

ARTICLE

Chromosome 22q12.1 microdeletions: confirmation of the *MN1* gene as a candidate gene for cleft palate

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We report on seven novel patients with a submicroscopic 22q12 deletion. The common phenotype constitutes a contiguous gene deletion syndrome on chromosome 22q12.1q12.2, featuring *NF2*-related schwannoma of the vestibular nerve, corpus callosum agenesis and palatal defects. Combining our results with the literature, eight patients are recorded with palatal defects in association with haploinsufficiency of 22q12.1, including the *MN1* gene. These observations, together with the mouse expression data and the finding of craniofacial malformations including cleft palate in a *Mn1*-knockout mouse model, suggest that this gene is a candidate gene for cleft palate in humans.

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INTRODUCTION

Orofacial clefts are the most common craniofacial human birth defect, affecting the lip only (CL), both the lip and the palate (CLP) or the palate alone (CP). CL and CLP can occasionally occur within the same pedigree. These defects are considered to arise from perturbations of a common pathway that underlies the growth or fusion of the maxillary, lateral and medial nasal processes, which form the upper lip and the primary palate.¹ CP is considered to be a distinct developmental entity, which arises from a defective growth, elevation or fusion of the palatal shelves. The palatal shelves emerge from the maxillary processes to form the secondary palate, which separates the nasal and oral cavities.^{1,2} Defective palatal shelf development can be evoked by genetic variants, environmental factors or a combination thereof.³ These hazards can be intrinsic to the palatal shelves, or can affect bones in the vicinity of the palatal shelves with a secondary effect on palatal development through mechanical hindrance (developmental deformation sequence). For example, palatal elevation depends on mandibular elongation.⁴ As the mandible elongates, the tongue is drawn forward and the palatal shelves are able to elevate and orientate horizontally. Therefore, the cleft palate can arise from primary defects of mandibular outgrowth, or from problems that are external to the mandibular skeleton but secondarily restrict its growth (eg, intrauterine constraint due to oligohydramnion).⁴ This triad of mandibular hypoplasia, glossoptosis and cleft secondary palate is referred to as Pierre–Robin sequence (PRS). Isolated PRS was found to be associated with variants in non-coding elements upstream of the *SOX9* gene,⁵ whereas common genetic causes for syndromic PRS include Stickler syndrome (*COL2A1*, *COL11A1*, *COL11A2*), Campomelic Dysplasia (*SOX9*), trisomy 18 and 22q11 microdeletion syndrome.

Most clefts are isolated (70% of CL or CLP and 50% of CP), sporadic and likely have a multifactorial cause. Orofacial clefts, associated with developmental delay, dysmorphic features or other major congenital anomalies, are defined syndromic. These mostly have a single genetic cause, either chromosomal or monogenic. Genetic causes of syndromic and nonsyndromic CL, CLP and CP were recently reviewed.^{6,7} Next to overt cleft lip or palate, the spectrum of orofacial clefts covers subclinical phenotypes, including microform clefts of the lip, defects of the musculus orbicularis oris,⁸ bifid uvula, submucous CP and velopharyngeal insufficiency. These subphenotypes may complicate genetic counseling and should be considered in genetic study design.

Palatal defects and PRS in association with schwannoma of the vestibular nerve have been recently described in patients with deletions of 22q12.⁹ Here we report on a further seven patients with a submicroscopic deletion involving chromosome 22q12, and presenting with multiple congenital anomalies, including a heterogeneous spectrum of palatal defects, vestibular schwannomas and corpus callosum agenesis (Table 1, Figure 1).

CASE REPORTS

Patient 1 – Decipher 287905

This 2.5-year-old boy was the second child of healthy, unrelated parents. Family history was unremarkable with regard to congenital anomalies or developmental delay. He was born at term after an uneventful pregnancy. His birth weight was 3.835 kg, his length was 52 cm and his head circumference was 36.5 cm (all at the 75th centile). At birth, he presented with facial dysmorphic features, including a large anterior fontanel, hypertelorism and a depressed nasal bridge. He had retrognathia and a hypoplastic soft palate with a cleft.

