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17q21.31 microduplication patients are characterized by behavioural problems and poor social interaction.

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ABSTRACT.

Background : Microdeletions at 17q21.31 have recently been shown to cause a novel syndrome. Here we identify the reciprocal 17q21.31 duplication syndrome in four patients.

Method : Patients with the 17q21.31 duplication were identified by screening a large cohort of patients (n=13070) with mental retardation and congenital malformation by CGH microarray. Parental origin was investigated in three patients by quantitative PCR and microsatellite genotyping.

Results : In three cases it was possible to show that duplication arose *de novo*. Intellectual skills range from normal to mild mental retardation. Patients are characterized by poor social interaction, with relationship difficulties, reminiscent of autistic spectrum disorders. Other features are rather variable with no striking common phenotypic features. Parental origin was investigated for 3 patients. In all cases duplication was of maternal origin either through interchromosomal (2 cases) or interchromatid (1 case) rearrangement. The three mothers are all carriers of the inverted H2 haplotype emphasizing the role of local genomic architecture alteration as a predisposing factor for this duplication.

Conclusion : Autistic features observed in our patients suggest that genes in the duplicated interval should be considered as candidates for disorders in the autistic spectrum. Other phenotypic observations are rather variable or aspecific. This adds 17q21.31 duplications to a growing group of recently identified genomic disorders with variable penetrance and expressivity.

INTRODUCTION.

The widespread use of CGH microarray has resulted in the identification of many new genomic disorders in patients with mental retardation with, or without, multiple congenital anomalies (MR/MCA) due to recurrent microdeletions. Many of these new syndromes including the 17q21.31 microdeletion were recently reviewed by Slavotinek *et al.* [1]. The 17q21.31 microdeletion, with a critical region of 424 kb in size, was the first to be identified by screening large cohorts of patients by CGH microarrays even prior to the detailed characterization of the associated clinical features. The clinical picture was recently documented for 22 patients. It included developmental delay, hypotonia, facial dysmorphism and usually a friendly and amiable disposition [2]. The microdeletion encompasses 6 genes : 2 ORF and 4 known genes (MAPT, IMP5, CRHR1 and STH). Three of these are good candidates to explain the neurological disturbance observed in these patients : MAPT (OMIM 157150), IMP5 (OMIM 608284) and CRHR1 (OMIM 122561). The microdeletion breakpoints were recurrently mapped within a low copy repeat [2-6]. Even if there is no formal proof that the underlying mechanism is non allelic homologous recombination (NAHR), it seems reasonable to postulate that NAHR between two directly oriented repeats is actually involved. Moreover, an inversion within 17q21.31, present in the Western European population at a frequency of 20% [7], was shown to be closely associated with this rearrangement. Inverted haplotypes (H2) differ notably from normal haplotypes (H1) by the orientation of critical repeat sequences. Such an orientation allows NAHR to occur either between H1 and H2 or between two H2 haplotypes.

The frequency of this microdeletion syndrome was estimated to be around 1 in 16000 live births [2]. Given the mechanisms involved, the reciprocal duplication is expected to occur in a ratio of roughly 1 duplication for 2 deletions [8]. However, to our knowledge only one patient with a 17q21.31 microduplication has been reported to date [9].

Here we report four additional patients with a 17q21.31 microduplication with extensive molecular and clinical data.

MATERIAL AND METHODS.

Patient clinical data.

Patient 1 (Figure 1 and Table 1).

This girl is the first child of unrelated parents. She was born at term after a pregnancy complicated by placental detachment in the first month of gestation. The birth weight was 3.570 kg (P50). She started to walk at 12 months but with a tendency to tiptoe-walking resulting in tendinous retraction. Language onset was normal but she developed difficulties in sentence formation and pronunciation which persist at 6 years of age. She was referred for genetic consultation for behavioural problems, hyperactivity and difficulties with social interaction. The intellectual quotient (IQ) was evaluated to be 105 (WPPSI-R : Wechsler Preschool and Primary Scale of Intelligence-Revised) with a verbal IQ of 108 and performance IQ of 100. The developmental quotient was 91 for fine mobility and 73 for global mobility. Brain magnetic resonance imaging was performed with normal results.

