

1 **17q21.31 microduplication patients are characterized by behavioural**
2 **problems and poor social interaction.**

3
4 Bernard Grisart^{1,*§}, Lionel Willatt^{2,*}, Anne Destrée¹, Jean-Pierre Fryns³, Katrina Rack¹,
5 Thomy de Ravel³, Jill Rosenfeld⁴, Joris Robert Vermeesch³, Christine Verellen-Dumoulin¹,
6 Richard Sandford².

7
8 ¹ Centre de Génétique Humaine, Institut de Pathologie et de Génétique, 25 Avenue Georges
9 Lemaître, B-6041 Charleroi, Belgium.

10 ² East Anglian Medical Genetics Service, Cytogenetics Laboratory, Box 108, Level 6,
11 Addenbrooke's Treatment Centre, Addenbrooke's Hospital, Cambridge CB2 0QQ, United
12 Kingdom.

13 ³ Center for Human Genetics, University Hospitals Leuven, Herestraat 49, 3000 Leuven,
14 Belgium.

15 ⁴ Signature Genomic Laboratories, LLC, 2820 North Astor Street, Spokane, WA 99207 USA.

16 * Both authors equally contributed to this work.

17 § Corresponding author : Phone number ##/32/71/447185, Fax Number : ##/32/71/471520, E-
18 mail : bernard.grisart@ipg.be.

19
20 **KEYWORDS :** 17q21.31 microduplication, MAPT, CRHR1, autism, behaviour.

21
22 Word count : 3434.

1
2 **ABSTRACT.**

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

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

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

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

22
23 **INTRODUCTION.**

24 The widespread use of CGH microarray has resulted in the identification of many new
25 genomic disorders in patients with mental retardation with, or without, multiple congenital
26 anomalies (MR/MCA) due to recurrent microdeletions. Many of these new syndromes
27 including the 17q21.31 microdeletion were recently reviewed by Slavotinek *et al.* [1]. The
28 17q21.31 microdeletion, with a critical region of 424 kb in size, was the first to be identified
29 by screening large cohorts of patients by CGH microarrays even prior to the detailed
30 characterization of the associated clinical features. The clinical picture was recently
31 documented for 22 patients. It included developmental delay, hypotonia, facial dysmorphism
32 and usually a friendly and amiable disposition [2]. The microdeletion encompasses 6 genes :
33 2 ORF and 4 known genes (MAPT, IMP5, CRHR1 and STH). Three of these are good
34 candidates to explain the neurological disturbance observed in these patients : MAPT (OMIM
35 157150), IMP5 (OMIM 608284) and CRHR1 (OMIM 122561). The microdeletion
36 breakpoints were recurrently mapped within a low copy repeat [2-6]. Even if there is no
37 formal proof that the underlying mechanism is non allelic homologous recombination
38 (NAHR), it seems reasonable to postulate that NAHR between two directly oriented repeats is
39 actually involved. Moreover, an inversion within 17q21.31, present in the Western European
40 population at a frequency of 20% [7], was shown to be closely associated with this
41 rearrangement. Inverted haplotypes (H2) differ notably from normal haplotypes (H1) by the
42 orientation of critical repeat sequences. Such an orientation allows NAHR to occur either
43 between H1 and H2 or between two H2 haplotypes.

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

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

1 **MATERIAL AND METHODS.**

2 **Patient clinical data.**

3 Patient 1 (Figure 1 and Table 1).

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

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

24
25 Patient 2 (Table 1).

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

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

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

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

3
4 Patient 3 (Figure 1 and Table 1).

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

24
25
26 Patient 4 (Table 1).

