Case Series: 2q33.1 Microdeletion Syndrome - Further delineation of the phenotype

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Case Series: 2q33.1 Microdeletion Syndrome - Further delineation of the phenotype

Running Title: 2q33.1 Microdeletion Syndrome: Expanding the phenotype

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ABSTRACT

Recurrent deletions of 2q32q33 have recently been reported as a new microdeletion syndrome, clinical features of which include significant learning difficulties, growth retardation, dysmorphic features, thin and sparse hair, feeding difficulties and cleft or high palate. Haploinsufficiency of one gene within the deleted region, *SATB2*, has been suggested to be responsible for most of the features of the syndrome. We describe seven previously-unreported patients with deletions at 2q33.1, all partially overlapping the previously-described critical region for the 2q33.1 microdeletion syndrome. The deletions ranged in size from 35 kb to 10.4 Mb, with the smallest deletion entirely within the *SATB2* gene. Patients demonstrated significant developmental delay and challenging behaviour, a particular behavioural phenotype that seems to be emerging with more reported patients with this condition. One patient in our cohort has a deletion entirely within *SATB2* and has a cleft palate, whereas several patients with larger deletions have a high-arched palate. In addition, one other patient has significant orthopaedic problems with ligamentous laxity. Interestingly, this patient has a deletion that lies just distal to *SATB2*. The orthopaedic problems have not been reported previously and are possibly an additional feature of this syndrome. Overall, our report provides further evidence that the *SATB2* gene is the critical gene in this microdeletion syndrome. In addition, because the individuals in our study range in age from 3 to 19 years, these patients will help define the natural progression of the phenotype in patients with this microdeletion.

KEYWORDS: 2q33.1 microdeletion, *SATB2*, ligamentous laxity, developmental delay, aCGH

INTRODUCTION

Previous to molecular characterisation, there were very few case reports of interstitial deletion of 2q reported [Al-Awadi *et al.*, 1983; Miyazaki *et al.*, 1988]. Recently, molecular cytogenetic techniques such as microarray analysis have allowed for the characterisation of microdeletion 2q33.1 syndrome. Van Buggenhout *et al.*, 2005 described four patients with an interstitial deletion of chromosome 2q32q33. The authors described similarities in clinical findings, including growth retardation, distinct facial dysmorphism, micrognathia, cleft or high palate and persistent feeding difficulties. All had significant learning difficulties. Since then, further case reports by de Ravel *et al.*, 2009, Urquhart *et al.*, 2010, and Rifai *et al.*, 2010 have described patients with this syndrome. Emerging evidence suggests that psychiatric problems, with a distinctive behavioural phenotype including hyperactivity, chaotic behaviour and happy personality with bouts of anxiety or aggression, are a significant part of the clinical features in some individuals [de Ravel *et al.*, 2009; Urquhart *et al.*, 2010].

Special AT-rich sequence binding protein 2 (*SATB2*; OMIM 608148), a DNA-binding protein that plays an important role in craniofacial patterning and brain development, has been identified as a candidate gene responsible for the craniofacial dysmorphism associated with deletions and translocations at 2q32-q33. This follows the identification of a locus for isolated cleft palate on chromosome 2q32, by Brewer *et al.*, 1999, and the identification of translocations that disrupted *SATB2* in individuals with isolated cleft palate [Fitzpatrick *et al.*, 2003]. Based upon several patients, with most of the features of 2q33.1 microdeletion syndrome and deletions within the gene, Rosenfeld *et al.*, 2009 suggested that haploinsufficiency of *SATB2* is responsible for several of the clinical features associated with 2q33.1 microdeletion syndrome.

We describe seven additional patients with the 2q33.1 microdeletion syndrome and provide genotype-phenotype correlation for some of the clinical features. We also describe several previously-unreported features in one or more individuals, thus expanding the phenotype. We also provide additional evidence that haploinsufficiency of *SATB2* causes some of the clinical features in this condition.

MATERIALS AND METHODS

Array-based Comparative Genomic Hybridisation (aCGH)

aCGH was performed on DNA extracted from peripheral blood from Patient (1) using both a Bluegenome Cytochip 1Mb BAC array version 1.1 and an Oxford Gene Technologies 105K version 2 oligonucleotide array. Both were processed according to manufacturer's instructions, using Promega pooled control DNA as a reference. Slides were scanned using an Axon 4100A microarray scanner and analysed using either Bluefuse Multi v 2.1 or OGT Cytosure v3.0.6 software depending on the array manufacturer.

aCGH was performed on DNA extracted from peripheral blood from Patient (4) using Oxford Gene Technologies 60 mer oligo-array printed in 8x60 K International Standard Cytogenomic Array (ISCA) Consortium configuration, according to manufacturer's instructions, using Promega pooled control DNA as a reference. Slides were scanned using an Agilent microarray scanner (G2539A) and analysed using Agilent CGHAnalytics (v4.0) microarray software.

