this figure. (b) Photographs showing facial features of individuals with a microduplication (cases 9—12) within 17p13.3. Subtle dysmorphic manifestations, including full tip of the nose and pointed chin, seem to be common in individuals with the microduplication. All individuals or their parents gave consent to publish the photographs in this figure.

Despite the close proximity (1.1 Mb) of these novel microdeletions to *PAFAH1B1*, the absence of lissencephaly implies that *PAFAH1B1* expression is not affected. This is notable in the light of reports of disturbance of gene expression within 2—6 Mb of a genomic copy number abnormality.[29] Although, cerebral MRI did not identify abnormal gyral patterns, mild structural changes with a global distribution affecting white matter were observed, resulting in wide perivascular spaces in our deletion patients. Hence, deletions of *YWHAE* and *CRK* may affect white matter and myelinisation. This is in line with the notion that the combined deletion of *YWHAE*, *CRK* and *PAFAH1B1* results in a more severe brain phenotype than the deletion of *PAFAH1B1*

Table 6 Precise breakpoint coordinates and associated genomic architecture

case	Genomic abnormality	Centromeric breakpoint	Telomeric breakpoint	Repetitive elements at breakpoints		BLAST2 alignment	Likely mechanism
1	Interstitial deletion	chr17: 1135022* (1 bp)	chr17: 2202835 * (1 bp)	*Alu*Y	*Alu*Y	94% homology	NAHR
2	Terminal deletion	chr17: 514 (terminal)	chr17: 2065626–2067096† (1,470 bp)	None	None	None	DSB with repair[36]
3	Interstitial deletion	chr17: 1066764–1067074 (310 bp)	chr17: 1394633–1395070 (437 bp)	None	*Alu*Sp	No homology	NHEJ or FoSTeS
4	Interstitial deletion	chr17: 841525–842389 (864 bp)	chr17: 2257857–2259115 (1,258 bp)	L2, *Alu*Jo, *Alu*Jo, *Alu*Y, *Alu*Jo	L1, *Alu*Sx, *Alu*Sg, *Alu*Y, L1	94% homology (overall). 92% homology between *Alu*Y and *Alu*Y	NAHR
5	Interstitial deletion	chr17: 1136244–1136270 (26 bp)	chr17: 2168155–2168814 (659 bp)	None	*Alu*Jb, *Alu*Y	No homology	NHEJ or FoSTeS
6	Interstitial deletion	chr17: 1254497–1,254,527 (30 bp)	chr17: 2164139–2165304 (1165 bp)	None	*Alu*Sp, *Alu*Y, *Alu*Sp, *Alu*Sg	No homology	NHEJ or FoSTeS
8	Terminal deletion with Concurrent duplication	chr17: 29169 (terminal) chr17: 2124174–2124215 (41 bp)	chr17: 2123816–2123915‡ (99 bp) chr17:3321560–3321582 (22 bp)	None MER1	*Alu*Sx, MER2 None	No homology No homology	FoSTeS‡
9	Interstitial duplication	chr17: 293620–294421 (801 bp)	chr17: 1371895–1373231 (1336 bp)	MIRb, MIR, *Alu*Sx, *Alu*Jo	FLAM_C, L2, *Alu*Yb8, *Alu*Sx, L2, L3	83% homology between *Alu*Sx and *Alu*Yb8, 82% homology between *Alu*Sx and *Alu*Sx	NAHR
10	Interstitial duplication	chr17: 738590–738991 (401 bp)	chr17: 2813518–2813531 (13 bp)	*Alu*Sq	None	No homology	NHEJ or FoSTeS
11	Interstitial duplication	chr17: 936235–936249 (14 bp)	chr17: 1573187–1573217 (30 bp)	None	None	No homology	NHEJ or FoSTeS
12	Interstitial duplication	chr17: 1287515–1287575 (60 bp)	chr17: 1558499–1558827 (328 bp)	None	*Alu*Sc	No homology	NHEJ or FoSTeS