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Table 1 Clinical and molecular data associated with 22q12.1 deletions in our patient cohort

	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6		Case 7	
	Decipher 287905		Decipher 256661		Decipher 290734		Decipher 269448		Decipher 274296		Decipher 758			
Gender/age	Male, 2.5 years		Female, 7 years		Female, 18 years		Male, 2 years		Female, 5 years and 9 months		Male, 6 years		Female, 18 years	
Array CGH platform	OGT array 180k		Affy Cytoscan 750k SNP array		Agilent 244k		Affy Cytoscan 750k SNP array		BlueGnome ISCA 8x60k		Agilent 60K		1 Mb BAC array	
Deletion size	4.3 Mb, <i>de novo</i>		3.58 Mb, <i>de novo</i>		3.59 Mb, <i>de novo</i>		3.04 Mb, <i>de novo</i>		2.17 Mb, <i>de novo</i>		0.93 Mb, NA		1.15 Mb	
Hg19 coordinates (bp)	25 689 977 – 30 038 041		27 266 032–30 848 647		26 727 554–30 313 733		26 980 074 – 30 016 888		26 857 719 – 29 029 963		27 375 542 – 28 307 008		29 281 852 – 30 429 113	
Growth	P75		P10		P25		P10		P75		P50		P25	
Head size	P75		P50–75		P10		P75		P3		P50		P90	
Cleft palate	Cleft soft palate, retrognathia		Cleft soft palate, retrognathia		High palate, nasal speech, retrognathia		Normal		Normal, nasal speech		Severe VPI, ogival palate		Cleft uvula, VPI	
Dysmorphism	Large anterior fontanel, hypertelorism, depressed nasal bridge		Large anterior fontanel, strabismus, wide forehead, short downslanting eyes, arched eyebrows, prominent nasal bridge		Short palpebral fissures, strabismus, hypertelorism prominent nose and maxilla, microtia		Yes		Epicanthus, webbed neck, narrow ear canals		Large dysplastic ears, prominent nose, short palpebral fissures		Amblyopia, mild hypertelorism, smooth philtrum, low-set ears	
Hearing loss	Conductive		No		Perceptive (A)		Normal		Conductive		Normal		Perceptive (A)	
MRI brain	Hypoplastic corpus callosum		Hypoplastic corpus callosum		Hypoglossal, bilateral vestibular and spinal schwannoma		NA		NA		Large ventricles, global atrophy		Bilateral acoustic schwannoma	
Developmental delay	Moderate		Mild global and severe speech delay		Mild delay and autism		Global and speech delay		Delayed speech, hypotonia		Absent language		Mild delay and hyperactive	
Heart defect	Normal		Atrial septal defect		Normal		Normal		Normal		Normal		Mild PS, PPS	
Other	Hypoplastic terminal phalanges		Ectopic kidney, long slender fingers, susceptible to infections		Unilateral choanal atresia, café au lait spots, susceptible to infections		Broad hallux, tapered fingers, laryngomalacia		Sublingual cysts leg length discrepancy, postural scoliosis		Vocal cord paralysis, arthrogryposis, feeding problems		Long fingers myopia	
Gene content	MN1, NF2?		MN1, NF2		MN1, NF2		MN1, NF2?		MN1		MN1		NF2	

Abbreviations: A, acquired; MRI, magnetic resonance imaging; NA, not available; PPS, peripheral pulmonary stenosis; PS, pulmonary valve stenosis; VPI, velopharyngeal insufficiency. All genome coordinates are according to NCBI human genome build 37 (hg19).