Oligonucleotide-based microarray analysis was performed on Patients (5) and (7) using a 105K-feature whole-genome microarray (SignatureChip Oligo Solution™ version 1.0, custom-designed by Signature Genomic Laboratories, made by Agilent Technologies, Santa Clara, CA), according to previously described methods [Ballif *et al.*, 2008].

Oligonucleotide-based microarray analysis was performed on Patients (2), (3) and (6) using a 135K-feature whole-genome microarray (SignatureChip Oligo Solution™ version 2.0, custom-designed by Signature Genomic Laboratories, made by Roche NimbleGen, Madison, WI), according to previously-described methods [Duker *et al.*, 2010]. Results were displayed using custom oligonucleotide aCGH analysis software (Genooglyphix; Signature Genomic Laboratories).

Fluorescence in situ hybridisation (FISH)

Deletions were confirmed by metaphase fluorescence *in situ* hybridization (FISH) using BAC clones from the regions found to be deleted by aCGH, as previously described [Traylor *et al.*, 2009], when whole blood samples were available (Patients (2), (4) and (6)). Parental samples, when available, were also assayed using metaphase FISH.

CLINICAL REPORTS

Patient (1)

Patient (1) aged 19 years, is the second child of healthy, non-consanguineous Caucasian parents, with no significant family history. The pregnancy was normal. She was a full-term, normal delivery with a birth-weight of 2.72 kilograms (9th-25th centile) and was

well immediately after birth. However, she had feeding problems and was bottle-fed with an orthodontic teat in the initial neonatal period. She was investigated for 'failure-to-thrive' as an infant, and no cause was found. She was noted to have breath-holding attacks as an infant, but her EEG was reported as normal. At two years of age, her weight was between the 3rd-10th centile, length just below the 50th centile, and weight: length ratio and body mass index (BMI) both <3rd centile.

Patient (1) had a normal brain-MRI at four years of age. Also, at this age she was diagnosed with conductive hearing loss and had grommets inserted. She was noted to be dysmorphic, with down-slanting palpebral fissures, prominent nose, slightly low-set ears, and a single palmar crease on the left hand (Figure 1a). Over time, the patient developed a 'marfanoid habitus' with arachnodactyly, bilateral claw toes with mild pes cavus, high-arched palate, and a long face (Figure 1b and c). She developed horizontal striae at the base of her back. She also has ligamentous laxity and bilateral mal-tracking patellae with subluxation in extension but no evidence of scoliosis. She has moderate to severe developmental delay, in particular speech and motor delay. She first walked at four years of age. She managed in mainstream school with one-to-one help. She has a deep, hoarse voice. Microlaryngoscopy showed normal vocal cords.

Homocystinuria was excluded, along with other metabolic investigations, which were reported as normal. She has challenging behaviour including hyperactivity, restlessness and has severe expressive and receptive language deficits. She remains under the Psychiatry services for management of her mood swings. She has significant problems with episodes of sub-luxation of her patellae and ligamentous laxity.

Patient (2)

Patient (2) is a five-year-old female, who was the first child of healthy non-consanguineous Jewish parents (Iraqi and Ashkenazi/Moroccan origin). There is no significant family history. She was born after a normal pregnancy, by spontaneous delivery at 39 weeks of gestation, with a birth weight of 3.425 kg (50th centile), length of 48.5 cm (25th centile) and head circumference of 36.7 cm (98th centile). She had feeding difficulties from birth and, at the age of four months, presented with failure to thrive: her weight was on the 10th centile and her height was on the 3rd centile.

Development was reported to be normal until the age of 12 months including, standing with support by the age of eleven months. At the age of eighteen months, psychomotor retardation became evident with generalised hypotonia, delayed first walking (25 months) and delayed language. At the age of 2.5 years, she was diagnosed with global developmental delay of about one year.

Dysmorphic features included sparse, thin hair, high forehead, down-slanting palpebral fissures, tented upper lip, downturned mouth corners, high-arched palate, crowded teeth and inverted nipples. She has mild conductive hearing loss on the left side. She was diagnosed with mild to moderate mental retardation. At five years of age, she started to run, has a few words and attends a special nursery. No behavioural or psychiatric problems were reported by the parents.

All metabolic investigations were normal in this patient, apart from a low serum carnitine level, which responded to supplementation and is likely to represent nutritional

carnitine deficiency. She had a normal cardiac echocardiogram. MRI-brain demonstrated dilated Virchow-Robin spaces in centrum semiovale. She had a normal EEG and eye examination. Her bone age was normal at two years of age.

