RESEARCH ARTICLE

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Complex Craniosynostosis Is Associated With the 2p15p16.1 Microdeletion Syndrome

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In a screening project of patients with (complex) craniosynostosis using genomic arrays, we identified two patients with craniosynostosis and microcephaly with a deletion in the 2p15p16.1 chromosomal region. This region has been associated with a new microdeletion syndrome, for which patients have various features in common, including microcephaly and intellectual disability. Deletions were identified using Affymetrix 250K SNP array and further characterized by fluorescence in situ hybridization (FISH) analysis and qPCR. The deletions in our two patients overlapped within the 2p15p16.1 microdeletion syndrome area and were 6.8 and 6.9 Mb in size, respectively. FISH and qPCR confirmed the presence of only one copy in this region. Finemapping of the breakpoints indicated precise borders in our patients and were further finemapped in two other previously reported patients. Clinical features of patients with deletions in the 2p15p16.1 region vary. Including data from our patients, now eight out of nine reported patients have microcephaly, one of the major features, and all had intellectual disability. The current reported two patients add different forms of craniosynostosis to the clinical spectrum of this recently recognized microdeletion syndrome.

Key words: chromosome 2p15p16.1; craniosynostosis; microcephaly; microdeletion; 250K SNP array

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INTRODUCTION

Genomic array studies have recently revealed a new microdeletion syndrome on chromosome 2p15p16.1 [Rajcan-Separovic et al., 2007; Chabchoub et al., 2008; de Leeuw et al., 2008; Liang et al., 2009; Felix et al., 2010; Prontera et al., 2011]. In total, seven patients have been described with overlapping deletions, varying from 570 kb to 5.7 Mb, using various comparative genomic hybridization (CGH) array analyses [bacterial artificial chromosome (BAC), oligo, or SNP-based techniques].

Of these seven patients, the one with the small 570 kb deletion seems to have a significantly milder phenotype compared to the other six with larger deletions varying from 3.2 to 5.7 Mb. In the latter six cases, common clinical features are moderate to severe developmental delay, mild to moderate intellectual disability, microcephaly and characteristic and recognizable facial dysmorphisms. In addition, five were known to have optic nerve hypoplasia, three had a hydronephrosis, two had cortical dysplasia, and hand/foot abnormalities were reported in four patients. Liang et al. [2009] proposed two critical regions: the distal 570 kb for

Additional supporting information may be found in the online version of this article.

Conflict of interest: none to report.

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developmental delay and some of the facial dysmorphisms and the proximal 2.1 Mb for autistic behavior, short stature, microcephaly, additional facial dysmorphisms, optic nerve hypoplasia, and hydronephrosis. The patient reported by Prontera et al. [2011], with a deletion of 3.5 Mb, had many clinical features overlapping with the other described patients, but that deletion encompasses only few genes.

Here, we report on two additional patients with overlapping de novo deletions in the same chromosomal area, detected by 250K SNP array analysis. These patients do not only show the common features of the 2p15p16.1 microdeletion syndrome, but additionally show a complex form of craniosynostosis. We have fine mapped the deletion breakpoints of our patients and the two previously reported patients described by de Leeuw et al. [2008] and Chabchoub et al. [2008] using qPCR. Additionally, we have further defined the published borders of the deletion breakpoints by mapping data in UCSC build 37.

MATERIALS AND METHODS

Clinical Assessment

All patients seen at the Dutch craniofacial center for syndromic forms of craniosynostosis undergo genetic screening for microscopic chromosomal abnormalities and/or *FGFR1*, *FGFR2*, *FGFR3* mutations, or *TWIST1* mutations or deletions. In the patients described in this article, who were suspected for a syndromic form of craniosynostosis, no such mutation was found. We performed SNP array analysis for these patients.

SNP Array Analysis

Whole genome analysis was performed by microarray analysis using Affymetrix 250K NspI SNP arrays. The assays were carried out according to the manufacturer's instructions (Affymetrix GeneChip Mapping assay: www.affymetrix.com). Genotype data analysis was performed in Nexus 5 (Biodiscovery, Hawthorne, CA) using the Rank Segmentation algorithm.

FISH Analysis

FISH analysis was performed on chromosomes of Patients 1 and 2 and their parents.

The BAC clones used were selected from the University of California Santa Cruz UCSC browser and purchased from BACPAC Resources.

DNA was digested (*Mbo*I) and labeled with Bio-16-dUTP or dig-11-dUTP by a Random Prime labeling system (Invitrogen, Carlsbad, CA). The FISH experiments were performed according to standard protocols with minor modifications. FISH slides were analyzed with a Axioplan 2 Imaging microscope (Zeiss, Sliedrecht, The Netherlands). Images were captured using the fluorescent software Isis (MetaSystems, Altlussheim, Germany).

Names and location (build 37) of the BAC clones used were:

RP11-373L24; 2p16.1; 61,073,681–61,282,740 bp.
RP11-772D22; 2p15; 62,167,828–62,350,291 bp.
RP11-355B11; 2p15; 61,660,124–61,819,815 bp.
CTB-8L3; 2p25.3; sub-telomeric chromosome 2 probe used as a control.