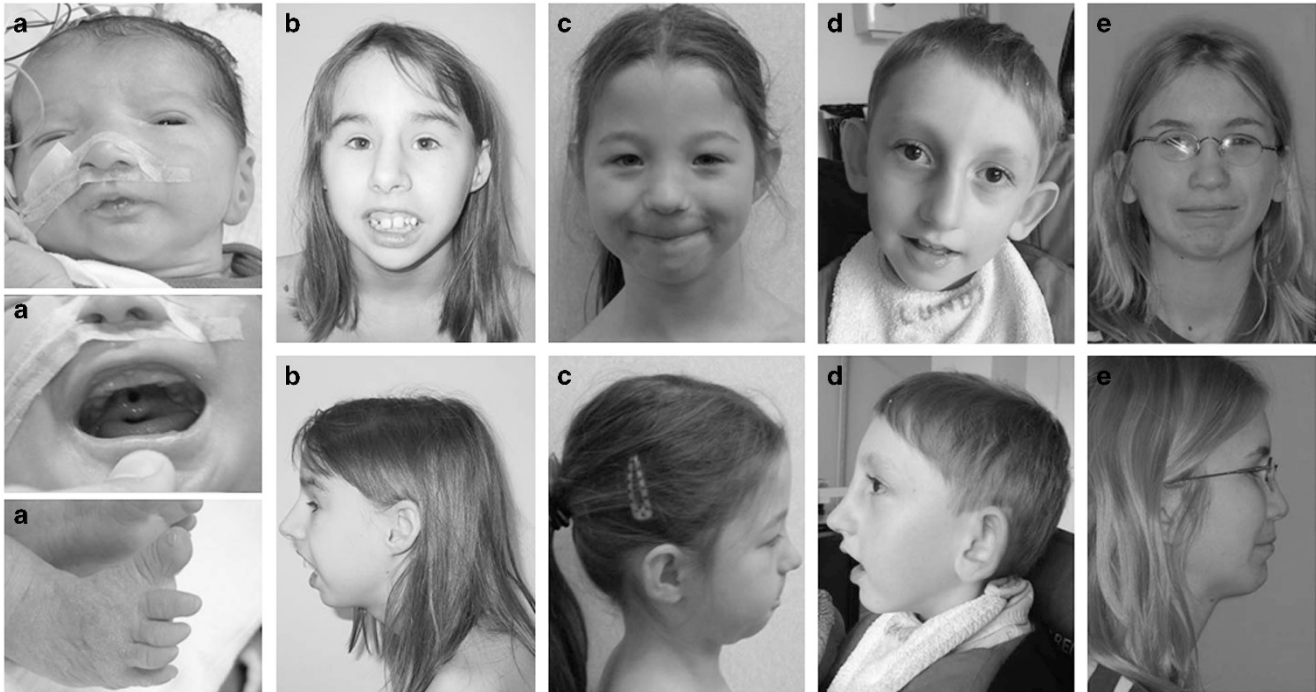


Figure 1 Facial dysmorphic features in patients with 22q12 deletions. (a) Patient 1 presented with a cleft soft palate and hypoplastic terminal phalanges. (b) Patient 3 aged 17 years. (c) Patient 5 at 5 years of age. (d) Patient 6 at the age of 6 years and 6 months. (e) Patient 7 at the age of 17 with postoperative facial nerve paralysis and mild dysmorphic features.

The terminal phalanges and the nails of the toes were hypoplastic, and the nipples were widely spaced. He had feeding problems, requiring tube feeding. He was hypotonic, with uncontrolled, irregular movements. No epileptic activity was recorded on EEG monitoring. Brain MRI revealed the presence of corpus callosum hypoplasia. Eye exam and cardiac ultrasound were normal. No other organ malformations were noted. At 7 months of age, transtympanic drains were inserted to treat for serous otitis media with bilateral conductive hearing loss. Surgery for cleft palate was performed at the age of 15 months. At 2.5 years he weighs 13.3 kg (25–50th centile), his length is 90 cm (10–25th centile), and his head circumference is 50.7 cm (50–75th centile). He presented with strabismus en thoracic kyphosis. Moderate motor delay was noted. The patient was able to sit and walk, independently, at 10 months and at 2 years, respectively. At 2.5 years his receptive and productive language skills are in accord with those of a 15-month-old (Figure 1a).

A 22q11q12.2 deletion was detected in this boy by means of comparative genome hybridization (CGH) using a 180 k oligo array platform (OGT CytoSure Syndrome Plus array, OGT Oxford, UK), performed according to the manufacturer's instructions. The deleted region spans maximally 4.3 Mb (25 689 977_30 038 041del) with the distal break point affecting the *NF2* gene. This deletion was not found in the parents using the same array platform.

Patient 2

This 7-year-old girl was born as the first child from healthy, nonconsanguineous parents, after a pregnancy which was complicated by hyperemesis and polyhydramnios. Her birth weight and length (52 cm, 75th centile) were normal. She presented with a large anterior fontanel, and a posterior cleft of the soft palate, which was operated twice. She had an atrial septal defect, which was closed percutaneously in infancy. A right pelvic ectopic kidney was detected on abdominal

ultrasound. At the age of 2, she underwent ophthalmic surgery for bilateral lateral rectus muscle palsy, causing strabismus. She was susceptible to recurrent respiratory infections. Brain MRI at 6 months revealed a thin corpus callosum with mild ventriculomegaly. No seizures were noted. Her motor development was severely delayed: head control, sitting without support and walking independently were obtained from the age of 1 year, 1.5 year and 3 years, respectively. She has an affable, friendly character, and uses simple words and three-word-sentences. She has had intensive physical and speech therapy. At the age of 7 years, she was unable to read or write.