FoSTeS, Fork Stalling and Template Switching; NHEJ, Non-homologous end joining; NAHR, Non-allelic homologous recombination; DSB, Double strand break.
*Breakpoint mapped to within 1 bp by sequencing of junction fragment.
†Mapped by multiplex ligation-dependent probe amplification.
‡Concurrent deletion-duplication interrupted by a 200 bp segment (three probes) of normal copy number.

alone, and the fact that both genes are involved in neuronal migration.[22] In addition, *CRK* has been shown to control neuronal positioning in the developing brain, both dependent on the Reelin pathway and independently of it: *Crk* knockout mice are smaller and have smaller brains.[30] *CRK* is also an attractive candidate for the subtle malformations of the hands and/or feet observed in six of the eight individuals with a microdeletion, and six of the seven individuals with co-locating microduplications (tables 2, 3 and 5). *CRK* is known to interact with *FLNA*, a gene involved in limb development.[31] Notably, case 3 (in Bi *et al*[12]) had no duplication of *CRK* and no limb abnormalities.

A single gene (*YWHAE*) maps within the duplication MRO (figure 1b). Duplication of *YWHAE* might have an effect on neuronal network development and maturation, as all seven cases with duplications involving this gene show delay in motor function and symptoms within the autism spectrum or developmental delay (table 2). This gene is thus likely to contribute to the neurodevelopmental symptoms in these individuals, although it has not previously been identified as a candidate gene for autism.[32–34] As the duplications in cases 12 and 13 are less likely to have a major phenotypic effect in these patients, it also indicates that the genes proximal to the duplication MRO are less likely to be involved in the duplication phenotype.

Interestingly, duplication of *PAFAH1B1* with concomitant duplication (case 10) or deletion (case 8) of *YWHAE* and *CRK* appears to result in a milder neurological phenotype than duplication of *PAFAH1B1* alone. These findings provide further evidence to support genetic interactions between *CRK*, *YWHAE* and *PAFAH1B1* in key molecular pathways controlling neuronal migration and cortical development.[22 35]

Our results refine the molecular and clinical description of patients carrying the recently described microdeletions and microduplications in 17p13.3. Review of all published cases indicates that deletions of *YWHAE* and *CRK*, not including *PAFAH1B1*, represent a clinically variable phenotype consisting of growth retardation, facial dysmorphism and developmental delay, but notably without lissencephaly. Autism and a tendency to overgrowth appear to be notable features of the co-locating class I microduplications, being less often present in the class II microduplication phenotype. *YWHAE* and *CRK*, in particular, have been identified as attractive candidate genes for autism (duplications) and facial dysmorphology manifestations (deletions), and the growth restriction (deletions) and limb malformations (deletions and duplications), respectively.

Author affiliations:
[1]Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Australia
[2]Department of Pediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Australia
[3]Clinical Genetics Unit, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
[4]Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[5]Department of Human Genetics, Emory University, Atlanta, Georgia, USA
[6]The National Birth Defects Center, Waltham, Massachusetts, USA
[7]University and University Hospital Antwerp, Antwerp, Belgium
[8]Department of Endocrinology, Clinic of Pediatrics, Karolinska University Hospital, Huddinge, Sweden
[9]Sachs' Children's Hospital, Södersjukhuset, Stockholm, Sweden
[10]INMED, INSERM U901, Université de la Méditerranee, Campus de Luminy, Marseille, France
[11]Department of Human Genetics, Radboud University, Nijmegen Medical Centre, Nijmegen, The Netherlands
[12]Mälar Hospital, Eskilstuna, Sweden

Acknowledgements We are grateful to all individuals and parents who participated in this study. This work was supported by grants from The Swedish Research Council (BMA), The Karolinska Institute foundation and Stockholm County Council (to JS, BMA), Perpetual Trustees Australia (to HRS), and the EU-funded AnEUploidy Project (to BBAdV and BvB) and the Netherlands Organisation for Health Research and Development (to BBAdV). We also thank Z Bowman, M Lagerberg, J Wincent and C Ngo for technical assistance and J Senior for critically reading the manuscript.

