npg

SHORT REPORT

A recurrent deletion syndrome at chromosome bands 2p11.2-2p12 flanked by segmental duplications at the breakpoints and including *REEP1*

Servi JC Stevens*, Eveline W Blom, Ingrid TJ Siegelaer and Eric EJGL Smeets

We identified an identical and recurrent 9.4-Mbp deletion at chromosome bands 2p11.2-2p12, which occurred *de novo* in two unrelated patients. It is flanked at the distal and proximal breakpoints by two homologous segmental duplications consisting of low copy repeat (LCR) blocks in direct orientation, which have >99% sequence identity. Despite the fact that the deletion was almost 10 Mbp in size, the patients showed a relatively mild clinical phenotype, that is, mild-to-moderate intellectual disability, a happy disposition, speech delay and delayed motor development. Their phenotype matches with that of previously described patients. The 2p11.2-2p12 deletion includes the *REEP1* gene that is associated with spastic paraplegia and phenotypic features related to this are apparent in most 2p11.2-2p12 deletion patients, but not in all. Other hemizygous genes that may contribute to the clinical phenotype include *LRRTM1* and *CTNNA2*. We propose a recurrent but rare 2p11.2-2p12 deletion syndrome based on (1) the identical, non-random localisation of the *de novo* deletion breakpoints in two unrelated patients and a patient from literature, (2) the patients' phenotypic similarity and their phenotypic overlap with other 2p deletions and (3) the presence of highly identical LCR blocks flanking both breakpoints, consistent with a non-allelic homologous recombination (NAHR)-mediated rearrangement.

European Journal of Human Genetics (2015) 23, 543–546; doi:10.1038/ejhg.2014.124; published online 2 July 2014

INTRODUCTION

Diagnostic implementation of oligoarray-based copy number variant (CNV) profiling in patients with intellectual disability and/or developmental delay has identified genomic regions that are recurrently prone to copy number change, as well as sporadic (ie, 'patient-unique') gains and losses.[1] Recurrent CNVs may be caused by non-allelic homologous recombination (NAHR). NAHR is driven by breakpoint-flanking low copy repeats (LCRs), which comprise 5–6% of the human genome and which can misalign in meiosis due to their sequence homology.[2,3] Genotype–phenotype relationships for such recurrent NAHR-mediated rearrangements have led to the definition of recurrent and syndromic genomic disorders, that is, microdeletion and duplication syndromes.[4–6]

Here, we report rare, recurrent 9.4-Mbp deletions occurring *de novo* in the proximal short arm of chromosome 2. These 2p11.2-2p12 deletion patients show a relatively mild phenotype, despite the large deletion size, with mild-to-moderate intellectual disability, a happy disposition, speech delay and delayed motor development as main features.

MATERIALS AND METHODS

Patients

Patient 1 was clinically evaluated at the age of 15 years and 8 months. He was born preterm after an uncomplicated pregnancy as a dizygotic twin, with a birth weight of 2080 g. The sole clinical phenotype apparent at time of birth was clubfeet. In his early youth, he showed developmental delay with moderate intellectual disability, normal growth parameters and a tendency to obesity. Cognitive assessment showed a verbal IQ of 49 and a performance IQ of 52 at age 9 years. He has a broad nasal bridge, relatively big nose, low set ears and

broad dental ridges but no overt facial dysmorphic features. He has hyperreflexia of the lower limbs and a hypotonic habitus as well as a varus deformity of both feet with toeing in and he is incontinent. The boy is of a very friendly disposition. His height at clinical evaluation was 175 cm (~50th percentile) and his weight was 75 kg (~80th percentile) and he has a slight tendency to overweight. Patient 2 was evaluated at the age of 5 years and 4 months. He was born after an uncomplicated pregnancy and developmental delay was noticed at 1.5 years of age, with delayed fine motor skills, language impairment and an IQ of 80. He is an easy-going, very friendly child. There were no overt facial dysmorphic features and no growth delay. He was hypermobile with his hands. His height was 114 cm (~60th percentile) and his weight was 21.9 kg (~75th percentile).