Patient (3)

Patient (3) is an eight year, eight month-old male with dysmorphic features, cleft palate, missing teeth, hypospadias, severe language impairment, and hypotonia (Figure 2a). He required nasogastric tube feeding in early childhood. He had a surgical repair of a cleft palate and hypospadias. Neurodevelopmental evaluation at the age of five years and seven months, using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI), showed a performance IQ of 51 (moderate mental retardation). He made vowel sounds, but no clear words, and was diagnosed with severe receptive and expressive language impairment with oral-motor dysfunction. He depends on non-verbal communication. Central nervous system dysfunction manifested as co-ordination and balance difficulties in gross motor skills and hypotonia. He has a happy disposition, but has some problems in school by inappropriately hugging others and touching their faces.

At the age of eight years and eight months, he was normocephalic with a triangular face, a high forehead and a prominent chin. Other dysmorphic features included hypertelorism, upturned nose, prominent and cupped pinnae, long philtrum, narrow palate, crowded dentition with missing front upper incisors, and slight pectus excavatum (Figure 2b). The hands were small with significant joint laxity to the fingers and normal palmar creases; they also had a “doughy” and soft consistency. The thumbs and great toes were

widened but not bifid. He has a clumsy, and wide-based gait with generalised hypotonia.

Patient (4)

Patient (4) is a nine year old male, the first child of non-consanguineous, White Caucasian, healthy parents with no significant family history. He was born after an uneventful pregnancy (normal antenatal scans), with no known teratogenic exposure at 35 weeks with a birth weight of 2.2 kilograms (25th centile). A submucous cleft palate was detected at 18 days of age, which was subsequently repaired. He had initial feeding problems with failure to thrive.

On examination aged 17 months, his head circumference, weight and length were below the 3rd centile. He was not walking at that age. On examination, he had a prominent forehead with prominent orbits, blue sclerae, small mouth, generalised hypotonia, 'myopathic' face and micrognathia (Figure 3a). He also had crowded teeth with alternating convergent strabismus. He had a heart murmur over the left sternal edge, but an echocardiogram did not reveal any structural anomalies of the heart. FISH for 22q11 deletion and Smith-Magenis syndrome, MRI-brain scan, metabolic tests, muscle biopsy, methylation for Angelman's syndrome and telomere analysis were normal. A review at eight years of age revealed low muscle tone, 'myopathic' face, severe learning difficulties and no speech (Figure 3b).

Patient (5)

Patient (5) was born at 38 weeks gestation to consanguineous Mexican parents, with normal birth-weight, length and head circumference. Pregnancy was complicated by gestational diabetes. At birth, he was found to have a cleft palate and remained in the hospital for ten days due to feeding problems. His cleft palate was repaired at one year of age. He was referred for a Genetics evaluation at 37 months, because of developmental delay. He reportedly sat at seven months and walked around two years of age. At 3 years of age, he has no speech and is not toilet-trained. Examination revealed normal weight, height, and head circumference. His lateral hairline protruded onto his forehead. He had a beaked nose with hypoplastic alae nasi and a high nasal bridge. His mouth was small with a short philtrum and micrognathia with long, narrow face and low-set ears. He has mild hearing loss and eye examination showed long lashes and intermittent exotropia. His feet have overlapping toes, prominent heels and tight heel cords and one café-au-lait spot was noted.

Patient (6)

Patient (6) is a four year-old male who first came to medical attention around two years of age with speech and language delay. There was no significant family history. He was born at term, following an unremarkable pregnancy, with a birth-weight of 3.4 kg (50th centile). Prenatal ultrasound was normal, apart from a two-vessel cord. Postnatal complications included a weak cry, pallor and feeding difficulties, which resolved with the use of a special teat.

On review at age four, he only had a 10-word vocabulary, did not join words together, and was able to sign approximately 50 words. He was unable to name colours. His initial words were at fifteen months of age. Motor development was normal. Behavioural concerns

included autistic-like features, easy distractibility, noise sensitivity (extremely frightened of loud noises), sensory perception issues (touches mother's hair all the time, puts mouth on the cat) and over-fixation on certain activities. Past medical history is significant for hyperopia in the right eye, esotropia in the left eye, recurrent otitis media (requiring placement of grommets), eczema and a heart murmur. MRI-brain was normal apart from multiple prominent peri-vascular spaces bilaterally and symmetrically.

On examination, he had a rectangular, prominent forehead, narrow and high-arched palate, dental crowding, narrow shoulder girdle, pectus carinatum and fifth toe clinodactyly. Neurological examination was notable for impaired cognition, non-specific vocalisation with inappropriate bouts of laughter, hyperactivity, excessive drooling, pale fundi, and bilateral Babinski response.