Funding Swedish Research Council, Karolinska Institute Foundation, Stockholm County Council, Perpetual Trustees Australia, EU-funded AnEUploidy Project, The Netherlands Organisation for Health Research and Development.

Competing interests None.

Ethics approval This study was conducted with the approval of the Karolinska Institute, Stockholm, Sweden.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Zahir F,** Friedman JM. The impact of array genomic hybridization on mental retardation research: a review of current technologies and their clinical utility. *Clin Genet* 2007;**72**:271—87.
2. **Schoumans J,** Ruivenkamp C, Holmberg E, Kyllerman M, Anderlid BM, Nordenskjold M. Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). *J Med Genet* 2005;**42**:699—705.
3. **Lupski JR.** Genomic rearrangements and sporadic disease. *Nat Genet* 2007;**39** (Suppl 7):S43—7.
4. **Koolen DA,** Pfundt R, de Leeuw N, Hehir-Kwa JY, Nillesen WM, Neefs I, Scheltinga I, Sistermans E, Smeets D, Brunner HG, van Kessel AG, Veltman JA, de Vries BB. Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. *Hum Mutat* 2009;**30**:283—92.
5. **Bruno DL,** Ganesamoorthy D, Schoumans J, Bankier A, Coman D, Delatycki M, Gardner RJ, Hunter M, James PA, Kannu P, McGillivray G, Pachter N, Peters H, Rieubland C, Savarirayan R, Scheffer IE, Sheffield L, Tan T, White SM, Yeung A, Bowman Z, Ngo C, Choy KW, Cacheux V, Wong L, Amor DJ, Slater HR. 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. *J Med Genet* 2009;**46**:123—31.
6. **Slavotinek AM.** Novel microdeletion syndromes detected by chromosome microarrays. *Hum Genet* 2008;**124**:1—17.
7. **Sharp AJ,** Mefford HC, Li K, Baker C, Skinner C, Stevenson RE, Schroer RJ, Novara F, De Gregori M, Ciccone R, Broomer A, Casuga I, Wang Y, Xiao C, Barbacioru C, Gimelli G, Bernardina BD, Torniero C, Giorda R, Regan R, Murday V, Mansour S, Fichera M, Castiglia L, Failla P, Ventura M, Jiang Z, Cooper GM, Knight SJ, Romano C, Zuffardi O, Chen C, Schwartz CE, Eichler EE. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet* 2008;**40**:322—8.
8. **Koolen DA,** Sharp AJ, Hurst JA, Firth HV, Knight SJ, Goldenberg A, Saugier-Veber P, Pfundt R, Vissers LE, Destree A, Grisart B, Rooms L, Van der Aa N, Field M, Hackett A, Bell K, Nowaczyk MJ, Mancini GM, Poddighe PJ, Schwartz CE, Rossi E, De Gregori M, Antonacci-Fulton LL, McLellan MD 2nd, Garrett JM, Wiechert MA, Miner TL, Crosby S, Ciccone R, Willatt L, Rauch A, Zenker M, Aradhya S, Manning MA, Strom TM, Wagenstaller J, Krepischi-Santos AC, Vianna-Morgante AM, Rosenberg C, Price SM, Stewart H, Shaw-Smith C, Brunner HG, Wilkie AO, Veltman JA, Zuffardi O, Eichler EE, de Vries BB. Clinical and molecular delineation of the 17q21.31 microdeletion syndrome. *J Med Genet* 2008;**45**:710—20.
9. **Hannes FD,** Sharp AJ, Mefford HC, de Ravel T, Ruivenkamp CA, Breuning MH, Fryns JP, Devriendt K, Van Buggenhout G, Vogels A, Stewart HH, Hennekam RC, Cooper GM, Regan R, Knight SJ, Eichler EE, Vermeesch JR. Recurrent reciprocal deletions and duplications of 16p13.11: The deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet* 2008;**46**:223—32.
10. **Brunetti-Pierri N,** Berg JS, Scaglia F, Belmont J, Bacino CA, Sahoo T, Lalani SR, Graham B, Lee B, Shinawi M, Shen J, Kang SH, Pursley A, Lotze T, Kennedy G, Lansky-Shafer S, Weaver C, Roeder ER, Grebe TA, Arnold GL, Hutchison T, Reimschisel T, Amato S, Geragthy MT, Innis JW, Obersztyn E, Nowakowska B, Rosengren SS, Bader PI, Grange DK, Naqvi S, Garnica AD, Bernes SM, Fong CT, Summers A, Walters WD, Lupski JR, Stankiewicz P, Cheung SW, Patel A. Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. *Nat Genet* 2008;**40**:1466—71.