Quantitative PCR Analysis

Relative copy numbers were determined by using real-time PCR analysis. Twenty-five nanogram of patient or control DNA was used in a 25 µl reaction containing 1× iTaq SYBR Green Supermix with ROX (Bio-Rad, Veenendaal, The Netherlands) and 200 nM of each primer. To verify results, three replicates were performed for each sample. Real-time PCR was performed on a 7300 Real Time PCR system (Applied Biosystems, Carlsbad, CA), cycle conditions: 10 min, 95°C initial denaturation, followed by 40 cycles, 15 sec denaturation; annealing/extension and data collection 1 min, 60°C. Data were analyzed using the software package 7300 System SDS Software RQ Study Application v1.2.3 (Applied Biosystems). Q-PCR Primer sequences used and their location on chromosome 2 are summarized in Supplementary eTable SI (See Supporting Information online).

RESULTS

Clinical Description of the Patients

We describe two patients who were referred to the Dutch Craniofacial Centre in Rotterdam.

Patient 1 is a boy who was born after 42 weeks of gestation with a weight of 3,240 g. At 3 months of age he had a small skull and a deviating skull circumference of −3 SD, based on curve "head circumference for age [growth analyzer, The Netherlands 1997]. He showed a protrusion of the central forehead and a triangular shaped head when viewed from above (Fig. 1A) and had a palpable metopic ridge, hypotelorism, and supraorbital retrusion, all characteristics in concordance with a combined trigonocephaly and microcephaly. The face showed strabismus, mild epicanthal folds, mild ptosis, short palpebral fissures, smooth and long philtrum, everted lower lip, and retrognathia. The hands showed no abnormalities; both feet show a clinodactyly of the 4th ray (Fig. 1A). A 3D CT scan image at the age of 3 months showed a synostosis of the metopic and the sagittal sutures (Fig. 1B). The coronal sutures and the lambdoid sutures were still open. Additionally the CT scan showed that the trigonocephaly caused the hypotelorism. Furthermore impressions of the inner table of the skull, mainly situated at the occipital and the parietal regions, were seen. The brain parenchyma and the ventricles showed a normal aspect. A MRI scan performed at the age of 2 years and 10 months (1.5 Tesla GE system, General Electric) showed a simplified gyral pattern in the supra tentorial region, a hypoplastic corpus callosum, and a rather small aspect of the cerebellum and the pons (Fig. 1C).

Patient 1 was operated for his craniosynostosis at the age of 7 months in our clinic. A parietal and frontal correction was performed without any complications. At the age of 3 years, his motor and mental developments were delayed. At the age of 22 months he started walking with support, which normally starts around the age of 13–15 months and there was no speech at all. His cognitive development was more severely retarded then his motor development. At the age of 33 months he had a mental development matching a 7.5-month-old child, and a motor development matching a 15-month-old child [mental and motor scale of Bayley Scales