Patient (7)

Patient (7) was one of twins, born to a 42-year-old mother at 40 weeks gestation, by Caesarean section, because of failure to progress. Her mother was on Enoxaparin and aspirin prenatally, because of a maternal clotting disorder. Birth-weight was 3.061kg (25-50th centile) and there were no neonatal complications reported. The patient was seen by a Gastroenterologist at six months of age for poor weight gain, at which time reflux was diagnosed and treated. She was also evaluated by a Neurologist for hypotonia. Hearing was normal.

Formal developmental evaluation at three years showed mild delay and receptive language that was much better than expressive language. Her speech included only a few

words, despite receiving speech and occupational therapy. She has global developmental delay. She rolled at 6-8 months, sat at 13 months, and walked at 19 months. Her twin sister was developing normally. On follow-up at four years, eleven months of age, she had about 4-5 words, and was using 7-8 signs. She was enrolled in a pre-school class with speech, occupational and physical therapy, as well as a sign language class. She was described as a friendly, social girl. Formal developmental evaluation at this age showed mild global developmental delay with more significant expressive language disorder.

Examination showed small, upturned nasal tip, long philtrum, and high palate (Figure 4a and b). She has fair skin and curly hair. Her gait was slightly wide-based. At four years and eleven months, her gait had improved, but was noted to still be slightly abnormal.

aCGH results

Oligonucleotide-based aCGH in patients 1-7 revealed deletions overlapping the previously-described critical region for the 2q33.1 microdeletion syndrome [Van Buggenhout *et al.*, 2005] (Figure 5-supplementary material). Deletions ranged in size from 35 kb to 10.4 Mb. The deletion in Patient (1) started just distal to *SATB2* (chr2:200,063,027-208,691,924, UCSC hg18 coordinates), approximately 20 kb 5' of the gene. The deletion in Patient (6) ended proximal to *SATB2* (chr2:195,531,681-199,625,499), approximately 220 kb 3' of the gene. The smallest deletion was found in Patient (5), whose deletion was entirely within *SATB2* (chr2:199,877,238-199,911,975). The remaining patients had deletions that encompassed *SATB2* (Patient 2, chr2:190,915,507-201,302,003; Patient 3, chr2:194,947,694-200,850,112; Patient 4, chr2:199,340,284-204,310,511; Patient 7, chr2:199,118,846-201,565,929). No other significant copy number changes were found in any individuals.

FISH in the parents of Patients (1) and (6) showed an apparent *de novo* origin; all other parents were unavailable for testing.

DISCUSSION

Small interstitial deletions of the distal portion of the long arm of chromosome 2 are not widely reported, the majority being terminal or much larger than the deletion detected in our patients. This case series will help further refine genotype-phenotype co-relation in 2q33.1 microdeletion syndrome, as patients in our cohort only include part of the critical region defined and may provide information on the critical candidate genes for the various phenotypes in this microdeletion syndrome. Figure 6 is a schematic representation that summarises the position of the reported deletions and the genes implicated in contributing to the phenotype. Patient (1) and (4) have a proximal deletion breakpoint that is more distal to the others reported and may therefore contribute to redefining the minimal deleted region for this syndrome.

In our case series, all patients have varying clinical features of 2q33.1 microdeletion syndrome (Table 1). Facial features in all reported patients with 2q33 microdeletion syndrome appear to be similar including, down-slanting palpebral fissures, low-set ears and prominent nasal bridge with a beaked nose. Most patients also have high-arched/cleft palate. Microcephaly or anatomical brain malformations on cranial imaging do not seem to be a consistent feature in reported patients. This would be consistent with our cohort of patients, as they all have similar facial dysmorphism to patients reported in the literature. In our opinion, there seems to be two distinct facial gestalts, one with up-turned nose, ‘myopathic’ appearance (Patients (3), (7), similar to Van Buggenhout *et al.*, 2005 Patients 2 and 3), and

the other with a prominent nose with down-slanting palpebral fissures (Patient (1), similar to Van Buggenhout *et al.*, 2005 Patient 4). Patient (3), (4) and (5) all have cleft palate while the others have a high-arched palate. All of the patients in our cohort have a high prominent forehead and crowded teeth with impaired dentition, which is also reported. Common manifestations include, significant feeding problems, in most cases requiring naso-gastric feeding and failure-to-thrive. Previous reports have alluded to strabismus and other eye manifestations, which is also seen in Patients (4), (5) and (6) in our series. Neurological features of hypotonia, broad-based gait, moderate to severe developmental delay and significant speech delay with no speech in two patients in this report is also in keeping with previously-published cases.