11. **Ben-Shachar S,** Ou Z, Shaw CA, Belmont JW, Patel MS, Hummel M, Amato S, Tartaglia N, Berg J, Sutton VR, Lalani SR, Chinault AC, Cheung SW, Lupski JR, Patel A. 22q11.2 distal deletion: a recurrent genomic disorder distinct from DiGeorge syndrome and velocardiofacial syndrome. *Am J Hum Genet* 2008;**82**:214—21.
12. **Bi W,** Sapir T, Shchelochkov OA, Zhang F, Withers MA, Hunter JV, Levy T, Shinder V, Peiffer DA, Gunderson KL, Nezarati MM, Shotts VA, Amato SS, Savage SK, Harris DJ, Day-Salvatore DL, Horner M, Lu XY, Sahoo T, Yanagawa Y, Beaudet AL, Cheung SW, Martinez S, Lupski JR, Reiner O. Increased LIS1 expression affects human and mouse brain development. *Nat Genet* 2009;**41**:168—77.
13. **Mignon-Ravix C,** Cacciagli P, El-Waly B, Moncla A, Milh M, Girard N, Chabrol B, Philip N, Villard L. Deletion of YWHAE in a patient with periventricular heterotopias and marked corpus callosum hypoplasia. *J Med Genet* 2009.
14. **Roos L,** Jonch AE, Kjaergaard S, Taudorf K, Simonsen H, Hamborg-Petersen B, Brondum-Nielsen K, Kirchhoff M. A new microduplication syndrome encompassing the region of the Miller-Dieker (17p13 deletion) syndrome. *J Med Genet* 2009;**46**:703—10.
15. **Sreenath Nagamani SC,** Zhang F, Shchelochkov OA, Bi W, Ou Z, Scaglia F, Probst FJ, Shinawi M, Eng C, Hunter JV, Sparagana S, Lagoe E, Fong CT, Pearson M, Doco-Fenzy M, Landais E, Mozelle M, Chinault AC, Patel A, Bacino CA, Sahoo T, Kang SH, Cheung SW, Lupski JR, Stankiewicz P. Microdeletions including YWHAE in the Miller-Dieker syndrome region on chromosome 17p13.3 result in facial dysmorphisms, growth restriction, and cognitive impairment. *J Med Genet* 2009.
16. **Cardoso C,** Leventer RJ, Ward HL, Toyo-Oka K, Chung J, Gross A, Martin CL, Allanson J, Pilz DT, Olney AH, Mutchinick OM, Hirotsune S, Wynshaw-Boris A, Dobyns WB, Ledbetter DH. Refinement of a 400-kb critical region allows genotypic differentiation between isolated lissencephaly, Miller-Dieker syndrome, and other phenotypes secondary to deletions of 17p13.3. *Am J Hum Genet* 2003;**72**:918—30.
17. **Jonsson G,** Bendahl PO, Sandberg T, Kurbasic A, Staaf J, Sunde L, Cruger DG, Ingvar C, Olsson H, Borg A. Mapping of a novel ocular and cutaneous malignant melanoma susceptibility locus to chromosome 9q21.32. *J Natl Cancer Inst* 2005;**97**:1377—82.
18. **Kok K,** Dijkhuizen T, Swart YE, Zorgdrager H, van der Vlies P, Fehrmann R, te Meerman GJ, Gerssen-Schoorl KB, van Essen T, Sikkema-Raddatz B, Buys CH. Application of a comprehensive subtelomere array in clinical diagnosis of mental retardation. *Eur J Med Genet* 2005;**48**:250—62.
19. **Atayar C,** Kok K, Kluiver J, Bosga A, van den Berg E, van der Vlies P, Blokzijl T, Harms G, Davelaar I, Sikkema-Raddatz B, Martin-Subero JI, Siebert R, Poppema S, van den Berg A. BCL6 alternative breakpoint region break and homozygous deletion of 17q24 in the nodular lymphocyte predominance type of Hodgkin's lymphoma-derived cell line DEV. *Hum Pathol* 2006;**37**:675—83.
20. **Baldwin EL,** Lee JY, Blake DM, Bunke BP, Alexander CR, Kogan AL, Ledbetter DH, Martin CL. Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. *Genet Med* 2008;**10**:415—29.