At 7 years of age, biometry was normal, with a relatively large head (52 cm, 50–75th centile), in comparison with height (at 10th centile). Clinical evaluation revealed facial dysmorphism: wide forehead with depressed supraorbital ridge, short downslanting palpebral fissures, arched eyebrows, a prominent wide nasal bridge, wide alae nasi, malar hypoplasia and low-set, posteriorly rotated ears. She showed an open mouth appearance with a high arched palate and full lips. She had multiple teeth decay and retrognathia, requiring orthodontic treatment. She presented with a narrow thorax and long, slender fingers and toes. Thus far, no hearing loss was found (Figure 1b). Follow-up by means of brain and spine imaging was planned.

High-resolution genome-wide DNA copy-number analysis, using platform CytoScan HD Array (Affymetrix, Santa Clara, CA, USA), was performed according to the manufacturer's protocol, and revealed a 3.58-Mb deletion in 22q12.1q12.2 (27 266 032_30 848 647del), comprising *NF2*. Data were evaluated with the Affymetrix Chromosome Analysis Suite software. Parental analysis by means of FISH analysis showed that the deletion occurred *de novo*.

Patient 3 – Decipher 256661

This 18-year-old girl was the second child of healthy, unrelated parents. Familial medical history was unremarkable. She was born at

term. Her birth weight and length were 2885 g (25th centile) and 46 cm (10th centile), respectively. During the first year, her development was delayed and at 9 months she had a serious apnea event. Clinical evaluation revealed a unilateral choanal atresia, which was corrected surgically. Facial dysmorphic features included short palpebral fissures, hypertelorism, a prominent nose and maxilla, a receding chin and small ears. She presented with nasal speech and a high palate without overt clefting. She has had a late eruption of teeth and strabismus. Her fingers and toes were long. She had four café au lait spots. Ultrasound of the heart and kidneys was normal. She has been susceptible to infections and had suffered from meningitis at several occasions. She walked independently at two and a half year of age. Although her hearing was normal, her speech development was delayed. She had been diagnosed with mild intellectual disability and high functioning autism. At age 17, she presented with hearing impairment, mainly on the right side. Brain MRI revealed a large vestibular schwannoma on the right side and a smaller tumor on the left (Figure 1c). One year later, a 12-mm schwannoma emerged from the right hypoglossal nerve and progression of both vestibular schwannomas was noticed. MRI imaging of the spine at the age of 18 years, revealed 1–2 mm schwannomas in the roots of the second and third cervical nerve, left and right, respectively. In addition, a schwannoma, sized 4 mm, was detected in the cauda equina at the level of lumbar 2.

Genetic evaluation with chromosome analysis, subtelomeric FISH screening and mutation analysis of *CHD7* were normal. Because of the combined picture of *NF2*-related symptoms with facial dysmorphism and developmental delay a chromosomal disorder was suspected. Array CGH, using the Agilent 244k platform (Agilent Technologies, Santa Clara, CA, USA), was performed according the manufacturer's instructions, and revealed a 3.59-Mb deletion in 22q12.1q12.2 (26 727 554_30 313 733del), encompassing the *NF2* gene. The deletion occurred *de novo*.

Patient 4 – Decipher 290734

This boy was born from healthy, nonconsanguineous parents, after an uneventful pregnancy. At birth, he presented with facial dysmorphic features, a broad hallux, tapered fingers and laryngomalacia. No palatal cleft nor velopharyngeal insufficiency were noted. Birth weight was normal. At the age of 2 years, biometry was normal, with a relatively short stature (84 cm, 9th centile), compared with his weight (13.7 kg, 75th centile) and head size 51.2 cm (75th centile). His development was globally delayed: he sat and walked independently since the age of 8 months and 18 months, respectively. At 2 years of age, he had a limited vocabulary, saying a handful of simple words. He showed copious drooling. His hearing was not impaired. Imaging of the brain has not been performed thus far.

Molecular karyotyping, using the Affy Cytoscan 750 k SNP genotyping array (Affymetrix), was performed according to the manufacturer's instructions. It revealed a 3.4-Mb deletion on 22q12.1q12.2, ranging from 26 980 074 to 30 016 888, hereby presumably affecting the promoter region and first exons of the *NF2* gene. Parental analysis, using the same array platform, showed that the deletion occurred *de novo* in the patient.