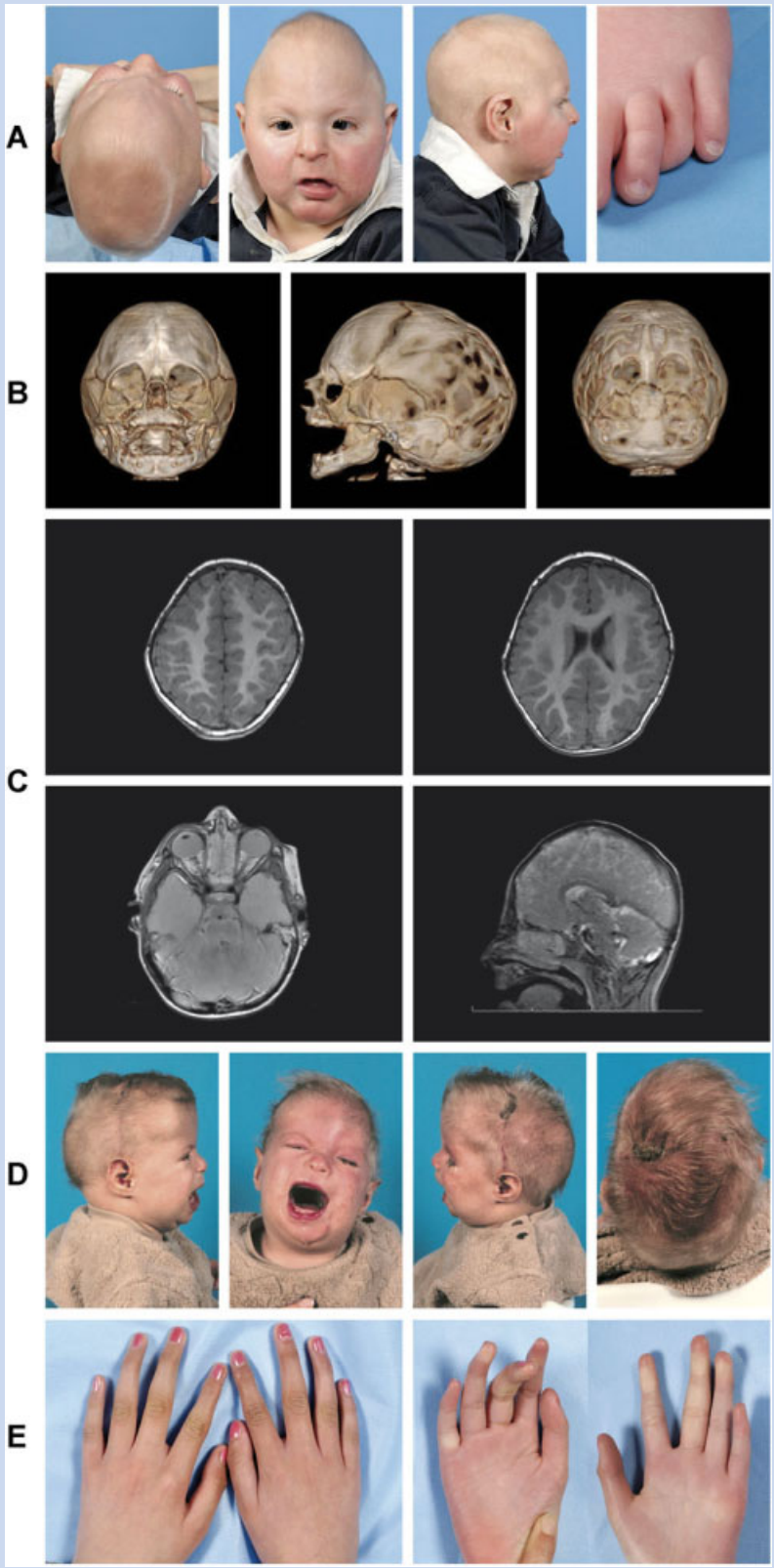


FIG. 1. Clinical data of Patients 1 and 2. A: Patient 1 at the age of six months. Note the trigonocephalic form of the skull and the small size of the head, the strabismus and epicanthal folds on both eyes and clinodactyly of the fourth ray on the feet. B: Three-dimensional CT scan of patient 1 at the age of 3 months showing a synostosis of the metopic and sagittal suture, hypotelorism and impressions at the occipital and parietal bones. C: MRI of Patient 1 at the age of 2 years and 10 months shows a simplified gyral pattern in the supra tentorial region and a hypoplastic corpus callosum. The cerebellum and pons are rather small. D: Patient 2 at the age of 6 months. Note the abnormal skull shape and small size of the head, a very broad nasal root and a flat philtrum, blepharophimosis, long eyelashes, and an expired helix fold of the ears. E: Hands of Patient 2 at the age of 12 years. Tapering of the fingers is clearly seen (in the dorsal view) as is the campodactyly of the third and fourth ray on the hands.

of Infant Development—Second Edition (BSID-II)]. Both parents had a normal phenotype.

Patient 2 was operated abroad and after that seen at our clinic at the age of 6 months. She was born after 36 weeks of gestational age with a weight of 2,150 g. Directly after birth different deformities were noticed (Fig. 1D). The head showed an abnormal skull shape, matching the sagittal suture and left coronal suture synostosis. The skull circumference at birth was 29 cm (<P3). The nose had a very broad nasal root and the philtrum was flat. Blepharophimosis and long downslanting eyelashes were noticed. The ears showed an hypoplastic helical rim, and the palate was flat. The hands showed tapering fingers and a camptodactyly of all the digits but mainly the third and fourth digit. The 5th finger was small on both hands (Fig. 1E). She had a very short neck and one café au lait spot on the left buttock. There was severe motor and mental developmental delay. Motor development at the age of 13 years was restricted to walking for several hours; climbing stairs was possible with support. Verbally the patient was restricted to signs and the use of single words. Psychological testing was performed at the age of 10 years and matched functioning at an age level of 17 months. Both parents and the brother and sister of the patient had a normal phenotype.

This study was approved by the medical ethical committee of the Erasmus MC, MEC-2005-273.

SNP Array Analysis and Validation With FISH Analysis

DNA of Patients 1 and 2 was analyzed on Affymetrix 250K Nsp1 SNP array. Both patients showed a deletion of respectively 6.9 and 6.8 Mb in chromosome 2, showing an overlap in the p15p16 area (Fig. 2A,B). The deletions were confirmed by FISH analysis with chromosome 2p15p16.1 and 2p25 BAC probes (Fig. 3A,B) in both patients. The deletions were absent in their parents (data not shown), indicating a de novo deletion in both children.

Defining Deletion Borders and Region

qPCR. Breakpoints of the deletions of Patients 1 and 2 were further mapped by qPCR. In addition, we have fine mapped the breakpoints for the chromosome 2 deletion patients described by Chabchoub et al. [2008] and de Leeuw et al. [2008] by qPCR, as the breakpoints in these patients were based on 1 Mb resolution BAC array data, with the BACs not covering the complete chromosomal 2p15p16.1 area. Primers including base pair positions for qPCR are indicated in Supplementary eTable SI (See Supporting Information online). Histogram displaying results of the qPCR are shown in supplementary eFigure S1 (See Supporting Information online).