Cytogenetic investigations and *in silico* analyses

Genome-wide copy number profiling was done using high-resolution Cyto-Scan HD arrays with 2.7 million markers, according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). CNVs were called using Chromosome Analysis Suite 1.2.2 Software (Affymetrix). Microarray nomenclature was designated according to HGVS.[7] Nucleotide positions were derived from the Genome Reference Consortium build GRCh37 (Ensembl release 68, July 2012). Genomic architecture of LCRs was analysed with the Human Genome Segmental Duplication Database (http://humanparalogy.gs.washington.edu/) based on human genome build 37 (February 2009) and as described previously.[8]

RESULTS

Microarray analysis of DNA isolated from peripheral blood of both patients 1 and 2 showed a diminished signal for 7368 markers in the proximal short arm of chromosome 2. Thus, they have an identical 9.4-Mbp deletion: chr2.hg19:g.(77,907,115_77,916,761)_(87,322,042_

Department Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands
*Correspondence: Dr SJC Stevens, Department Clinical Genetics, Maastricht University Medical Center, PO Box 5800, 6202 AZ Maastricht, The Netherlands.
Tel: +31 43 387 1625; Fax: +31 43 387 7901; E-mail: servi.stevens@mumc.nl

87,330,965)del (Figure 1). Karyotyping of both parents of patient 1 showed that the deletion had occurred *de novo*. Analysis of informative SNP genotypes in the 2p11.2-2p12 region showed that the deletion had occurred *de novo* on the maternal chromosome in patient 2 (ie, only the paternal haplotype was present). Finally, metaphase FISH on the available parental blood samples, using probe RP11-301O19 specific for band 2p11.2, excluded balanced insertional translocations.

The breakpoints of the deletions are flanked by two paired blocks of homologous LCRs, which are in direct orientation relative to each other. The first LCR at the distal breakpoint in band 2p12 (position chr2: 77,894,189–77,901,295) is 7106 bp in size and has 99.50% sequence identity to an LCR block at the proximal breakpoint in 2p11.2, sized 7435 bp (position chr2: 87,338,168–87,345,604). The second LCR block in band 2p12 (chr2: 77,901,296–77,916,981) has 99.23% sequence identity to a 16 114 bp region near the proximal breakpoint of the deletion in band 2p12 (chr2: 87,322,054–87,338,168). Figure 2 gives a schematic representation of the genomic architecture of the LCRs at both breakpoint regions and the genomic localisation of the deletions.

DISCUSSION

We identified identical, recurrent 9.4-Mbp deletions at chromosome bands 2p11.2-2p12, which occurred *de novo* in two unrelated patients. A very similar deletion was reported recently,[9] which has an identical proximal breakpoint (Figure 2). The distal breakpoints differ by a mere 5 kbp, which is within experimental variation of the array. Both breakpoints are directly flanked by two LCR blocks, which have

>99% sequence identity and are in same orientation (Figure 2). Such genomic architecture is consistent with an NAHR-mediated rearrangement.[2–5] Owing to their high sequence identity, LCRs can be substrates for meiotic misalignment, in an inter- or intrachromosome or intrachromatid manner, leading to either deletion or duplication of the intervening region.

The clinical phenotype matches with that of other patients with overlapping deletions in proximal 2p (incl. MIM 613564).[9–12] All show mild-to-moderate intellectual disability, a very happy disposition, speech delay, delayed motor development and minor facial anomalies such as high forehead, broad nasal bridge and large low set ears (Table 1). Despite the very large size of the del(2)(p11.2p12), the clinical phenotype is relatively mild when compared generally with similar-sized deletions reported in literature. This fits with previous observations that deletion of the major part of chromosome band 2p12, a gene-poor G-dark band, has minimal phenotypic impact.[12] On the basis of the striking phenotypic resemblance of patients, the identical, non-random breakpoints and the presence of highly identical LCR blocks at both breakpoints, we propose here a previously unrecognised, recurrent 2p11.2-2p12 deletion syndrome.