Rosenfeld *et al.*, in 2009 described three patients with smaller microdeletions within 2q33.1. The results of this study showed that haploinsufficiency of *SATB2* causes some of the clinical features associated with this condition. Patient (5) represents the fourth reported individual with an intragenic *SATB2* deletion. He had a cleft palate repaired, developmental delay and similar facial dysmorphism to other reported patients with the 2q33.1 microdeletion syndrome. He did not have any ectodermal features (Table 1). It is important to note that he has significant speech delay, more obvious than delay in other milestones and no reported behavioural problems, which supports the contribution of other genes to the behavioural phenotype in the 2q33.1 microdeletion syndrome. However, abnormal behaviour was reported in the previous cases with small *SATB2* deletions [Rosenfeld *et al.*, 2009]. Patient (5) provides further support that *SATB2* haploinsufficiency contributes to the developmental delay, cleft palate, and other dysmorphic features seen in the 2q33.1 microdeletion syndrome.

A characteristic feature reported in some individuals with 2q32q33 deletions is an ectodermal dysplasia-like phenotype, with thin, transparent skin and abnormalities of the hair and teeth (Table 1). The skin is reported to be similar to the Wrinkly Skin Syndrome (WSS) and is suggested to be due to haploinsufficiency for *COL3A1* and *COL5A2* [Buggenhout *et al.*, 2005; Rifai *et al.*, 2010]. However, other case reports of 2q32 microdeletions of these genes have alluded to thick hair and eyebrows in some patients [Mencarelli *et al.*, 2007; Prontera *et al.*, 2009]. Furthermore, Patients (2) and (7) in our series have thin, fine and sparse hair, and their deletions do not include these collagen genes, which indicates that there are other genes in the region contributing to the ectodermal phenotype. For example, *SATB2* haploinsufficiency apparently contributes to tooth abnormalities, though other ectodermal features are not observed in patients with deletions limited to *SATB2* [Rosenfeld *et al.*, 2009].

Although orthopaedic problems have been reported in a few individuals with the 2q33.1 microdeletion syndrome, Patient (1) has a Marfanoid habitus, ligamentous laxity, recurrent patellar dislocation and on-going problems with the alignment of her toes. All the other patients in this series have delayed gross motor skills and difficulty with mobility. Further case reports are required to identify if this is an additional feature in the phenotype of patients with the 2q33.1 microdeletion syndrome. This may be explained by a deletion of a gene outside of the critical region described previously in this syndrome.

The behavioural phenotype in patients with 2q33.1 microdeletion syndrome include: hyperactivity, chaotic behaviour, generally ‘happy’ personalities, but with bouts of anxiety or aggression. ‘Happy disposition’ as a younger child seems to be a consistently-reported observation in reported patients and was also seen in the patient reported by Urquhart *et al.*, 2010 and in Patients (3), (4) and (7) in our cohort. Older children with this syndrome are said

to develop bouts of hyperactivity and aggression, which was seen in the patients reported by Van Buggenhout *et al.*, 2005 (Patients 1-3 in their series) and de Ravel *et al.*, 2009. Sleep disorder with frequent awakening also seems to be a feature in previously-reported patients and in Patient (1) in our cohort. In Patient (1), there are significant behavioural problems and she remains under the Psychiatry services. Similarly, Patient (3) and (6) have a specific behavioural phenotype, including ‘autistic-like’ features and inappropriate behaviour. However, the deletions in Patients (1) and (6) do not overlap, suggesting that multiple genes in this region contribute to the behavioural abnormalities seen in these patients. Candidate genes deleted in Patient (1) include *CREB1* (OMIM *123810) and *ADAM23* (OMIM *603710), which have been implicated as possible candidate genes in autism [Pescucci *et al.*, 2003; Valverde *et al.*, 2004], and hyperactivity candidates *ABI2*, *CYP20A1* and *ALS2* [www.genetics.med.ed.ac.uk/suspects/]. Other candidate genes for the neurologic phenotypes seen in individuals with deletions in this region include *ZNF533* (180.01-180.43Mb) and *MYO1B* (191.81-191.99Mb), both of which are involved in neuronal function [Mencarelli *et al.*, 2007; Rifai *et al.*, 2010]. The *GLS* gene (191.45-191.53 Mb) was also suggested as a candidate for the behavioural phenotype, as it is involved in glutamate production [Mencarelli *et al.*, 2007]. In our cohort, *GLS* and *MYO1B* are deleted in Patient (2) (Figure 6). Therefore, while these genes are unlikely to be the critical genes for the phenotype of del 2q33, behavioural abnormalities and significant developmental delays are seen in patients with non-overlapping deletions in this region, so many of these candidate genes may cumulatively contribute to the neurologic phenotype. This region on chromosome 2q has linkage evidence for an autism candidate region, and it is possible that there are multiple genes in the region linked to autism and the behavioural component in this syndrome [Rabionet *et al.*, 2004; Mencarelli *et al.*, 2007; Prontera *et al.*, 2009; Cocchella *et al.*, 2010].