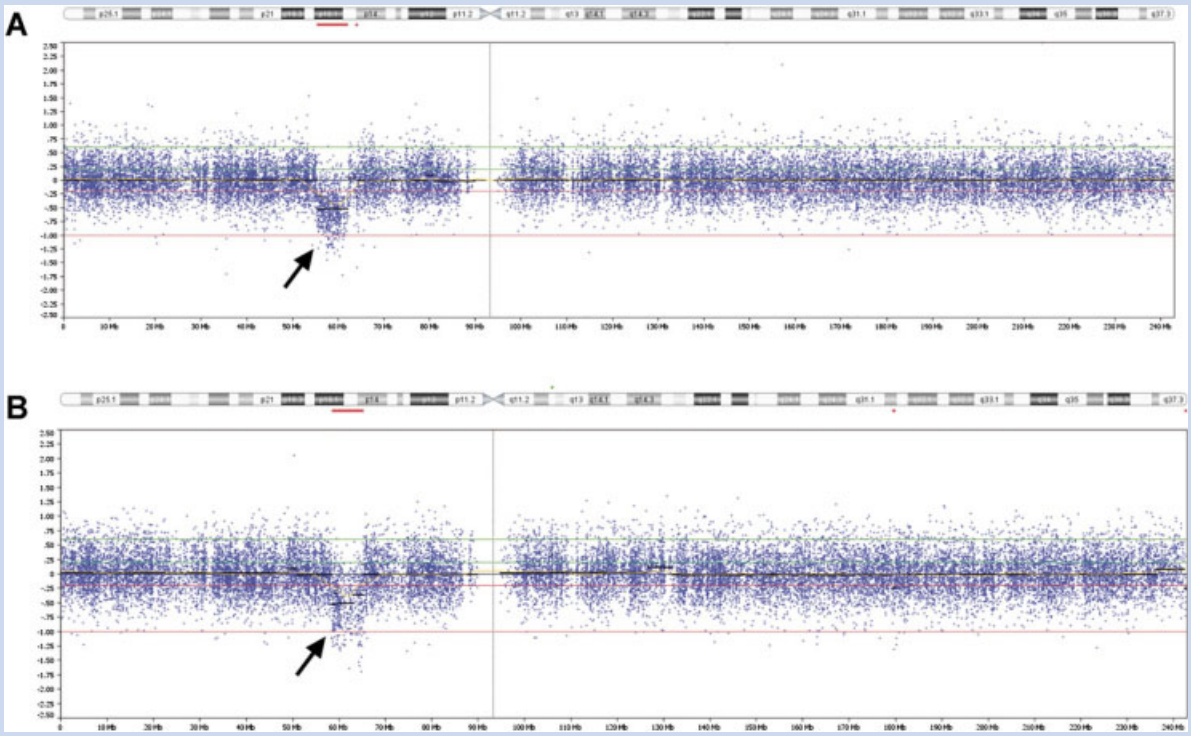


FIG. 2. Identification of the 2p15p16 deletions by analysis of Affymetrix 250 K NSPI SNP data in Nexus 5 (Biodiscovery) in (A) Patient 1 (6.9 Mb) and (B) Patient 2 (6.8 Mb). Blue dots represent the log2 ratio's of SNP probe intensities of experiment and control samples. Arrows indicate the presence of copy number loss at 2p15p16.

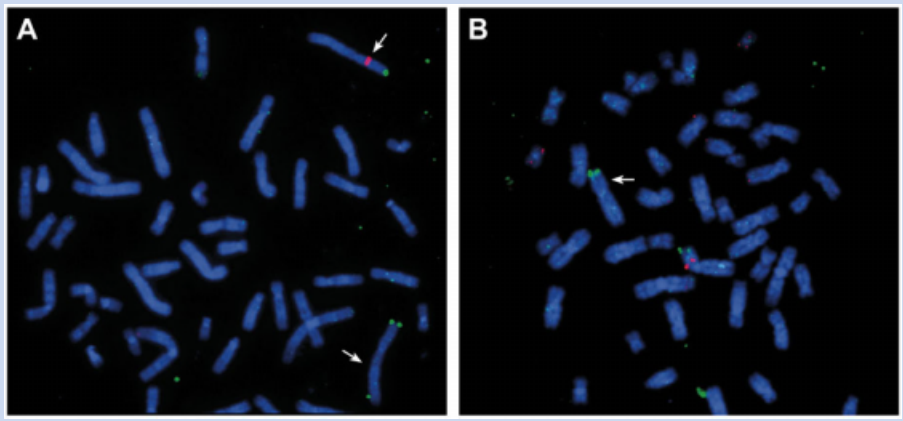


FIG. 3. Fluorescence in situ hybridization analysis on metaphases obtained from cultured lymphocytes of Patient 1 (A) and Patient 2 (B). A: BAC probe RP11-373L24 (2p16) in red, 1 copy present and CTB-8L3 (2p25.3, control probe) in green, 2 copies present. B: BAC probe RP11-772D22 (2p15) in red, 1 copy present and CTB-8L3 (2p25.3, control probe) in green, 2 copies present.

Results are indicated in Table I. The distal breakpoint in our patient 1 is between *MTIF2* (2 copies; primerset cr2_s13_F2/R2) and *CCDC88A* (1 copy; cr2_s15_F1/R1). The proximal breakpoint is between *COMMD1* (1 copy; primerset cr2_s5_COMMD1_Fp1/Rp1) and *B3GNT2* (2 copies; cr2_s14_F1/R1). The deletion area encompasses 35 genes (excluding the pseudogenes). The distal breakpoint in patient 2 is between *FANCL* (2 copies; primerset cr2_p1_F/R) and *FLJ30838* (1 copy, primerset cr2_p2_F/R). The proximal breakpoint is between *RAB1A* (1 copy; primerset cr2_s13_F4/R4) and *ACTR2* (2 copies; primerset cr2_s13_F5/R5) with the deletion encompassing 43 genes (excluding pseudogenes). The deletion overlap for these two patients with microcephaly and craniosynostosis encompasses 23 genes, from *FLJ30838* to *COMMD1*.