The deletion includes the *REEP1* gene in band 2p11.2. Nonsense variants in *REEP1* causing haploinsufficiency/loss of function are responsible for autosomal dominant hereditary spastic paraplegia (HSP)-type SPG31 (OMIM 610250).[13,14] HSP-related features were seen in patient 1, the previous patient with the 9.4-Mbp deletion[2] and other patients with *REEP1* deletions.[10,11] The age distribution for SPG31 shows a bimodal pattern of <20 and >30 years of age[13,14] leaving a possibility that HSP is not yet manifest in patient 1.
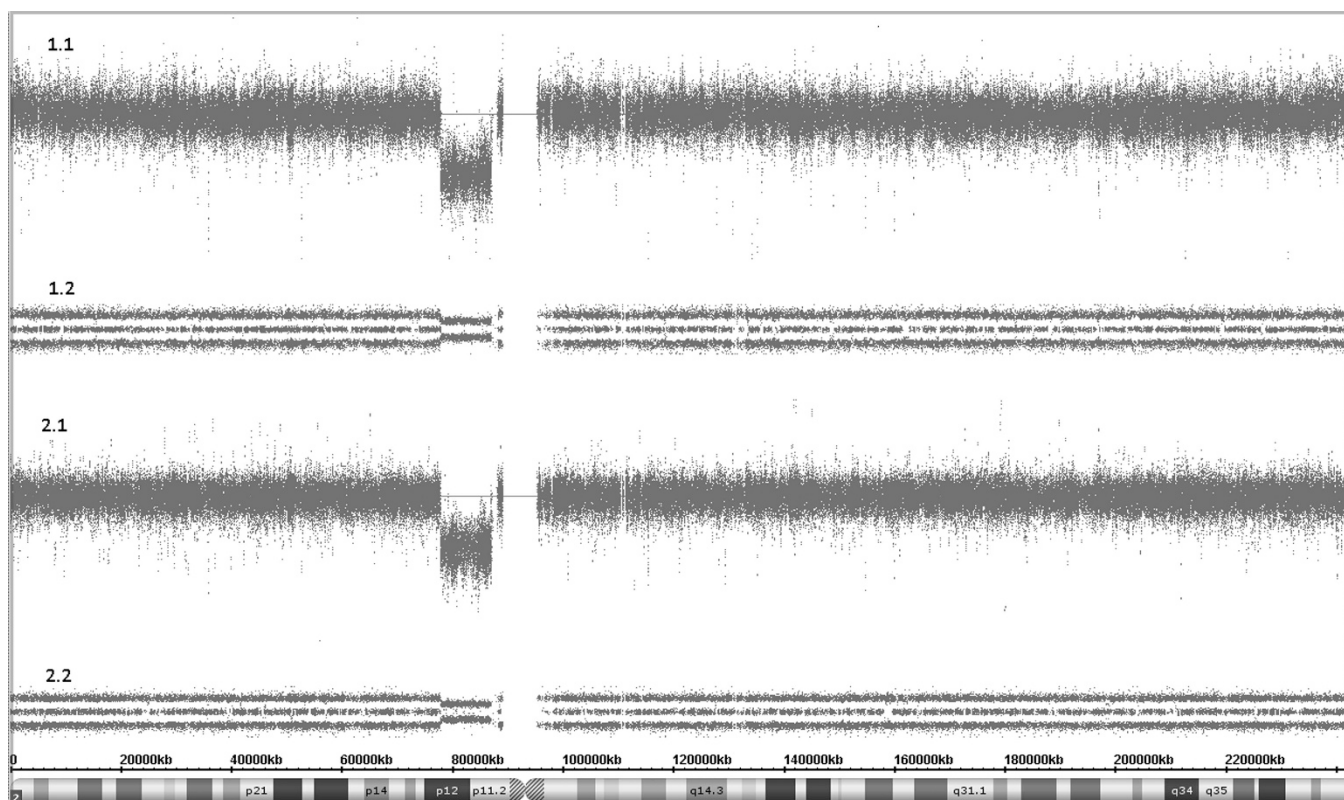
Figure 1 Interstitial deletion 2p11.2→2p12 in patients 1 and 2 as identified by high-resolution Cytoscan HD array. The log2ratio of the hybridisation signal of patient *vs* references (1.1 and 2.1 for patients 1 and 2, respectively) shows a diminished signal in bands 2p11.2 and 2p12. The 'allele peak' analysis track (1.2 and 2.2 for patients 1 and 2, respectively) shows absence of heterozygous calls in the deleted region, whereas three 'bands' are seen outside of the deletion, representing homozygous calls (top and bottom bands) and heterozygous calls (middle band).
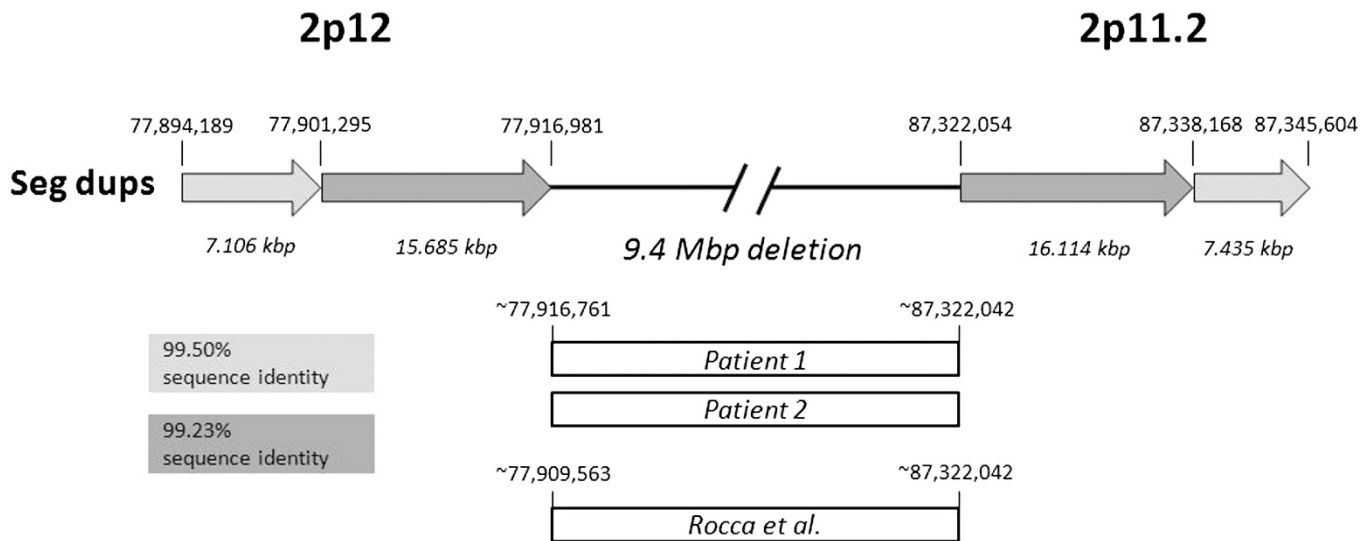
Figure 2 Genomic organisation of segmental duplications (Seg dups) flanking the deletion breakpoints in chromosome bands 2p11.2 and 2p12, their orientation and sequence identity. Figure is based on data obtained from UCSC human genome built 37 and from http://humanparalogy.gs.washington.edu/.