At six years, growth parameters were in the normal range : height was at the 90th centile (126 cm) and weight at the 50th centile (23.3 kg). Microcephaly was observed with head circumference at -2SD (49 cm). Some facial dysmorphic features were noticed with synophrys, puffy eyelids, short philtrum, thin upper lip, retrognathia and dysplastic ears. Slight hirsutism was observed on the back. Clinodactyly of the fifth finger, single palmar crease on right hand and partial bilateral syndactyly between the 2^d and 3^d and 3^d and 4th toes was also noted. Hormonal blood measurements (FSH, LH, DHEA sulphate, 17-hydroxyprogesterone) were in the normal range. These patient data were submitted in DECIPHER database (<https://decipher.sanger.ac.uk/application/>) with identification number LEM249002.

Patient 2 (Table 1).

This girl is the only child of unrelated parents. The pregnancy was uncomplicated. Birth weight was 3.5 kg (P50), length 53 cm (P90) and head circumference 34.2 cm (P25-50). Meconium aspiration occurred during delivery which resulted in no apparent sequelae. Feeding and early milestones were normal. She started to walk at the age of 2 when hypotonia was noticed. At age 3 she demonstrated obsessive behaviour characterized by an adherence to ritual in performing various common tasks. She had difficulty with toilet training. She was very passive, often played by herself and did not demand affection. First clinical evaluation was carried out at age of 6. She had significant behavioural problems at school and home, poor social interaction with peers, disruptive behaviour and occasional aggressive outbursts. Her IQ was evaluated at 78 (WPPSI-R). Verbal skills and vocabulary were normal. She has normal reasoning and comprehension but she has some difficulty following instructional language. Fine motor skills, spatial awareness and non-verbal communication were evaluated as poor. She was very rule driven preferring routine tasks. At the age of 11 some dysmorphic features were noted including epicanthal folds, large posteriorly rotated ears, short upturned nose, short philtrum, flat midface, high arched palate, prominent upper incisors, low posterior hairline, thick hair, mild truncal obesity and tapering fingers.

The behavioural problems persisted with difficulties maintaining friendships and occasional inappropriate responses to other people. Fine motor skills remain poor and her obsessional behaviour is still problematic. Growth parameters are in the normal range with height and weight at the 91th centile and head circumference between the 50th and the 75th centile.

The common 17p11.2 deletion responsible for hereditary neuropathy with liability to pressure palsies (HNPP, OMIM 162500) was also identified but she is asymptomatic for this syndrome. This rearrangement was inherited from her mother. Therefore patient 2 has both an HNPP deletion and a 17q21.31 duplication with a maternal origin for both (see FISH

analysis in results section). DECIPHER identification number for this patient is CAM249012.

Patient 3 (Figure 1 and Table 1).

This boy is the second child of non-consanguineous healthy parents. His sister is healthy. The pregnancy and delivery occurred without complications and he was born at term with a birth weight of 2.890 kg (P3), length of 50 cm (P50) and head circumference of 34 cm (P10). He underwent bilateral inguinal herniorrhaphy between the ages of 10 and 12 months. He also had an orchidectomy due to torsion. His early milestones were considered normal : he could walk independently at the age of 14 to 16 months and said his first words at 12 to 14 months. He received speech therapy at the age of 3.5 years as he was socially anxious and his verbal communication was poor at nursery school. He also began tiptoe-walking and physiotherapy was initiated. Assessment with the WPPSI-R at the age of 4 years and 9 months indicated a total IQ of 81 (verbal IQ = 80, performance IQ = 87). His auditory memory, visuomotor interaction and word finding capacities were poor. Due to his socio-communicative difficulties, certain elements of the autism spectrum disorder (ASD) were recognised in him. Investigations showed a normal hearing test, normal magnetic resonance imaging of the brain and normal electromyogram. Clinical examination at the age of 7 years and 4 months revealed height of 127 cm (50th centile), weight of 25.3kg (50th centile) and head circumference of 50.7cm (10th centile). He has brachycephaly, an open square facies with a pointed chin. A congenital hypopigmented 3x3 cm mark was found on his left flank and there was no hirsutism. He has normal symmetric reflexes and shortened gastrocnemius muscles (right more than left). DECIPHER identification number for this patient is CHG249083.

Patient 4 (Table 1).