The distal breakpoint for the patient described by Chabchoub et al. [2008] is between *REL* (2 copies, primerset cr2_s12_F3/R3) and *PUS10* (1 copy, primerset cr2_s8_PUS10_Fp1/Rp1). The proximal breakpoint is between *SNORA70B* (1 copy, primerset cr2_s9_Fp3/Rp3) and *LOC647077* (2 copies, primerset cr2_betweenXPO + FAMM_F/R), possibly in the *XPO1* gene based on the published BAC data [Chabchoub et al., 2008]. By qPCR we could not determine the copy number in the area between *SNORA70B* and *LOC647077* due to normal copy number variation in this area (Database of Genomic Variants; http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg19). The distal breakpoint for the patient described by de Leeuw et al. [2008] is between *CCDC85A* (2 copies, primerset cr2_betweenVRK2 + LOC1001F/R) and the *VRK2* gene (1 copy, primerset cr2_s12_F1/R1)[2]. The proximal

TABLE I. Results Breakpoint Analysis by qPRC: Deletion Areas Are Indicated in Gray

Name primer pair forward/reverse	Patient 1	Patient 2	Chabchoub	de Leeuw	Gene
1-2: cr2_s13_F2/R2	2				MTIF2
3-4: cr2_s15_F1/R1	1				CCDC88A
5-6: cr2_s13_F3/R3					
7-8: cr2_betweenVRK2+LOC1001_F/R				2	CCDC85A
9-10: cr2_s12_F1/R1				1	VRK2
11-12: cr2_p1_F/R		2			FANCL
13-14: cr2_p2_F/R		1			FLJ30838
15-16: cr2_s12_F3/R3			2		REL
17-18: cr2_s8_Pus10_Fp1/Rp1			1		PUS10
19-20: cr2_s9_Fp3/Rp3			1	1	SNORA70B
21-22: cr2_betweenXPO+FAMM_F/R			2	2	LOC647077
23-24: cr2_s5_COMMD1_Fp1/Rp1	1				COMMD1
25-26: cr2_s14_F1/R1	2				B3GNT2
27-28: cr2_s13_F4/R4		1			RAB1A
29-30: cr2_s13_F5/R5		2			ACTR2

breakpoint is the same as for the patient described by Chabchoub et al. [2008].

Deletions of all patients, encompassing genes in the area, are indicated in Figure 4.

Enhancers

In the region from 55,463,756 to 65,498,387, 42 Human Vista enhancer elements are located [Visel et al., 2007]. These are highly conserved elements with possible gene distant-acting enhancer activity. These 42 are all between *FANCL* and *PAPOLG*, the region that is deleted in seven and partially deleted in one of the patients (Supplementary eFigure S2—See Supporting Information online). Fourteen of these elements have expression as assessed in transgenic mice, in amongst others brain (hs394,hs399,hs779,hs975,hs1076,hs1119,hs1535), facial mesenchym (hs836) eye (hs393), and ear (hs1071). This indicates that these enhancers possibly play a role in the observed phenotype and have a function in these tissues in regulating other genes.

DISCUSSION

We describe two patients with overlapping deletions in the 2p15p16.1 region, an area that is recently recognized as a new microdeletion syndrome [Rajcan-Separovic et al., 2007; Chabchoub et al., 2008; de Leeuw et al., 2008; Liang et al., 2009; Felix et al., 2010; Prontera et al., 2011]. These patients have the largest deletions in this area described so far, but the common overlapping deleted region is similar to the deletions described in the patients by Liang et al. [2009] and Felix et al. [2010]. Notably, the current patients add craniosynostosis as a new clinical characteristic to this novel microdeletion syndrome (Table II), though both patients have different forms. Patient 1 has a synostosis of the metopic and the sagittal suture in addition to a trigonocephalic head, whereas Patient 2 has a synostosis of the left coronal and sagittal suture. Patient 1 additionally showed brain abnormalities in the form of a simplified gyral pattern in the supra tentorial region, a hypoplastic corpus callosum and a small cerebellum and pons. Because Patient 2 was operated in another center abroad, no original MRI or CT data were available. We propose that the craniosynostosis in these patients is primary, and not secondary to the microcephaly, as this last would generally result in pansynostosis rather than sagittal, coronal or metopic synostosis. Except for the craniosynostosis, clinically both patients do resemble the 2p15p16.1 microdeletion syndrome. However, for some clinical features, for example, optic nerve hypoplasia and hydronephrosis, the children may still be too young to express these abnormalties (Table II).