Table 1 Clinical findings in patients with 2p11.2→2p12 deletions presented in this report and in literature

	Tzschach et al[10]	Writzl et al[11]	Rocca et al[9]	Patient 1	Patient 2
Deletion size (Mbp)	11.4	10.4	9.4	9.4	9.4
Sex	F	M	F	M	M
Inheritance	de novo	de novo	de novo	de novo	de novo
Age at examination	5 years	5 years, 9 mo.	9 years	15 years, 8 mo.	5 years, 4 mo.
Intellectual disability	+	?	mild	moderate	mild
Short stature	+	+	+	−	−
Speech delay	+	+	+	+	+
Delayed motor development	+	+	+	+	+
Hypertonia	−	+	−	−	−
Ataxia	−	+	+	−	−
High forehead	+	+	+	+	+
Broad high nasal bridge	+	+	+	+	+
Low set ears	+	+	+	+	+
Large ears	+	+	+	+	+
Feet anomalies	Bilateral clubfeet	Turned outwards	−	Varus deformity	−
Microcephaly	+	-	-	-	-
Happy disposition	+	+	+	+	+
Digital abnormalities	+	+	-	-	-
Other phenotypic characteristics	Pectus carinarum, hyperlordosis, mild syndactyly	Vesicoureteral reflux, clinodactyly	Incomplete myelination of white matter, hyperlaxity	Hyperreflexia lower limbs, clumsy gait	Hypermobile hands

Previous publications on overlapping deletions are by Tzschach et al[11] and Writzl et al,[12] whereas a near identical deletion was reported by Rocca et al.[9]

Furthermore, most patients with 2p11.2p12 deletion exhibit feet deformities (Table 1), which is also described in patients with REEP1 variants/SPG31.[13]

Candidate genes for the intellectual disability phenotype are *LRRTM1* and *CTNNA2*. The LRRTM1 protein is part of a trans-synaptic adhesion network involved in axon guidance, synapse formation, synaptic strength regulation and stabilisation of neuronal circuits. LRRTMs have been implicated in neuropsychiatric disorders such as autism and schizophrenia.[15–17] CTNNA2 is a neuronal-specific αN-catenin expressed in the prefrontal cortex. It functions in stabilising synaptic contacts and is a schizophrenia candidate gene.[18–20] Moreover, mouse *CTNNA2* knock-outs show defective neuron migration and nuclear organisation.[21]

Recurrent deletion 2p11.2-2p12 is exceedingly rare, given the very limited literature on this aberration to date.[9] Its scarcity may be related to the very large intervening segment that is flanked by the LCRs (Figure 2) as the physical proximity of LCRs may be positively related to the chance of non-allelic synapsis in meiosis.[22,23] The percentage identity of the two misaligning LCRs determines NAHR chance and for the 2p11.2 and 2p12 breakpoint-flanking LCRs this is extremely high (>99% identity). However, the size of the segmentally duplicated LCR sequences is just over 20 kbp in total, which is comparable to LCRs flanking other rare microdeletion syndromes, for example, that at 17q23.1q23.2. However, this is much smaller than the size of LCRs mediating more frequent microdeletion syndromes such as the 22q11 DiGeorge deletion.[22,24] A 2p11.2p12 duplication

reciprocal to the deletion is expectable, in similarity with other opposite microdeletions/microduplications.[4,22] Still, 2p11.2-2p12 duplication has not been identified to date in literature or ISCA or DECIPHER databases. Like the deletion presented here, it may be extremely rare.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Carvill GL, Mefford HC: Microdeletion syndromes. *Curr Opin Genet Dev* 2013; **23**: 232–239.

2 Mefford HC, Eichler EE: Duplication hotspots, rare genomic disorders, and common disease. *Curr Opin Genet Dev* 2009; **19**: 196–204.

3 Cheung VG, Nowak N, Jang W *et al*: BAC Resource Consortium. Integration of cytogenetic landmarks into the draft sequence of the human genome. *Nature* 2001; **409**: 953–958.

4 Shaw CJ, Lupski JR: Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease. *Hum Mol Genet* 2004; **13**:Spec No 1 R57–R64.

5 Mefford HC: Genotype to phenotype-discovery and characterization of novel genomic disorders in a "genotype-first" era. *Genet Med* 2009; **11**: 836–842.