This man was born after an uncomplicated pregnancy by caesarean section. He is the second child of unrelated healthy parents. Birth weight was 5.1 kg (>97th centile) and length 58 cm (>97th centile). Mild hypotonia and nasal congestion was noted but feeding was normal. An inguinal hernia was repaired at age 6 months. All major developmental milestones were delayed. He walked at 27 months and speech was significantly delayed. A muscle biopsy performed for hypotonia was reported as normal. A diagnosis of Asperger's Syndrome was made by a clinical psychologist at age 11 years. The history also included a diagnosis of depression and attention deficit hyperactivity disorder for which he receives long-term medication. He required educational support in all subjects and a formal IQ was in the low-normal range. He was also described as a loner and established no close friendships. Eye contact and social interaction were poor. His behaviour was occasionally complicated by rages but these were not associated with self-harm. His height and weight were noted to be <3rd centile but no cause was identified. Puberty was delayed. LH/FSH and testosterone levels were all reported as low. A brain MRI was also reported as normal. At age 18, height and head circumference were between 10th -25th centiles and weight remained <3rd centile. He was dysmorphic with thin arched eyebrows, deep-set eyes and down slanting palpebral fissures. Mild malar hypoplasia was also noted. The ears were simple and elongated (>+2SD). Mild pectus excavatum, 5th finger clinodactyly, 2-3 toe syndactyly and a sandal gap were also present. Secondary sexual characteristics were absent and he had a slender body habitus with a generalised lack of body fat. The remainder of the physical examination was normal. DECIPHER identification number for this patient is LEM249014.

Microarray analysis

Patients' DNA were analysed with 4 different CGH technologies. For patient 1, CGH microarray analysis was performed using a 44K oligonucleotide microarray (Agilent, Santa Clara, CA, USA). Patient 2 was analysed using a 250K Affymetrix Nsp SNP array and Nexus software. Patient 3 was investigated using a 1 Mb BAC array as previously reported [10] and confirmed using a whole genome tiling-path array. Patient 4 was studied using the Signature Genomics BAC/PAC array.

Parental origin of the duplication

Quantitative PCR analysis.

Quantitative PCR was used to confirm the rearrangement. Primer design and real-time PCR amplification were performed as described by Hoebeeck *et al.* [11]. ABgene SYBRgreen mastermix was used according to the manufacturers (ABgene, Epsom, UK) recommendations with 250 nM final primers concentration and 10 ng of DNA in a volume of 25 µl. Ct values obtained from PCR amplification of exon 1 of MAPT (GCCGTCTTCCGCCAAGAG, GGAGCCGATCTTGGACTTGACATT) were normalized by two reference genes (primers for GPR15 : GGTCCCTGGTGGCCTTAATT and TTGCTGGTAATGGGCACACA and primers for P53 : CCCAAGCAATGGATGATTTGA and GAGCTTCATCTGGACCTGGGT).

Microsatellite and haplotype analysis.

Microsatellites were used to determine the parental origin of the duplication. Primers are reported in supplementary Table 1. Two microsatellites (D17S810 and DG17S920) located outside the duplication were genotyped as well as 3 microsatellites (VNTR17_1, VNTR17_4 and D17GS142) inside the duplication. PCR were performed using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) in semi-quantitative conditions by running only 25 cycles of amplification to allow semi-quantitative analysis of number of inherited alleles based on the analysis of relative height of alleles observed on the electrophoretic data.

The H2 specific 238 bp deletion [12] was genotyped using primers GGAAGACGTTCTCACTGATCTG and AGGAGTCTGGCTTCAGTCTCTC, followed by electrophoresis on a 1% agarose gel.

FISH analysis.

BAC probes were used to confirm the rearrangement observed in patient 2. BAC RP11-64B12 at PMP22 locus (17p12) was used in conjunction with RP11-100C5 and RP11-413P22 at the 17q21.31 microduplication locus.