27 This man was born after an uncomplicated pregnancy by caesarean section. He is the second
28 child of unrelated healthy parents. Birth weight was 5.1 kg (>97th centile) and length 58 cm
29 (>97th centile). Mild hypotonia and nasal congestion was noted but feeding was normal. An
30 inguinal hernia was repaired at age 6 months. All major developmental milestones were
31 delayed. He walked at 27 months and speech was significantly delayed. A muscle biopsy
32 performed for hypotonia was reported as normal. A diagnosis of Asperger's Syndrome was
33 made by a clinical psychologist at age 11 years. The history also included a diagnosis of
34 depression and attention deficit hyperactivity disorder for which he receives long-term
35 medication. He required educational support in all subjects and a formal IQ was in the low-
36 normal range. He was also described as a loner and established no close friendships. Eye
37 contact and social interaction were poor. His behaviour was occasionally complicated by
38 rages but these were not associated with self-harm. His height and weight were noted to be
39 <3rd centile but no cause was identified. Puberty was delayed. LH/FSH and testosterone
40 levels were all reported as low. A brain MRI was also reported as normal. At age 18, height
41 and head circumference were between 10th -25th centiles and weight remained <3rd centile.
42 He was dysmorphic with thin arched eyebrows, deep-set eyes and down slanting palpebral
43 fissures. Mild malar hypoplasia was also noted. The ears were simple and elongated
44 (>+2SD). Mild pectus excavatum, 5th finger clinodactyly, 2-3 toe syndactyly and a sandal gap
45 were also present. Secondary sexual characteristics were absent and he had a slender body
46 habitus with a generalised lack of body fat. The remainder of the physical examination was
47 normal. DECIPHER identification number for this patient is LEM249014.

48
49

1 **Microarray analysis**

2 Patients' DNA were analysed with 4 different CGH technologies. For patient 1, CGH
3 microarray analysis was performed using a 44K oligonucleotide microarray (Agilent, Santa
4 Clara, CA, USA). Patient 2 was analysed using a 250K Affymetrix Nsp SNP array and
5 Nexus software. Patient 3 was investigated using a 1 Mb BAC array as previously reported
6 [10] and confirmed using a whole genome tiling-path array. Patient 4 was studied using the
7 Signature Genomics BAC/PAC array.

8
9 **Parental origin of the duplication**

10 **Quantitative PCR analysis.**

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

20
21 **Microsatellite and haplotype analysis.**

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

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

32
33 **FISH analysis.**

34 BAC probes were used to confirm the rearrangement observed in patient 2. BAC RP11-
35 64B12 at PMP22 locus (17p12) was used in conjunction with RP11-100C5 and RP11-413P22
36 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,

1 known to be polymorphic in normal individuals. Interestingly, each patient with the
2 duplication investigated with non-targeted arrays (Patients 1-3 and Kirchhoff's patient) show
3 an amplified copy number for this CNV indicating either that one of the parents is a carrier of
4 a multi-copy allele for this CNV or that the duplication involves a more distal breakpoint.

5 In three of four cases reported here, the microduplication could be demonstrated to be of
6 maternal origin whereas a paternal origin was established for the first patient described by
7 Kirchhoff *et al.* [9]. In the three cases tested, the parent of origin was either heterozygote or
8 homozygote for the H2 haplotype. In patient 3 the duplication arose through interchromatid
9 rearrangement whereas, for the 2 others, an interchromosomal mechanism was discovered.
10 This again emphasizes the role of the inverted H2 haplotype in this microdeletion /
11 microduplication syndrome as recently investigated in detail in the human and great apes
12 lineage [29].

13 In conclusion, we report four additional cases with 17q21.31 microduplication (for 3 of which
14 *de novo* occurrence could be assessed) which contribute to documenting the molecular and
15 clinical features associated with this recently discovered syndrome. Whilst the microdeletion
16 associated phenotype is now clearly established, the microduplication is associated with a
17 very variable phenotype, of which behavioural problems and poor social interaction seem to
18 be the most consistent.

21 **ACKNOWLEDGMENT.**

22 We would like to thank Mélanie D'Amico and Dr Pascale Hilbert for their support with
23 microsatellites analysis. This work was funded by a grant from the "Institute de Recherche
24 Scientifique en Pathologie et en Génétique" and by grants to JV from the Catholic University
25 of Leuven (GOA/2006/12 and Centre of Excellence SymbioSys, Research Council
26 K.U.Leuven EF/05/007) and the IWT (IWT 60848). We are grateful to the MicroArray
27 Facility, Flanders Interuniversity Institute for Biotechnology (VIB) for their help in the
28 spotting of the arrays and the Mapping Core and Map Finishing groups of the Wellcome Trust
29 Sanger Institute for the initial clone supply and verification. This work was also funded by a
30 Biomedical Research grant from the National Institute for Health Research (NIHR), UK.