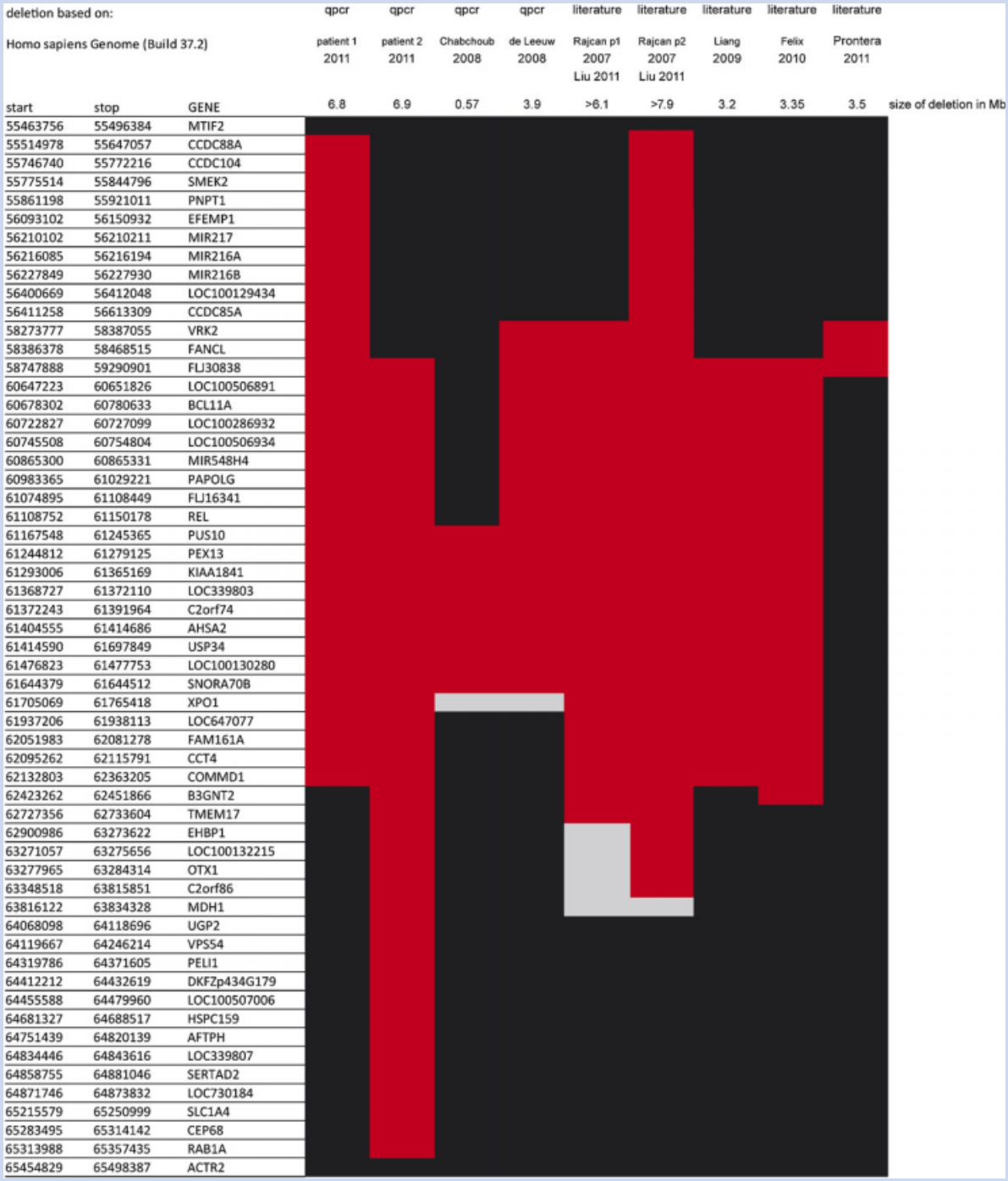
Rajcan-Separovic et al. [2007] reported on brachycephaly as a dysmorphic feature in their patients, although it is not clear what the underlying cause of this feature in these patients is. In addition to our Patient 1, the two patients from Rajcan-Separovic et al. also showed brain abnormalities; perisylvian migration disorder (Patient 1 [Rajcan-Separovic et al., 2007]) and generally thickened cortex with hyperintense subcortical tissue suggesting dysmyelination or cortical dysplasia; enlarged 4th ventricle, mild hypoplasia of the inferior cerebellar vermis, and small anterior pituitary and pons (Patient 2 [Rajcan-Separovic et al., 2007]). These partly overlap with the brain features in our patient.

We have determined the deletion borders in our two patients and the patients described by Chabchoub et al. [2008] and de Leeuw et al. [2008] by qPCR. The data of these authors were based on BAC data, and therefore, the borders were not determined precisely. According to de Leeuw et al. [2008], *XPO1* is outside their deletion area, according to Chabchoub et al. [2008], *XPO1* is included in their deletion area. However, by qPCR we have not been able to resolve whether *XPO1* is deleted in the two patients described by these authors. This is likely due to copy number variation in that area (Database of Genomic Variants; http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg19).

The patient described by Chabchoub et al. [2008], with the smallest deletion, is also intellectually disabled, similar to the other patients with a deletion in this area. However, he is the only one without microcephaly. Additionally, several other clinical features observed in the majority of 2p15p161 microdeletion patients, for example, some facial dysmorphisms, optic nerve hypoplasia, and hydronephrosis, could not be observed in this patient (Table II). This milder phenotype is in line with the small size of its deletion which is only 570 kb versus the 3.2–6.9 Mb in all the other so far reported cases. The patient reported by Prontera et al. [2011], with a deletion of 3.5 Mb, has many clinical features overlapping with the other described patients, including microcephaly and intellectual disability, but no craniosynostosis or structural brain abnormalities and that deletion encompasses only one uncharacterized and two known genes. Additionally, this patient has two other chromosomal rearrangements that could possibly influence the clinical phenotype. All nine patients have moderate to severe mental disability, though not all deletions overlap, indicating that genes in different regions can have influence on mental disability. For the microcephaly, the only gene that seems left, is the uncharacterized gene *LOC100506891*. Considering the presence of craniosynostosis in our two patients, we propose that the candidate region for this feature lies within the region between *FANCL* and *B3GNT2*. Additionally, features may depend on the different sizes of deletions found in the different patients and may show variable expression depending on genetic background [Girirajan et al., 2010]. Also the enhancer elements present in the area may play a role.