1

2 Table 1 : List of clinical observations reported for 5 patients with 17q21.31 duplication.

Clinical observation	Kirchhoff <i>et al.</i> , 2007	Patient 1 (female)	Patient 2 (female)	Patient 3 (male)	Patient 4 (male)	Frequency
Pregnancy	Uneventful 42 weeks	Placental detachment At term	Normal At term	Normal At term	Normal At term	
Birth						
Weight (kg)	3.070	3.570 (P50)	3.500 (P50)	2.890 (-2SD)	5.100 (+3SD)	
Length (cm)	50	ND	53 (P90)	50 (P50)	58 (+3SD)	
OFC (cm)	34	ND	34.2 (P25-50)	34 (P10)	ND	
Walk (month)	60	12	24	14-16	27	
Psychomotor development						
Hypotonia	+	-	+	-	+	3/5
Hyperactivity	+	+	-	ND	+	3/4
Passivity	ND	ND	+	ND	ND	
Obsessive behaviour	ND	ND	+	ND	-	1/2
Social interaction	ND	Poor	Poor	Poor, anxious	Poor	4/4
Aggressiveness (without self injury)	ND	ND	+	ND	+	2/2
Intelligence						
Psychomotor retardation	Severe	Mild	Mild	Mild	Mild	5/5
IQ	ND	105	78	81	ND	
Verbal skills	Very Poor	Limited	Normal	Poor	Normal	3/5
Motor skills	ND	Poor	Poor	ND	Normal	2/3
Tiptoe-walking	ND	+	-	+	-	2/4
Dysmorphism						
Microcephaly	+	Mild	-	-	-	1-2/5
Synophrys	ND	+	Bushy eyebrows	-	-	2/4
Epicanthal folds	ND	-	+	-	-	1/4
Dysplastic ears	+	+	+	-	+	4/5
Nose	Short	Upturned	Short, Upturned	-	-	2-3/5
Philtrum	Smooth	Short	Short	-	-	2-3/5
Midface	ND	-	Flat	-	Flat	2/4
Palate	High arched	-	High arched	-	-	2/5
Prominent incisor	+	+	+	-	-	3/5
Micrognathia	+	ND	-	ND	-	1/3
Retrognathia	ND	+	ND	ND	-	1/2
Finger	Broad	Clinodactyly of 5 th	Tapering	ND	Clinodactyly 5 th	2/4
Palmar crease	ND	Single on right hand	-	ND	ND	1/2
Feet	Broad	Partial syndactyly	-	ND	2-3 syndactyly	2/3
Others						
Hirsutism	On the back	On the back	low post-hairline	-	-	2-3/5
Failure to thrive	At 7 months	-	-	-	ND	
Cysts on liver & spleen	+	ND	ND	ND	ND	
Hypogonadism	ND	ND	ND	ND	+	

3 ND : not determined : no data reported in the clinical file.

4 “+” : Trait present in the patient.

5 “-” : Trait not observed in the patient.

6

7 **RESULTS.**8 **Microarray results.**

9 The size of the duplication in the three patients investigated with non-targeted array (patient
10 1-3) ranged from 585 kb to 763 kb. An overview of the breakpoints is given in Figure 2
11 where the duplications are aligned with the recurrent deletion [2] and CNV of region
12 17q21.31. Exact breakpoint positions are listed as a supplementary Table 2. The difference
13 in the breakpoints likely reflects the different resolution of the four array types used.

14

15 **Parental origin of the duplication.**16 **Quantitative PCR analysis.**

17 Quantitative PCR was performed on patient and parental DNA to determine the origin of the
18 duplication using a PCR system developed in exon 11 of MAPT. In patients 1 to 3 the
19 duplication appeared de novo (data not shown). *De novo* occurrence in patient 4 could not be
20 determined due to lack of parental DNA.

21

Microsatellite and haplotype analysis

To investigate the origin of the duplication, the probands and their parents were genotyped for 5 microsatellites which were phased in order to identify transmitted haplotypes (see supplementary Table 1). In all cases the duplication occurred on the maternal chromosome. Detailed analysis of genotypic data shows that the duplication occurred by interchromosomal rearrangement (probably NAHR) between H1 and H2 haplotypes in patient 1 and 2 whereas it occurs by interchromatid rearrangement (probably NAHR) between two H2 haplotypes in patient 3. An illustration of electrophoretic data obtained from a capillary sequencer, as well as detailed interpretation of these microsatellite data, is given in supplementary Figure 1. An H2 specific deletion of 238 bp was also genotyped by PCR (supplementary Table 2 and supplementary Figure 2). Patient 4 could not be tested for parental origin because parental DNAs were unavailable.

FISH analysis

Patient 2 was investigated by FISH given the presence of two rearrangements on chromosome 17. Co-hybridization of HNPP locus specific probes (RP11-64B12) and 17q21.31 specific probes (RP11-100C5 and RP11-413P22) demonstrated that the 17q21.31 duplication occurs on the same chromosome as the HNPP microdeletion (data not shown).