Patient 5 – Decipher 269448

This girl was born from healthy, unrelated parents with facial dysmorphic features, including epicanthic folds, a webbed neck and narrow ear canals. Birth weight was normal (3.997 kg). At birth, she presented with sublingual cysts, which resolved spontaneously. No overt cleft palate was noted. This girl developed a bilateral

conductive hearing loss, which was attributed to bilateral glue ear. Hearing aids, as well as speech and language therapy, were introduced to improve speech, as she presented with nasal speech and speech delay, performing at the level of a 3-year old at the age of 5. She showed significant improvement with speech therapy, but today she still misses off ends of words and still has a nasal speech. Aged 5, normal motor and social development was observed. Weight and length were at the 25th and 75th centile, respectively. Head size was 49.5 cm (3rd centile). She was hypotonic and hypermobile, and presented with a 1-cm leg length discrepancy and a postural scoliosis. No brain imaging has been performed.

Molecular karyotyping was performed by means of the BlueGnome ISCA 8 × 60 k OligoArray v2.0 and analyzed with Bluefuse Multi software (BlueGnome Ltd, Cambridge, UK). A 2.17 Mb deletion on 22q12.1 was detected, ranging from 26 857 719 to 29 029 963. The deletion occurred *de novo*.

Patient 6 – Decipher 274296

At birth, this boy presented with neonatal vocal cord paralysis and arthrogryposis. In addition, he featured large dysplastic ears, an ogival palate and severe velopharyngeal insufficiency. He suffered from feeding problems and required a gastrostomy for enteral nutrition. Biometric parameters were all at the 50th centile. At 5 years of age, vocal language was totally absent, but he could communicate using pictograms and signs, and he understood simple orders (Figure 1e). Brain MRI revealed large ventricles and global cortical atrophy. His mother is healthy. Paternal data could not be retrieved.

A 22q12.2 deletion was detected by means of array CGH, using the Agilent 60 K array platform (SurePrint G3 Human CGH Microarray 8 × 60 K) and Agilent CytoGenomics software (Agilent Technologies), according to the manufacturer's instructions, and was confirmed by FISH analysis. The deletion spans 0.93 Mb (27 375 542_28 307 008del), and comprises *MNI*, but not the *NF2* gene. The deletion was not found in the mother by means of FISH analysis. The father was not available for further testing.

Patient 7 – Decipher 758

This girl was the third child of healthy, nonconsanguineous parents. Family history was negative with regard to malformations or mental handicap. She was born at term with birth weight 4 kg (90th centile), length 52 cm (75th centile) and head circumference of 35.5 cm (75th centile). She had feeding problems, with nasal regurgitation. Mild pulmonary valve stenosis and peripheral pulmonary stenosis were observed, which did not require any intervention. She had a cleft uvula. At the age of 3 years, she had surgery for strabismus. She had developed amblyopia, with residual vision of 1/10 at the left eye. She had a high myopia of – 5 and – 8 Diopter.

At the age of 4 years 8 months, she showed a normal growth. Her weight was 16.7 kg (at the 25th centile), her length was 104 cm (at the 25th centile) and head circumference was 52.5 cm (at the 90th centile). Facial features were not remarkable, with mild hypertelorism and smooth philtrum. She had long fingers and toes, with shorter 5th toes. The uvula was bifid. Speech was hypernasal due to velopharyngeal insufficiency, for which a pharyngoplasty was performed at age 5.

She walked at the age of 18 months, but especially her language development was delayed. At the age of 4 years and 9 months her total, verbal and performance IQ were 83, 85 and 84, respectively (Wechsler scales). She followed special education for children with mild learning difficulties. In addition, she was followed by a children's psychiatrist because of hyperactivity. Brain MRI at 3 years of age was

normal. When re-examined at the age of 14 years, she experienced no medical problems. Biometry was normal.