21. **Zhang ZF,** Ruivenkamp C, Staaf J, Zhu H, Barbaro M, Petillo D, Khoo SK, Borg A, Fan YS, Schoumans J. Detection of submicroscopic constitutional chromosome aberrations in clinical diagnostics: a validation of the practical performance of different array platforms. *Eur J Hum Genet* 2008;**16**:786—92.
22. **Toyo-oka K,** Shionoya A, Gambello MJ, Cardoso C, Leventer R, Ward HL, Ayala R, Tsai LH, Dobyns W, Ledbetter D, Hirotsune S, Wynshaw-Boris A. 14-3-3epsilon is important for neuronal migration by binding to NUDEL: a molecular explanation for Miller-Dieker syndrome. *Nat Genet* 2003;**34**:274—85.
23. **Batzer MA,** Deininger PL. Alu repeats and human genomic diversity. *Nat Rev Genet* 2002;**3**:370—9.
24. **Deininger PL,** Batzer MA. Alu repeats and human disease. *Mol Genet Metab* 1999;**67**:183—93.
25. **Gu W,** Zhang F, Lupski JR. Mechanisms for human genomic rearrangements. *Pathogenetics* 2008;**1**:4.
26. **Kolomietz E,** Meyn MS, Pandita A, Squire JA. The role of Alu repeat clusters as mediators of recurrent chromosomal aberrations in tumors. *Genes Chromosomes Cancer* 2002;**35**:97—112.
27. **Lee JA,** Carvalho CM, Lupski JR. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell* 2007;**131**:1235—47.
28. **Mei D,** Lewis R, Parrini E, Lazarou LP, Marini C, Pilz DT, Guerrini R. High frequency of genomic deletions—and a duplication—in the LIS1 gene in lissencephaly: implications for molecular diagnosis. *J Med Genet* 2008;**45**:355—61.
29. **Stranger BE,** Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, Redon R, Bird CP, de Grassi A, Lee C, Tyler-Smith C, Carter N, Scherer SW, Tavare S, Deloukas P, Hurles ME, Dermitzakis ET. Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science* 2007;**315**:848—53.
30. **Park TJ,** Curran T. Crk and Crk-like play essential overlapping roles downstream of disabled-1 in the Reelin pathway. *J Neurosci* 2008;**28**:13551—62.
31. **Robertson SP,** Twigg SR, Sutherland-Smith AJ, Biancalana V, Gorlin RJ, Horn D, Kenwrick SJ, Kim CA, Morava E, Newbury-Ecob R, Orstavik KH, Quarrell OW, Schwartz CE, Shears DJ, Suri M, Kendrick-Jones J, Wilkie AO. Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nat Genet* 2003;**33**:487—91.
32. **Glessner JT,** Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, Imielinski M, Frackelton EC, Reichert J, Crawford EL, Munson J, Sleiman PM, Chiavacci R, Annaiah K, Thomas K, Hou C, Glaberson W, Flory J, Otieno F, Garris M, Soorya L, Klei L, Piven J, Meyer KJ, Anagnostou E, Sakurai T, Game RM, Rudd DS, Zurawiecki D, McDougle CJ, Davis LK, Miller J, Posey DJ, Michaels S, Kolevzon A, Silverman JM, Bernier R, Levy SE, Schultz RT, Dawson G, Owley T, McMahon WM, Wassink TH, Sweeney JA, Nurnberger JI, Coon H, Sutcliffe JS, Minshew NJ, Grant SF, Bucan M, Cook EH, Buxbaum JD, Devlin B, Schellenberg GD, Hakonarson H. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature* 2009;**459**:569—73.
33. **Sebat J,** Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimaki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M. Strong association of de novo copy number mutations with autism. *Science* 2007;**316**:445—9.
34. **Szatmari P,** Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB, Skaug JL, Thompson AP, Senman L, Feuk L, Qian C, Bryson SE, Jones MB, Marshall CR, Scherer SW, Vieland VJ, Bartlett C, Mangin LV, Goedken R, Segre A, Pericak-Vance MA, Cuccaro ML, Gilbert JR, Wright HH, Abramson RK, Betancur C, Bourgeron