32 **Competing interests :** J Rosenfeld is employee of Signature Genomic.

34 **Patient consents :** Obtained.

36 **Statement :** The Corresponding Author has the right to grant on behalf of all authors and
37 does grant on behalf of all authors, an exclusive licence (or non exclusive for government
38 employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article to be
39 published in Journal of Medical Genetics and any other BMJ PGL products and sublicences
40 such use and exploit all subsidiary rights, as set out in our licence
41 (<http://JMG.bmj.com/misc/ifora/licenceform.shtml>).

44 **REFERENCE.**

- 46 1 Slavotinek AM. Novel microdeletion syndromes detected by chromosome microarrays.
47 Hum Genet. 2008;**124**:1-17.
- 49 2 Koolen DA, Sharp AJ, Hurst JA, Firth HV, Knight SJL, Goldenberg A, Saugier-veber P,
50 Pfundt R, Vissers LELM, Destrée A, Grisart B, Rooms L, Van der Aa N, Field M,

- 1 Hackett A, Bell K, Nowaczyk MJM, Mancini GMS, Poddighe PJ, Schwartz CE, Rossi E,
2 De Gregori M, Antonacci-Fulton LL, McLellan II MD, Garrett JM, Wiechert MA, Miner
3 TL, Crosby S, Ciccone R, Rauch A, Zenker M, Aradhya S, Manning MA, Strom TM,
4 Wagenstaller J, Krepischi-Santos AC, Vianna-Morgante AM, Rosenberg C, Price SM,
5 Stewart H, Shaw-Smith C, Brunner HG, Wilkie AOM, Veltman JA, Zuffardi O, Eichler
6 EE, de Vries BBA. Clinical and molecular delineation of the 17q21.31 microdeletion
7 syndrome. *J Med Genet.* Published Online First: 17 July 2008.
8 doi:10.1136/jmg.2008.058701.
9
- 10 3 Koolen DA, Vissers LE, Pfundt R, de Leeuw N, Knight SJ, Regan R, Kooy RF, Reyniers E,
11 Romano C, Fichera M, Schinzel A, Baumer A, Anderlid BM, Schoumans J, Knoers NV,
12 van Kessel AG, Sistermans EA, Veltman JA, Brunner HG, de Vries BB. A new
13 chromosome 17q21.31 microdeletion syndrome associated with a common inversion
14 polymorphism. *Nat Genet* 2006;**38**:999-1001.
15
- 16 4 Sharp AJ, Hansen S, Selzer RR, Cheng Z, Regan R, Hurst JA, Stewart H, Price SM, Blair E,
17 Hennekam RC, Fitzpatrick CA, Segraves R, Richmond TA, Guiver C, Albertson DG,
18 Pinkel D, Eis PS, Schwartz S, Knight SJ, Eichler EE. Discovery of previously
19 unidentified genomic disorders from the duplication architecture of the human
20 genome. *Nat Genet* 2006;**38**:1038-1042.
21
- 22 5 Shaw-Smith C, Pittman AM, Willatt L, Martin H, Rickman L, Gribble S, Curley R,
23 Cumming S, Dunn C, Kalaitzopoulos D, Porter K, Prigmore E, Krepischi-Santos AC,
24 Varela MC, Koiffmann CP, Lees AJ, Rosenberg C, Firth HV, de Silva R, Carter NP.
25 Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with
26 developmental delay and learning disability. *Nat Genet* 2006;**3**:1032-1037.
27
- 28 6 Varela MC, Krepischi-Santos AC, Paz JA, Knijnenburg J, Szuhai K, Rosenberg C,
29 Koiffmann CP. A 17q21.31 microdeletion encompassing the MAPT gene in a mentally
30 impaired patient. *Cytogenet Genome Res* 2006;**114**:89-92.
31
- 32 7 Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, Baker
33 A, Jonasdottir A, Ingason A, Gudnadottir VG, Desnica N, Hicks A, Gylfason A,
34 Gudbjartsson DF, Jonsdottir GM, Sainz J, Agnarsson K, Birgisdottir B, Ghosh S,
35 Olafsdottir A, Cazier JB, Kristjansson K, Frigge ML, Thorgeirsson TE, Gulcher JR, Kong
36 A, Stefansson K. A common inversion under selection in Europeans. *Nat Genet*
37 2005;**37**:129-137.
38
- 39 8 Turner DJ, Miretti M, Rajan D, Fiegler H, Carter NP, Blayney ML, Beck S, Hurles ME.
40 Germline rates of de novo meiotic deletions and duplications causing several genomic
41 disorders. *Nat Genet* 2008;**40**:90-95.
42
- 43 9 Kirchhoff M, Bisgaard AM, Duno M, Hansen FJ, Schwartz M. A 17q21.31
44 microduplication, reciprocal to the newly described 17q21.31 microdeletion, in a girl with
45 severe psychomotor developmental delay and dysmorphic craniofacial features. *Eur J*
46 *Med Genet* 2007;**50**:256-263.
47
- 48 10 Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van
49 Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen
50 P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR. Emerging patterns of