A tentative diagnosis of 22q11 deletion syndrome could not be confirmed by means of fluorescence *in situ* hybridization. This patient was described previously in a set of 60 patients with idiopathic syndromic heart defects, which were screened for submicroscopic anomalies by means of array CGH with an estimated resolution of 1Mb.¹⁰ A *de novo* microdeletion was detected on chromosome 22q12.2. The deletion break points were further delineated using a 180k oligo array platform, performed as described above (OGT CytoSure Syndrome Plus array). The deletion spans 1.15 Mb (29 281 852_30 429 113del) and comprises the *NF2* gene. As haploinsufficiency of *NF2* predisposes to neurofibromatosis type 2, brain MRI was repeated at 14 years of age, and revealed the presence of bilateral acoustic schwannomata, before clinical manifestation. A spine MRI was normal. Rapid tumor growth with progressive perceptive hearing loss at the age of 17 years necessitated for a resection of the left-sided acoustic schwannoma, which was complicated by a postoperative facial nerve paralysis (Figure 1d).

DISCUSSION

We report on seven patients with submicroscopic deletions within the 22q12.1q12.2 region. Informed consent was obtained from all patients or their legal representatives to share clinical data and pictures. Molecular and clinical details are summarized in Table 1, and were submitted to DECIPHER, a public database for chromosomal variants (<https://decipher.sanger.ac.uk/>). Deletion sizes range from 1.15 to 4.3 Mb. All deletion break points are unique, advocating non-homologous end-joining as the underlying pathogenic mechanism (Figure 2). Five out of seven patients present with palatal malformation or dysfunction, ranging from a high arched palate to overt cleft palate. Overt clefting of the secondary palate is observed in two patients. Mandibular hypoplasia is associated in three patients. Other

recurrent features include schwannoma of the auditory vestibular nerve and agenesis of the corpus callosum.

Palatal defects, including PRS, have been described in three patients harboring a 22q12.1q12.2 deletion^{9,11,12} and in one Decipher patient (Decipher 4110)⁹ with a complex chromosomal rearrangement on chromosome 22, including a *de novo* 2 Mb deletion on 22q12.2 and a typical duplication of the diGeorge region on 22q11. Clinical and molecular details are provided in Table 2 and Figure 2. Review of the literature for 22q12.2 deletions reveals a further three patients with vestibular schwannoma but without palatal defects,^{13,14} and one additional Decipher patient (Decipher 999)⁹ with poor clinical data (Supplementary Table 1). Combining our data with those provided in literature, we discern 22q12.2 deletions in six patients with an overt cleft secondary palate (Figure 2), and in three with minor palatal defects, including velopharyngeal insufficiency, high arched palate and cleft uvula. The overlapping region for overt CP covers 1.47 Mb (chr22: 27 411 505 - 28 885 620) and contains three genes: *MN1*, *PITPNB* and *TTC28*, of which *MN1* has been suggested as a candidate for CP and PRS before.⁹ *MN1*, an oncogene in murine hematopoiesis, is considered a candidate gene for sporadic meningioma¹⁵ and is involved in human myeloid leukemia.^{16–18} *Mn1* is shown to be crucial in the development of membranous bones in the murine skull. In homozygous *Mn1*-knockout mice, cranial bone development is severely affected with decreased mineralization, hypoplasia or agenesis of several bones. In addition, palatal shelf elevation, particular in the middle and posterior regions, is impaired. This results in cleft secondary palate, leading to death of *Mn1* null mice shortly after birth. Heterozygous *Mn1* ± mice present an intermediate phenotype, including hypoplasia of the maxillary and palatal shelves and cleft secondary palate with incomplete penetrance.¹⁹ The role for *MN1* in cleft palate development is further supported by the finding of an increased allele frequency (although not significant) at SNP rs5752638 of *MN1* in patients with submucous cleft palate compared to control