Some genes in the deletion region have a known function in brain. *BCL11A* (*CTIP1*) [OMIM:606557], a gene that plays a role in globin gene regulation [Sankaran et al., 2008] is also highly expressed in brain [Kuo and Hsueh, 2007] and thought to be involved in axon outgrowth and branching [Kuo et al., 2010]. *BCL11A* is deleted in seven of the nine patients. As information on brain development on MRI is available in six of the patients, with brain abnormalities in three of them, it is at this point not possible to conclude whether absence of this gene plays a major role in the formation of brain abnormalities seen in these patients. *XPO1* (or *CRM1*) [OMIM:602559] is evolutionary conserved and expressed in the developing brain and proposed to be involved in motor neuron development and survival [Kolle et al., 2000]. Additionally it is implicated to have a role during mitosis [Hutten and Kehlenbach, 2007].

Notably, REL [OMIM:164910] binds to CREBBP [OMIM:600140] and EP300 [OMIM:602700], two genes involved in Rubinstein–Taybi



deletion based on:			qpcr	qpcr	qpcr	qpcr	literature	literature	literature	literature	literature	
Homo sapiens Genome (Build 37.2)			patient 1 2011	patient 2 2011	Chabchoub 2008	de Leeuw 2008	Rajcan p1 2007 Liu 2011	Rajcan p2 2007 Liu 2011	Liang 2009	Felix 2010	Prontera 2011	
start	stop	GENE	6.8	6.9	0.57	3.9	>6.1	>7.9	3.2	3.35	3.5	size of deletion in Mb
55463756	55496384	MTIF2										
55514978	55647057	CCDC88A										
55746740	55772216	CCDC104										
55775514	55844796	SMEK2										
55861198	55921011	PNPT1										
56093102	56150932	EFEMP1										
56210102	56210211	MIR217										
56216085	56216194	MIR216A										
56227849	56227930	MIR216B										
56400669	56412048	LOC100129434										
56411258	56613309	CCDC85A										
58273777	58387055	VRK2										
58386378	58468515	FANCL										
58747888	59290901	FLJ30838										
60647223	60651826	LOC100506891										
60678302	60780633	BCL11A										
60722827	60727099	LOC100286932										
60745508	60754804	LOC100506934										
60865300	60865331	MIR548H4										
60983365	61029221	PAPOLG										
61074895	61108449	FLJ16341										
61108752	61150178	REL										
61167548	61245365	PUS10										
61244812	61279125	PEX13										
61293006	61365169	KIAA1841										
61368727	61372110	LOC339803										
61372243	61391964	C2orf74										
61404555	61414686	AHSA2										
61414590	61697849	USP34										
61476823	61477753	LOC100130280										
61644379	61644512	SNORA70B										
61705069	61765418	XPO1										
61937206	61938113	LOC647077										
62051983	62081278	FAM161A										
62095262	62115791	CCT4										
62132803	62363205	COMMD1										
62423262	62451866	B3GNT2										
62727356	62733604	TMEM17										
62900986	63273622	EHBP1										
63271057	63275656	LOC100132215										
63277965	63284314	OTX1										
63348518	63815851	C2orf86										
63816122	63834328	MDH1										
64068098	64118696	UGP2										
64119667	64246214	VPS54										
64319786	64371605	PELI1										
64412212	64432619	DKFZp434G179										
64455588	64479960	LOC100507006										
64681327	64688517	HSPC159										
64751439	64820139	AFTPH										
64834446	64843616	LOC339807										
64858755	64881046	SERTAD2										
64871746	64873832	LOC730184										
65215579	65250999	SLC1A4										
65283495	65314142	CEP68										
65313988	65357435	RAB1A										
65454829	65498387	ACTR2										

FIG. 4. Microdeletion area chromosome 2p15p16. Deletions (in red) are indicated in our Patients 1 and 2 and in all patients described in literature until now. Genes in the region with base pair positions (build 37, hg19) are indicated on the left. The light-gray area around *Xpo1* could not be resolved by q-PCR, due to normal copy number variation in this region. The minimal distal deletion borders in the two subjects from Rajcan-Separovic et al. are indicated as described in Liu et al. [2011]. Unresolved areas are indicated in light-gray. Basepair positions from the deletions described in the literature were all converted to build 37, hg 19. The deletion from Prontera et al. seems small in the figure, because in their 3.5 Mb deletion area, only three genes are located (distances are not drawn to scale).