6 Cooper GM, Coe BP, Girirajan S *et al*: A copy number variation morbidity map of developmental delay. *Nat Genet* 2011; **43**: 838–846.

7 den Dunnen JT, Antonarakis SE: Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000; **15**: 7–12.

8 She X, Jiang Z, Clark RA *et al*: Shotgun sequence assembly and recent segmental duplications within the human genome. *Nature* 2004; **431**: 927–930.

9 Rocca MS, Fabretto A, Faletra F *et al*: Contribution of SNP arrays in diagnosis of deletion 2p11.2-p12. *Gene* 2012; **492**: 315–318.

10 Tzschach A, Graul-Neumann LM, Konrat K *et al*: Interstitial deletion 2p11.2-p12: report of a patient with mental retardation and review of the literature. *Am J Med Genet A* 2009; **149A**: 242–245.

11 Writzl K, Lovrecić L, Peterlin B: Interstitial deletion 2p11.2-p12: further delineation. *Am J Med Genet A* 2009; **149A**: 2324–2326.

12 Barber JC, Thomas NS, Collinson MN *et al*: Segmental haplosufficiency: transmitted deletions of 2p12 include a pancreatic regeneration gene cluster and have no apparent phenotypic consequences. *Eur J Hum Genet* 2005; **13**: 283–291.

13 de Bot ST, van de Warrenburg BP, Kremer HP, Willemsen MA: Child neurology: hereditary spastic paraplegia in children. *Neurology* 2010; **75**: e75–e79.

14 Beetz C, Schüle R, Deconinck T *et al*: REEP1 mutation spectrum and genotype/phenotype correlation in hereditary spastic paraplegia type 31. *Brain* 2008; **131**: 1078–1086.

15 Soler-Llavina GJ, Arstikaitis P, Morishita W, Ahmad M, Südhof TC, Malenka RC: Leucine-rich repeat transmembrane proteins are essential for maintenance of long-term potentiation. *Neuron* 2013; **79**: 439–446.

16 Soler-Llavina GJ, Fuccillo MV, Ko J, Südhof TC, Malenka RC: The neurexin ligands, neuroligins and leucine-rich repeat transmembrane proteins, perform convergent and divergent synaptic functions in vivo. *Proc Natl Acad Sci USA* 2011; **108**: 16502–16509.

17 Grabrucker S, Proepper C, Mangus K *et al*: The PSD protein ProSAP2/Shank3 displays synapto-nuclear shuttling which is deregulated in a schizophrenia-associated mutation. *Exp Neurol* 2014; **253**: 126–137.

18 Smith A, Bourdeau I, Wang J, Bondy CA: Expression of Catenin family members CTNNA1, CTNNA2, CTNNB1 and JUP in the primate prefrontal cortex and hippocampus. *Brain Res Mol Brain Res* 2005; **135**: 225–231.

19 Chu TT, Liu Y: An integrated genomic analysis of gene-function correlation on schizophrenia susceptibility genes. *J Hum Genet* 2010; **55**: 285–292.

20 Abe K, Chisaka O, van Roy F, Takeichi M: Stability of dendritic spines and synaptic contacts is controlled by αN-catenin. *Nat Neurosci* 2004; **7**: 357–363.

21 Uemura M, Takeichi M: αN-Catenin deficiency causes defects in axon migration and nuclear organization in restricted regions of the mouse brain. *Dev Dynamics* 2006; **235**: 2559–2566.

22 Stankiewicz P, Lupski JR: Genomic architecture, rearrangements and genomic disorders. *Trend Genet* 2002; **18**: 74–82.

23 Dittwald P, Gambin T, Gonzaga-Jauregui C *et al*: Inverted low-copy repeats and genome instability—a genome-wide analysis. *Hum Mutat* 2013; **34**: 210–220.

24 Ballif BC, Theisen A, Rosenfeld JA *et al*: Identification of a recurrent microdeletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. *Am J Hum Genet* 2010; **86**: 454–461.