- 1 cryptic chromosomal imbalance in patients with idiopathic mental retardation and
2 multiple congenital anomalies: a new series of 140 patients and review of published
3 reports. *J Med Genet* 2006;**43**:625-33.
4
- 5 11 Hoebeeck J, Speleman F, Vandesomepele J. Real-time quantitative PCR as an alternative
6 to Southern blot or fluorescence in situ hybridization for detection of gene copy number
7 changes. *Methods Mol Biol* 2007;**353**:205-226.
8
- 9 12 Baker M, Litvan I, Houlden H, Adamson J, Dickson D, Perez-Tur J, Hardy J, Lynch T,
10 Bigio E, Hutton M. Association of an extended haplotype in the tau gene with
11 progressive supranuclear palsy. *Hum Mol Genet*. 1999;**8**:711-5.
12
- 13 13 Cantor RM, Kono N, Duvall JA, Alvarez-Retuerto A, Stone JL, Alarcón M, Nelson SF,
14 Geschwind DH. Replication of autism linkage: fine-mapping peak at 17q21. *Am J Hum*
15 *Genet* 2005;**76**:1050-1056.
16
- 17 14 Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB,
18 Skaug JL, Thompson AP, Senman L, Feuk L, Qian C, Bryson SE, Jones MB, Marshall
19 CR, Scherer SW, Vieland VJ, Bartlett C, Mangin LV, Goedken R, Segre A, Pericak-
20 Vance MA, Cuccaro ML, Gilbert JR, Wright HH, Abramson RK, Betancur C, Bourgeron
21 T, Gillberg C, Leboyer M, Buxbaum JD, Davis KL, Hollander E, Silverman JM,
22 Hallmayer J, Lotspeich L, Sutcliffe JS, Haines JL, Folstein SE, Piven J, Wassink TH,
23 Sheffield V, Geschwind DH, Bucan M, Brown WT, Cantor RM, Constantino JN, Gilliam
24 TC, Herbert M, Lajonchere C, Ledbetter DH, Lese-Martin C, Miller J, Nelson S,
25 Samango-Sprouse CA, Spence S, State M, Tanzi RE, Coon H, Dawson G, Devlin B, Estes
26 A, Flodman P, Klei L, McMahon WM, Minshew N, Munson J, Korvatska E, Rodier PM,
27 Schellenberg GD, Smith M, Spence MA, Stodgell C, Tepper PG, Wijsman EM, Yu CE,
28 Rogé B, Mantoulan C, Wittmeyer K, Poustka A, Felder B, Klauck SM, Schuster C,
29 Poustka F, Bölte S, Feineis-Matthews S, Herbrecht E, Schmötzer G, Tsiantis J,
30 Papanikolaou K, Maestrini E, Bacchelli E, Blasi F, Carone S, Toma C, Van Engeland H,
31 de Jonge M, Kemner C, Koop F, Langemeijer M, Hijmans C, Staal WG, Baird G, Bolton
32 PF, Rutter ML, Weisblatt E, Green J, Aldred C, Wilkinson JA, Pickles A, Le Couteur A,
33 Berney T, McConachie H, Bailey AJ, Francis K, Honeyman G, Hutchinson A, Parr JR,
34 Wallace S, Monaco AP, Barnby G, Kobayashi K, Lamb JA, Sousa I, Sykes N, Cook EH,
35 Guter SJ, Leventhal BL, Salt J, Lord C, Corsello C, Hus V, Weeks DE, Volkmar F,
36 Tauber M, Fombonne E, Shih A, Meyer KJ. Mapping autism risk loci using genetic
37 linkage and chromosomal rearrangements. *Nat Genet* 2007;**39**:319-328.
38
- 39 15 Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S,
40 Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT,
41 Puura K, Lehtimäki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra
42 V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K,
43 Wigler M. Strong association of de novo copy number mutations with autism. *Science*
44 2007;**316**:445-449.
45
- 46 16 Pittman AM, Fung H-C, de Silva R. Untangling the tau gene association with
47 neurodegenerative disorders. *Hum Mol Genet* 2006 ;**15**:R188-R195.
48