TABLE II. Overview Clinical Features of Patients With Chromosome 2p15-p16.1 Deletions

	Present (Patient 1)	Present (Patient 2)	Chabchoub et al.	de Leeuw et al.	Rajcan-Separovic et al. (subject 1)	Rajcan-Separovic et al. (subject 2)	Liang et al.	Félix et al.	Prontera et al.	Total
	6.9	6.8	0.57	3.9	4.5	5.7	3.2	3.35	3.5	
General										
Age (years) at time of assessment	3 months/ 4 years	6 months/ 13 years	16	32	8	6	4,5	4	9	
Gender	M	F	M	M	F	M	F	F	F	
IUGR	NA	NA	NM	−	+	−	+	+	NM	3/9
Short stature	−	−	−	+	−	+	+	NM	NM	3/9
Developmental delay	+	+	NM	+	+	+	NM	+	+	7/9
Intellectual disability	Moderate	Severe	Mild	Moderate/ Severe	Moderate	Moderate	Moderate	Moderate	Moderate	9/9
Autistic features/confirmed autism spectrum disorder	NA	NA	−	NM	+	+	NM	−	NM	2/9
Attention deficit hyperactivity disorder	NA	NA	NM	NM	+	+	+	NM	+	4/9
Delayed language skills	±	±	Mild	+	Severe	Severe	+	Severe	+	9/9
Feeding problems			NM	+	+	+	−	+	+	
Microcephaly	+	+	−	+	+	+	+	+	+	8/9
Craniosynostosis	+	+	−	−	−	−	−	−	−	2/9
Structural brain abnormalities	+	Unknown	−	Unknown	+	+	−	−	−	3/7
Facial features										
Bitemporal narrowing	+	+	NM	+	+	+	−	NM	+	6/9
Receding short forehead	−	±	−	+	+	+	−	+	−	5/9
Strabismus	+	−	−	+	+	−	−	−	−	3/9
Ptosis	+	+	−	+	+	+	+	+	+	8/9
Telecanthus	+	+	+	+	+	+	+	+	+	9/9
Widened inner canthal distance	+	+	−	+	+	+	+	NM	+	7/9
Short palpebral fissures	−	−	−	+	+	+	+	+	+	6/9
Down slanting palpebral fissures	−	−	−	+	+	+	−	NM	−	3/9
Epicanthal folds	+	+	+	+	−	+	+	NM	+	7/8
Broad/high nasal root	+	+	+	+	+	+	±	+	+	9/9
Prominent nasal tip	−	−	+	−	+	+	−	NM	+	4/9
Long, straight eyelashes	−	+	NM	+	+	+	−	+	−	5/9
Long, thin eyebrows	−	+	NM	−	−	+	−	NM	−	2/9
Large ears	+	−	+	−	+	+	−	+	+	6/9
Smooth and/or long philtrum	+	+	−	+	+	+	+	+	+	8/9
Smooth upper vermillion border	−	−	+	+	+	+	−	NM	+	5/9
Everted lower lip	−	−	+	+	+	+	−	NM	+	5/9
High narrow palate	−	−	+	+	+	+	−	+	+	6/9
Retrognathia	−	−	NM	+	−	+	+	Micrognathia	−	3/9
Other physical features										

(*Continued*)

TABLE II. (Continued)

	Present (Patient 1)	Present (Patient 2)	Chabchoub et al.	de Leeuw et al.	Rajcan-Separovic et al. (subject 1)	Rajcan-Separovic et al. (subject 2)	Liang et al.	Félix et al.	Prontera et al.	Total
Widened internipple distance	−	−	NM	+	+	+	−	+	+	5/9
Extra nipple	−	−	NM	−	−	+	−	NM	−	1/9
Camptodactyly digit(s)	+	+	−	−	+	+	−	+	−	5/9
Metatarsus abductus	−	−	−	−	+	+	+	+	−	4/9
Spasticity legs	−	−	−	−	+	+	+	NM	+	4/9
Other										
Optic nerve hypoplasia	−	−	NM	+	+	+	+	−	−	4/9
Disturbed vision	−	−	NM	+ (myopia)	−	+ (hyperopia)	NA	+ mild hypermetropia	NM	3/9
Hearing loss	−	+	NM	−	−	+	−	−	−	2/9
Frequent upper respiratory infections	−	−	NM	+	+	−	−	−	−	2/9
Laryngomalacia	−	−	NM	−	−	+	−	NM	−	1/9
Hydronephrosis	−	−	−	+	+	+	−	−	−	3/9
Hypogonadism	−	−	NM	−	−	+	−	−	−	1/9

NM, not mentioned; NA, not assessed.

syndrome [OMIM:180849 and 61384], where intellectual disability and microcephaly are part of the phenotypic characteristics, and structural brain abnormalities have been reported in a minority of cases [Roelfsema and Peters, 2007].

Array CGH and SNP array screening has led to the characterization of many heterozygous (novel) microdeletion and microduplication syndromes [Slavotinek, 2008]. Intellectual disability is often a feature of these syndromes, as is microcephaly [Slavotinek, 2008; Walczak-Sztulpa et al., 2008; Reddy et al., 2009; Girirajan et al., 2010]. Additionally, microcephaly is found as an isolated feature, but can also be associated with many syndromes [Abuelo, 2007]. Also, disruption of one gene can lead to a combination of phenotypic features including microcephaly and intellectual disability [Mukhpadhyay et al., 2010; Williams et al., 2010; Campbell et al., 2011]. For most microdeletion syndromes it is difficult to pinpoint one clinical feature to the absence of a specific gene in a deletion encompassing many genes [Kumar, 2008; Klaassens et al., 2009; Masurel-Paulet et al., 2010; Stankiewicz and Lupski, 2010; Vissers et al., 2010], and this is supported by comparing the deletions with the clinical phenotype in this study. Mouse models carrying targeted deletions of genes within these regions may help to elucidate the function of the individual genes [Abrams and Jiao, 2009]. Deletions in the 2p15p16.1 area show variable clinical expression and may lead to microcephaly, intellectual disability and additionally to craniosynostosis, depending on the size and extension of the deletion combined with genetic background.

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