DISCUSSION.

The 17q21.31 microduplication described here is a novel syndrome characterized by features of the autism spectrum disorder. All patients have behavioural problems with social interaction and communication difficulties and mild psychomotor retardation suggesting that some of the genes within the duplication interval may be candidates for the autistic spectrum, even if at this stage this hypothesis requires formal testing. A linkage study of autistic families [13] has identified strong linkage within 17q21. However this association was not confirmed by a larger linkage study reported by the Autism Consortium [14] nor in the CNV (copy number variant) analysis of autistic families reported by Sebat *et al.* [15]. The duplicated interval contains 6 genes (2 ORFs and 4 known genes). Some of these are good candidates for the impaired cognitive function or motor skills observed in our patients. Some mutations of MAPT (microtubule associated protein TAU) altering the biochemistry of tau protein have been reported to cause a greater abundance of unbound tau species and formation of insoluble tau inclusions in frontotemporal dementia and parkinsonism (reviewed in Pittman *et al.* [16]) which could therefore be considered as a “toxic gain of function mutation”. Increased accumulation could be expected from dosage sensitive overexpression of the duplicated MAPT gene observed in our patients. However MAPT gene duplications have not been identified in cases of frontotemporal lobar degeneration [17]. Patients 1, 2 and 4 have had a brain scan with no sign of neurodegeneration. However it should be mentioned that our patients are between 6 and 20 whereas the first signs of degeneration in frontotemporal dementia usually becomes apparent around 45-55 years of age [16]. Then a follow up of these patients for this particular aspect is certainly advisable to determine their evolution with age. CRHR1 (Corticotropin Releasing Hormone Receptor 1) is expressed in the embryonic and postnatal cerebellum and could play a role in central nervous system development by affecting neurone survival through a depolarization mechanism [18]. Duplication of the gene coding for CRHR1 could explain the impaired motor skills reported for at least two patients (patient 1 and 2). Moreover experimental evidence suggests that CRHR1 is involved in stress response and anxiety related behaviour [19]. CRHR1 is one of the preferred targets for depression, anxiety and stress related therapy [20]. Again duplication of this gene could contribute to the behavioural troubles and poor social interaction observed in 4 of the 5 patients reported with 17q21.31 microduplication. Finally, IMP5 encodes a gene highly

1 homologous to the presenilin 1 and 2 genes, which are involved in APP (Amyloid Precursor
2 Protein) cleavage. Interaction of IMP5, or other presenilin analogues, with APP has not been
3 formally established but experimental evidence suggests that protease others than presenilin 1
4 and 2 are involved in APP cleavage [21, 22] since APP cleavage still occurs in presenilin 1
5 and 2 double knock-out mouse fibroblasts.

6 The other phenotypic aspects are very variable and much milder. Other common features
7 displayed by these four cases and by the patient reported by Kirchhoff *et al.* [14] include :
8 dysplastic ears (4/5), hypotonia (3/5), hyperactivity (3/4), poor verbal skills (3/5), prominent
9 incisors (3/5), poor motor skills (2/3), tiptoe-walking (2/4) and short nose (2/5). More cases
10 are required to accurately define the clinical spectrum associated with this microduplication as
11 many features described are mild and non-specific. Patient 2 was noted to share a number of
12 dysmorphic features with the case described by Kirchhoff including hypotonia, poor motor
13 skills, dysplastic ears, short nose, short philtrum, high-arched palate, prominent incisors and
14 hirsutism. The role of the duplication in the aetiology of the hypogonadism in case 4 is
15 unclear and will await identification of further cases. However it suggests that all cases of
16 17q21.31 microduplication should be assessed for this potential complication. A patient
17 identified by Hunter Genetics in Australia has been recently reported in the DECIPHER
18 database (<https://decipher.sanger.ac.uk/application/>). This patient was reported to have
19 mental retardation, abnormal shaped teeth and tapering fingers.

20 No phenotypic similarities or differences between deletion and duplication carriers are
21 obvious. Deletion carrier seems to be more affected (moderate to severe mental retardation)
22 than duplication carriers (mild mental retardation in our 4 patients). It's also interesting to
23 note that, whereas deletion carriers have a friendly and amiable behaviour, duplication
24 carriers have on contrary some traits reminiscent of the autistic spectrum. This opposite
25 phenotypic situation should certainly be kept in mind in the future when additional patients
26 are clinically assessed.