T, Gillberg C, Leboyer M, Buxbaum JD, Davis KL, Hollander E, Silverman JM, Hallmayer J, Lotspeich L, Sutcliffe JS, Haines JL, Folstein SE, Piven J, Wassink TH, Sheffield V, Geschwind DH, Bucan M, Brown WT, Cantor RM, Constantino JN, Gilliam TC, Herbert M, Lajonchere C, Ledbetter DH, Lese-Martin C, Miller J, Nelson S, Samango-Sprouse CA, Spence S, State M, Tanzi RE, Coon H, Dawson G, Devlin B, Estes A, Flodman P, Klei L, McMahon WM, Minshew N, Munson J, Korvatska E, Rodier PM, Schellenberg GD, Smith M, Spence MA, Stodgell C, Tepper PG, Wijsman EM, Yu CE, Roge B, Mantoulan C, Wittemeyer K, Poustka A, Felder B, Klauck SM, Schuster C, Poustka F, Bolte S, Feineis-Matthews S, Herbrecht E, Schmotzer G, Tsiantis J, Papanikolaou K, Maestrini E, Bacchelli E, Blasi F, Carone S, Toma C, Van Engeland H, de Jonge M, Kemner C, Koop F, Langemeijer M, Hijmans C, Staal WG, Baird G, Bolton PF, Rutter ML, Weisblatt E, Green J, Aldred C, Wilkinson JA, Pickles A, Le Couteur A, Berney T, McConachie H, Bailey AJ, Francis K, Honeyman G, Hutchinson A, Parr JR, Wallace S, Monaco AP, Barnby G, Kobayashi K, Lamb JA, Sousa I, Sykes N, Cook EH, Guter SJ, Leventhal BL, Salt J, Lord C, Corsello C, Hus V, Weeks DE, Volkmar F, Tauber M, Fombonne E, Shih A, Meyer KJ. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat Genet* 2007;**39**:319—28.

35. **Chen K,** Ochalski PG, Tran TS, Sahir N, Schubert M, Pramatarova A, Howell BW. Interaction between Dab1 and CrkII is promoted by Reelin signaling. *J Cell Sci* 2004;**117**(Pt 19):4527—36.
36. **Yatsenko SA,** Brundage EK, Roney EK, Cheung SW, Chinault AC, Lupski JR. Molecular mechanisms for subtelomeric rearrangements associated with the 9q34.3 microdeletion syndrome. *Hum Mol Genet* 2009;**18**:1924—36.