- 1 17 Lladó A, Rodríguez-Santiago B, Antonell A, Sánchez-Valle R, Molinuevo JL, Reñé R,
2 Pérez-Jurado LA. MAPT gene duplications are not a cause of frontotemporal lobar
3 degeneration. *Neurosci Lett* 2007;**424**:61-65.
4
- 5 18 King JS, Bishop GA. Localization of the type 1 corticotropin releasing factor receptor
6 (CRF-R1) in the embryonic mouse cerebellum. *J Neurocytol* 2003;**32**:305-316.
7
- 8 19 Hauger RL, Risbrough V, Brauns O, Dautzenberg FM. Corticotropin Releasing Factor
9 (CRF) Receptor Signaling in the Central Nervous System: New Molecular Targets. *CNS*
10 *Neurol Disord Drug Targets* 2006;**5**:453-479.
11
- 12 20 Kehne JH. The CRF1 receptor, a novel target for the treatment of depression, anxiety, and
13 stress-related disorders. *CNS Neurol Disord Drug Targets*. 2007;**6**:163-182.
14
- 15 21 Armogida M, Petit A, Vincent B, Scarzello S, Alves da Costa C, Checler F. Endogenous -
16 amyloid production in presenilin-deficient embryonic mouse fibroblasts. *Nat Cell Biol*
17 2001;**3**:1030-1033.
18
- 19 22 Grigorenko AP, Moliaka YK, Korovaitseva GI, Rogaev EI. Novel Class of Polytopic
20 Proteins with Domains Associated with Putative Protease Activity. *Biochemistry*
21 (Moscow) 2002;**67**:826-834.
22
- 23 23 Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, Summers AM,
24 Meschino WS, Anyane-Yeboah K, Kashork CD, Shaffer LG, Lupski JR. Molecular
25 mechanism for duplication 17p11.2-the homologous recombination reciprocal of the
26 Smith-Magenis microdeletion. *Nat Genet* 2000;**24**:84-87.
27
- 28 24 Kriek M, White SJ, Szuhai K, Knijnenburg J, van Ommen GJ, den Dunnen JT, Breuning
29 MH. Copy number variation in regions flanked (or unflanked) by duplicons among
30 patients with developmental delay and/or congenital malformation; detection of reciprocal
31 and partial Williams-Beuren duplication. *Eur J Hum Genet* 2006;**14**:180-189.
32
- 33 25 Ou Z, Berg JS, Yonath H, Enciso VB, Miller DT, Picker J, Lenzi T, Keegan CE, Sutton
34 VR, Belmont J, Chinault AC, Lupski JR, Cheung SW, Roeder E, Patel A.
35 Microduplications of 22q11.2 are frequently inherited and are associated with variable
36 phenotypes. *Genet Med* 2008;**10**:267-277.
37
- 38 26 Grisart B, Rack K, Vidrequin S, Hilbert P, Deltenre P, Verellen-Dumoulin C, Destrée A.
39 NF1 microduplication first clinical report: association with mild mental retardation, early
40 onset of baldness and dental enamel hypoplasia? *Eur J Hum Genet* 2008;**16**:305-311.
41
- 42 27 Ben-Shachar S, Ou Z, Shaw CA, Belmont JW, Patel MS, Hummel M, Amato S, Tartaglia
43 N, Berg J, Sutton VR, Lalani SR, Chinault AC, Cheung SW, Lupski JR, Patel A. 22q11.2
44 distal deletion: a recurrent genomic disorder distinct from DiGeorge syndrome and
45 velocardiofacial syndrome. *Am J Hum Genet* 2008;**82**:214-221.
46
- 47 28 Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, Buysse K, Huang S, Maloney VK,
48 Crolla JA, Baralle D, Collins A, Mercer C, Norga K, de Ravel T, Devriendt K, Bongers
49 EM, de Leeuw N, Reardon W, Gimelli S, Bena F, Hennekam RC, Male A, Gaunt L,
50 Clayton-Smith J, Simoncic I, Park SM, Mehta SG, Nik-Zainal S, Woods CG, Firth HV,