SATB2 is a DNA-binding protein that regulates chromatin organisation and gene expression. It is expressed in the developing brain and is the regulatory determinant of cortico-cortical connections in the developing cerebral cortex [Alcamo *et al.*, 2008]. Haploinsufficiency of the *SATB2* gene causes cleft palate. It has also been shown to be the important determinant in regulating skeletal development, particularly cranio-facial patterning and osteoblast differentiation [Dobrev *et al.*, 2006]. *SATB2* is also a target for SUMOylation (small ubiquitine relative modifier), a reversible modification of the protein that modulates its activity as a transcription factor [Dobrev *et al.*, 2003]. Murine *in vivo* experiments have suggested that *SATB2* haploinsufficiency affects jaw development [Britanova *et al.*, 2006]. Nonsense mutation of *SATB2* has been described in a patient with cleft palate, osteoporosis, severe learning difficulties, epilepsy and cognitive defects [Leoyklang *et al.*, 2007]. The presence of cleft/high-arched palate in all of the patients reported is in concordance with the proposal by Fitzpatrick *et al.*, 2003 that *SATB2* located at 2q32-q33 (199.84-200.03Mb) is a candidate gene for cleft palate. Therefore, there is accumulating evidence of the significance of *SATB2* gene in human development and its role in the developing brain. In the 2q33.1 microdeletion syndrome, it is likely that haploinsufficiency of *SATB2* gene accounts for the developmental delay, cleft/high-arched palate and possibly even the facial dysmorphism and psychiatric problems. Given the role of *SATB2* in osteoblast differentiation, more experiments need to be done to identify if this could account for the skeletal features in the 2q33.1 microdeletion syndrome. Interestingly, another gene in the region, *PPIL3* has been implicated in cleft palate and is known to share isomerise activity with *EBP*, which in humans is known to cause X-linked cleft palate and chondrodysplasia punctata [Ikegawa *et al.*, 2000].

The seven individuals described in this study refine and expand the 2q33.1 microdeletion syndrome phenotype and highlight the psychiatric problems which may need to be managed at an earlier stage. Our results further suggest *SATB2* is the candidate gene for most of the clinical phenotype in this condition. However, given the variability of the phenotype, the study of additional patients may help elucidate the phenotypic effects of other gene/s in this region.

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COMPETING INTERESTS

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Table 1: Comparison of patients reported with 2q33.1 microdeletion syndrome