27 The phenotypic variability is striking and adds this syndrome to a growing group of
28 chromosomal imbalances with variable expressivity. Such variability is observed especially
29 in association with microduplication genomic disorders : duplications of the SMS [23],
30 Williams-Beuren [24], 22q11 [25] and NF1 [26] loci result in a heterogeneous phenotype
31 ranging from normal to severe. Distal deletion and duplication of the 22q11 locus has been
32 reported in patients with highly variable symptoms especially for the duplication carriers [25,
33 27]. Another recently identified microdeletion/duplication genomic disorder of 1.35 Mb at
34 1q21.1 also escapes syndromic classification due to large phenotypic variability such that
35 identification of carriers probably precludes a phenotypic-based evaluation [28].

36 The broad variability of phenotypes associated with specific chromosomal rearrangements
37 suggests that many mildly affected individuals remain undiagnosed. This generates an
38 ascertainment bias in the evaluation of the frequency of such rearrangements. The cohort
39 investigated here was made of 13070 patients. A total of 24 deletion carriers were observed.
40 Based on a frequency 2% for individuals presenting mental retardation in the human
41 population, the prevalence of 17q21.31 deletion could be evaluated to 1 in 55000 in our
42 cohort, as explained in Koolen *et al.* [2], which is lower than the value of 1 in 16000 reported
43 by Koolen *et al.* [2]. This certainly reflects a bias in the selection of patients. Similarly the
44 prevalence of duplication can be evaluated to 1 in 327000 which is less than half the
45 prevalence of deletion which would be mechanistically expected from the model proposed by
46 Turner *et al.* [8] for deletion and duplication resulting from NAHR.

47 Koolen *et al.* [2] were able to map the proximal breakpoint to a 500 bp interval (position
48 41046729-41047168 bp on chromosome 17) corresponding to a L2 LINE element. This is
49 comparable to the proximal breakpoints in the four patients with the microduplication.
50 However, as mentioned by Koolen *et al.* [2], the distal breakpoint falls within a CNV region,

known to be polymorphic in normal individuals. Interestingly, each patient with the duplication investigated with non-targeted arrays (Patients 1-3 and Kirchhoff's patient) show an amplified copy number for this CNV indicating either that one of the parents is a carrier of a multi-copy allele for this CNV or that the duplication involves a more distal breakpoint. In three of four cases reported here, the microduplication could be demonstrated to be of maternal origin whereas a paternal origin was established for the first patient described by Kirchhoff *et al.* [9]. In the three cases tested, the parent of origin was either heterozygote or homozygote for the H2 haplotype. In patient 3 the duplication arose through interchromatid rearrangement whereas, for the 2 others, an interchromosomal mechanism was discovered. This again emphasizes the role of the inverted H2 haplotype in this microdeletion / microduplication syndrome as recently investigated in detail in the human and great apes lineage [29]. In conclusion, we report four additional cases with 17q21.31 microduplication (for 3 of which *de novo* occurrence could be assessed) which contribute to documenting the molecular and clinical features associated with this recently discovered syndrome. Whilst the microdeletion associated phenotype is now clearly established, the microduplication is associated with a very variable phenotype, of which behavioural problems and poor social interaction seem to be the most consistent.

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Competing interests : J Rosenfeld is employee of Signature Genomic.

Patient consents : Obtained.

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Figure legend

Figure 1 : Pictures of patient 1 (a) and patient 3 (b). Consents to publish these pictures were obtained from the parents of the patients.

Figure 2 : Schematic representation of genomic duplication in Kirschhoff's patient [9] and in the 4 patients presented here. Array used in each cases is noticed at the right. Duplicated genomic regions are showed as white rectangle. A thin horizontal line on both side illustrate the break point regions. The exact distal position of the duplication was not available in Kirschhoff's paper (thin dotted line). The regions of overlap of normal and duplicated BAC in patient 3 are shown as a thin horizontal line even if these regions do not actually correspond to breakpoints regions. The minimal recurrent deletion reported by Koolen *et al.* [2] is depicted as a shaded rectangle. Dotted rectangles illustrates known CNV. Positions of the 4 genes mentioned in the discussion are depicted as black rectangles. The thin vertical line localize the position of the recurrent proximal breakpoint [2].



a



b