- 1 Parkin G, Fichera M, Reitano S, Lo Giudice M, Li KE, Casuga I, Broomer A, Conrad B,
2 Schwerzmann M, Räber L, Gallati S, Striano P, Coppola A, Tolmie JL, Tobias ES, Lilley
3 C, Armengol L, Spyschaert Y, Verloo P, De Coene A, Goossens L, Mortier G, Speleman
4 F, van Binsbergen E, Nelen MR, Hochstenbach R, Poot M, Gallagher L, Gill M,
5 McClellan J, King MC, Regan R, Skinner C, Stevenson RE, Antonarakis SE, Chen C,
6 Estivill X, Menten B, Gimelli G, Gribble S, Schwartz S, Sutcliffe JS, Walsh T, Knight SJ,
7 Sebat J, Romano C, Schwartz CE, Veltman JA, de Vries BB, Vermeesch JR, Barber JC,
8 Willatt L, Tassabehji M, Eichler EE. Recurrent rearrangements of chromosome 1q21.1
9 and variable pediatric phenotypes. *N Engl J Med.* 2008;**359**:1685-1699.
10
- 11 29 Zody MC, Jiang Z, Fung HC, Antonacci F, Hillier LW, Cardone MF, Graves TA, Kidd
12 JM, Cheng Z, Abouelleil A, Chen L, Wallis J, Glasscock J, Wilson RK, Reily AD,
13 Duckworth J, Ventura M, Hardy J, Warren WC, Eichler EE. Evolutionary toggling of the
14 MAPT 17q21.31 inversion region. *Nat Genet* 2008;**40**:1076-1083.
15

1 **Figure legend**

2
3 Figure 1 : Pictures of patient 1 (a) and patient 3 (b). Consents to publish these pictures were
4 obtained from the parents of the patients.

5
6 Figure 2 : Schematic representation of genomic duplication in Kirschhoff's patient [9] and in
7 the 4 patients presented here. Array used in each cases is noticed at the right.
8 Duplicated genomic regions are showed as white rectangle. A thin horizontal line
9 on both side illustrate the break point regions. The exact distal position of the
10 duplication was not available in Kirschhoff's paper (thin dotted line). The regions
11 of overlap of normal and duplicated BAC in patient 3 are shown as a thin
12 horizontal line even if these regions do not actually correspond to breakpoints
13 regions. The minimal recurrent deletion reported by Koolen *et al.* [2] is depicted
14 as a shaded rectangle. Dotted rectangles illustrates known CNV. Positions of the
15 4 genes mentioned in the discussion are depicted as black rectangles. The thin
16 vertical line localize the position of the recurrent proximal breakpoint [2].



a



b