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	Brewer <i>et al.</i> , (1999)* Patient 1	Brewer <i>et al.</i> , (1999)* Patient 2	Buggenhout <i>et al.</i> , (2005) Patient 1	Buggenhout <i>et al.</i> , (2005) Patient 2	Buggenhout <i>et al.</i> , (2005) Patient 3	Buggenhout <i>et al.</i> , (2005) Patient 4	Tegay <i>et al.</i> , (2009)*	De Ravel <i>et al.</i> , (2009)	Rosenfeld <i>et al.</i> , (2009) Patient 1	Rosenfeld <i>et al.</i> , (2009) Patient 2
Gender	F	F	M	M	M	M	M	M	F	M
Age	10 y	8 y	11 y	20 y	3 y, 8 m	11 y, 11m	<1 y	20 y	9 y, 6m	21 y
Height	50 th	75 th -90 th	3 rd -10 th	<3 rd	<<2 nd	<<3 rd	50 th	<<3 rd	25 th - 50 th	10 th
Weight	75 th	10 th	<<3 rd	<3 rd	<2 nd	3 rd -10 th	50 th	<3 rd	50 th -75 th	5 th -10 th
OFC	50 th	NS	<3rd	75 th	<2 nd	<3 rd	<3 rd	<3 rd	75 th	>95 th
Feeding Difficulties	NS	NS	+, NG feeding	+, NG feeding	+	+, NG feeding	+, NG feeding	NS	-	+
Cranio-facial features	NS	Long, narrow face	Long, high forehead	Pierre-Robin sequence	Asymmetry	Flat occiput	Pierre-Robin sequence	High forehead	Long, narrow face, frontal bossing	Proportionately large head
Ears	Normal	Normal	Low-set	Low-set, dysplastic	Frequent otitis media	Dysplastic	Low-set	Low-set	Slightly large	Normal
Eyes	NS	Convergent squint	Dacryocystitis	Deep-set, down-slanting	Dacryocystitis, coloboma	Down-slanting palpebral fissures	Puffy	NS	Normal	Normal
Nose	Prominent nasal bridge	Prominent nasal bridge	High nasal bridge	High & broad nasal bridge	NS	Prominent, beaked nose	Small nose	NS	Normal	Broad
Upper Lip/Philtrum	Small mouth with distinctive upper lip	Small mouth with distinctive upper lip	Short philtrum	NS	NS	Long philtrum, thin lip	NS	Microstomia	Smooth upper lip	NS
Cleft Palate	+	+	+	+	-; high-arched	-; high-arched	+	high-arched	-	+
Teeth	NS	NS	Short & broad	Short & broad	Abnormal	Absent adult teeth	NA	Broad	Class II malocclusion	Crowded teeth
Chin	NS	Mild micrognathia	Micrognathia	Micrognathia	NS	NS	Severe micrognathia	Micrognathia, protruding	Micrognathia	Small mandible
Digits	Long, slender	Long, slender, 5 th finger clinodactyly	Club feet	Small hands & feet	NS	NS	5 th finger clinodactyly	NS	Nodules from chewing	Broad thumb and big toe
Skeletal	NS	NS	Club feet	NS	NS	NS	Short neck, osteopenia with fractures	Bowed tibiae, bent knees and hips, lumbar lordosis	NS	Prominent heels
Heart	NS	NS	NS	NS	Small VSD	NS	NS	NS	Murmur	Normal
Genitalia	NS	NS	Small, undescended testes	Small testes	NS	Micropenis	NS	NS	Normal	Small testes
Speech	Delayed	Delayed	None	NS	'Donald Duck'	'Donald Duck'	NS	None	20 words	Single words only
Dev. Outcome	Moderate delay	Mild	Severe	Severe	Severe	Severe	Severe delay	Severe delay	Severe delay	Severe delay
Behaviour	NS	NS	Chaotic, aggressive	Difficult, self-mutilation	Mood changes, hyperactive	None	NS	Aggressive behaviour	Repetitive, sociable	Happy, over-eating, aggressive at times
Neurology	NS	NS	NS	Spasticity in lower limbs, broad-based gait, seizures	Severe motor restlessness	Hypotonia	Seizures, obstructive sleep apnoea	Hypotonia	-	Hypertonia
Ectodermal Features	NS	Thin, sparse hair	Thin hair	Thin, sparse hair	Thin, sparse hair	Thin, white hair	NS	No subcutaneous fat, varicose veins	-	-

+, present; -, absent; NS, not specified; NA, not applicable *- Balanced translocation involving 2q

	Rosenfeld <i>et al.</i> , (2009) Patient 3	Urquhart <i>et al.</i> , (2010)	Rifai <i>et al.</i> , (2010)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Gender	F	F	F	F	F	M	M	M	M	F
Age	6 years	5.5 years	19y	19 y	5 y	8.5 y	9 y	3 y	4 y	3.5 y
Height	75 th	10 th	-3.5SD	75 th -91 st	10 th	Normal	<0.4 th	Normal	<5 th	75 th
Weight	50 th -75 th	3 rd	-3SD	9 th -25 th	10 th	Normal	<0.4 th	Normal	<5 th	80 th
OFC	2 nd	91 st -98 th	-4.5SD	9 th -25 th	50 th	Normal	<0.4 th	Normal	50-95 th	40 th
Feeding Difficulties	-	+	+	+, NG feeding	+	+, NG feeding	+	+	+	+
Cranio-facial features	Brachycephalic	Broad, high forehead	Asymmetric face, high forehead	Long, narrow face	High forehead	High fore-head	Prominent forehead	Long, narrow face	High forehead	Normo-cephalic
Ears	Normal	Normal	Low-set, cupped	Low-set, over-turned helices	Mild unilateral HL	Cupped, prominent	Low-set, recurrent otitis media	Low-set; mild HL	Normal	Normal
Eyes	Normal	Deep-set	Down-slanting palpebral fissures	Down-slanting palpebral fissures	Down-slanting palpebral fissures	Hypertelorism	Prominent orbits	Intermittent exotropia	Esotropia; hypermetropia, blond fundus	Normal
Nose	Normal	Normal	Thin, prominent tip	Prominent	Normal	Up-turned	Small, up-turned	Beaked nose, hypoplastic alae nasi	Normal	Upturned nasal tip
Upper Lip/Philtrum	NS	Thin, flat philtrum	Straight upper lip	Thin	Tented upper lip	Long	Thin upper lip, long philtrum	Microstomia, short philtrum	Normal	Thin, long philtrum
Cleft Palate	-	+	++; short; bifid uvula	-; high-arched	-; high-arched	++; narrow	+	+	-; high arched, narrow palate	High palate
Teeth	Fused mandibular central incisors	Missing lateral incisors	Oligodontia with 7 missing teeth	Crowded teeth	Crowded teeth	Crowded, missing front upper incisors	Crowded teeth	-	Crowded teeth	Normal
Chin	Normal	Small	Prominent	Small	Normal	Prominent	Micrognathia	Micrognathia	Normal	Normal
Digits	NS	Broad thumb and big toes, short	Short thumbs, bilat. camptodactyly	Arachnodactyly, claw toes	Normal	Broad thumbs & big toes	?Normal	Overlapping toes	fifth toe clinodactyly	Normal
Skeletal	NS	Generalised joint laxity	Bilateral talipes	Ligamentous laxity, mal-tracking patellae	Normal	Joint laxity; pectus excavatum	Tip-toe walking	Tight heel-cords	pectus carinatum, narrow shoulder girdle	Normal
Heart	Normal	NS	NS	Normal	Normal	Normal	Normal	NS	Murmur	Normal
Genitalia	Normal	NS	Inguinal hernia of ovary	Normal	Normal	Hypospadias	Normal	Normal	Normal	Normal
Speech	No words	Few words	No speech	Severe delay	Few words	No words	No speech	None	Single words only	Few words and signs
Dev. Outcome	Severe delay	Delay	Severe delay	Severe delay	Mild-moderate delay	Moderate delay	Severe delay	Moderate delay	Severe delay	Mild global delay
Behaviour	No sleeping throughout night until age 5	Sociable disposition	NS	Hyperactivity, restless, mood swings	None	Inappropriate hugging, laughter	Violent bursts of aggression	NS	Hyperactivity; autistic features	Friendly, social with occasional tantrums
Neurology	-	Febrile seizures	Hypotonic, broad-based gait	-	-	Hypotonia, balance difficulties, wide-based gait	Hypotonia	-	-	Hypotonia
Ectodermal Features	-	Mottling of skin	Thin, lipotrophic	Fine hair	Sparse, thin hair	-	-	-	Bilateral extensor plantars	Fair skin, curly hair

+, present; -, absent; NS, not specified; NA, not applicable

FIGURE LEGENDS

Figure 1a and b: Facial appearance of Patient (1) at 5 and 10 years of age demonstrating down-slanting palpebral fissures, prominent nose and long face.

Figure 1c and d: Facial appearance of Patient (1) at 19 years of age and arachnodactyly

Figure 2a and b: Facial appearance of Patient (3) demonstrating down-slanting palpebral fissures, absent dentition

Figure 3a and b: Facial appearance in Patient (4) demonstrating prominent forehead, myopathic face

Figure 4a and b: Facial appearance of Patient (7) demonstrating down-slanting palpebral fissures, thin upper lip and long philtrum

Figure 5A1, 2, B and C [Supplementary Material]: Microarray and FISH characterization of deletions within 2q32.2q33.1. A1- Microarray results on Patient 1. A-2- Oligonucleotide-based microarray characterization of deletions within 2q32.2q33.1. Probes are arranged with the most proximal 2q32.2 probes on the left and the most distal 2q34 probes on the right. Results are displayed using custom aCGH analysis software (Genoglyphix; Signature Genomic Laboratories). Analysis of Patient (2) showed a single-copy loss of 656 oligonucleotide probes, 10.39 Mb in size; Patient (3), a single-copy loss of 483 oligonucleotide probes, 5.90 Mb in size; Patient (5), a single-copy loss of 5 oligonucleotide probes, 35 kb in size; Patient (6), a single-copy loss of 331 oligonucleotide probes, 4.09 Mb

in size; Patient (7), a single-copy loss of 57 oligonucleotide probes, 2.45 Mb in size. All genes in the region annotated within the Reference Sequence database are shown as blue and grey boxes.

(B) FISH showing a deletion at 2q33.1 in Patient (2). BAC clone RP11-404F23 from 2q33.1 is labeled in red, and the chromosome 2 centromere probe is labeled in green as a control.

The presence of one red signal indicates deletion of 2q33.1 on one homologue (arrow). (C)

FISH showing a deletion at 2q32.3 in Patient (6). BAC clone RP11-259B3 from 2q32.3 is labeled in red, and the chromosome 2 centromere probe is labeled in green as a control. The presence of one red signal indicates deletion of 2q33.1 on one homologue (arrow).

Figure 6: ArrayCGH results in current cohort and patients in the published literature. Green bars represent minimal deletion sizes, and horizontal lines extend through gaps in coverage to show maximum deletion sizes. Genes of note within region are represented by blue boxes.

PATIENT 1

PATIENT 3

PATIENT 4

PATIENT 7

**Figure
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B.

Patient 12

C.

Patient 15