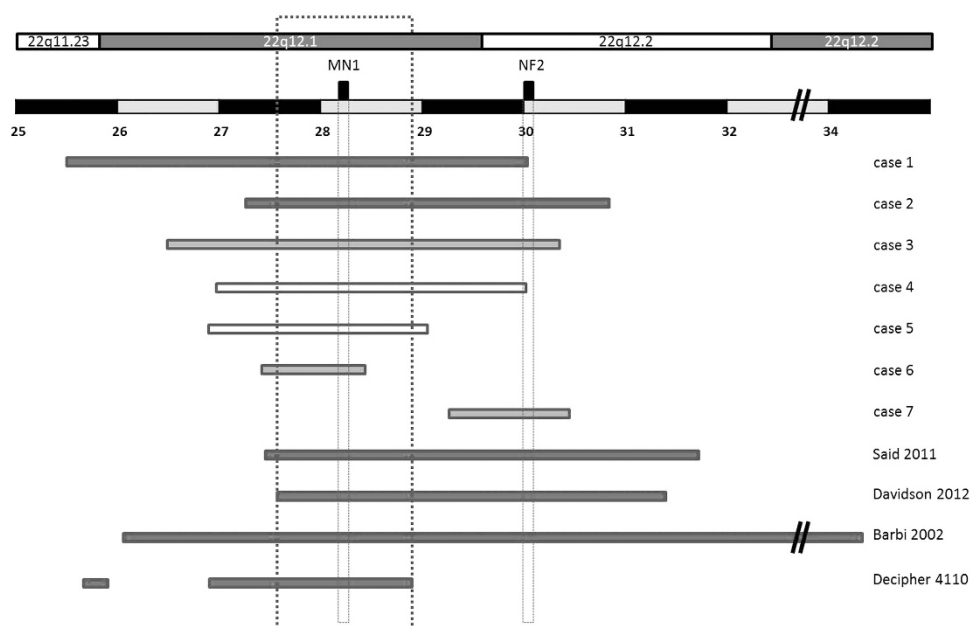


Figure 2 Overview of 22q12 deletions in our patient cohort, complemented with four previously reported cases^{9,11,12} and one Decipher patient (Decipher 4110) with a complex chromosomal rearrangement on chromosome 22, including a *de novo* 2 Mb deletion of 22q12.2 and a typical duplication of the diGeorge region on 22q11. Deletions associated with overt cleft palate are depicted in deep purple, those associated with minor palatal defects (high arched palate or velopharyngeal insufficiency) in light purple, and those without palatal malformations in white. All genome coordinates are according to NCBI human genome build 37 (hg19, February 2009). A full color version of this figure is available at the *European Journal of Human Genetics* journal online.

Table 2 Clinical and molecular data previously described in patients with 22q12.1 deletions

	Said, 2011 <i>Decipher 252737</i>	Davidson, 2012	Barbi, 2002	<i>Decipher 4110</i>
Gender/age	Female, 2 years	Female, 15 years	Female, 8 months	NA
Array CGH platform	1 Mb BAC array	Affymetrix SNP 6.0	FISH analysis	NA
Deletion size	4.3 Mb	3.7 Mb	8.16 Mb, <i>de novo</i>	2.01 Mb ^p
Hg19 coordinates (bp)	27 411 505–31 712 873	27 607 541–31 305 842	26 043 590–34 210 916	26 877 538–28 885 620
Growth	P3	NA	<P3	NA
Head size	P3	NA	<P3	NA
Cleft palate	Cleft uvula, soft and hard palate, microretrognathia	Cleft palate, micrognathia	Cleft palate, microretrognathia	Submucous cleft palate
Dysmorphism	Large anterior fontanel, low anterior hairline, frontal bossing, hypertelorism, anteverted nares, long columella, downturned mouth, low-set dysplastic ears	Hypertelorism, bulbous nose tip, dysplastic ears	Depressed nasal root, hypertelorism, short downslanting palpebral fissures, short philtrum, tented upper lip, low-set ears	Proptosis, dysplastic ears, preauricular pit
Hearing loss	NA	Conductive at preschool age, mixed at age 14	NA	Hearing impairment not further defined
MRI brain	Corpus callosum agenesis large cisterna magna	Bilateral acoustic schwannoma, extramedullary intra- & extradural neurofibroma	Ventriculomegaly, pachygyria, corpus callosum hypoplasia	NA
Developmental delay	Moderate	Yes	Severe	Yes
Congenital heart defect	Atrial septal defect, VSD, PDA	NA	Dextrocardia, interrupted aortic arch, subaortic stenosis, VSD, bilateral superior caval veins	NA
Other	Single palmar crease, accessory nipple, pectus excavatum, hypoplastic terminal phalanges	Ataxia	Situs inversus, duodenal stenosis with annular pancreas	Tooth abnormalities, cataract, hypoplasia of terminal phalanges
Gene content	<i>MN1, NF2</i>	<i>MN1, NF2</i>	<i>MN1, NF2</i>	<i>MN1</i>

Abbreviations: FISH, fluorescence *in situ* hybridization; MRI, magnetic resonance imaging; NA, not available; PDA, patent ductus arteriosus; VSD, ventricular septum defect.
p: *Decipher* patient 4110 was found to have a *de novo* duplication of the *clGeorge* region on 22q11 (18 895 226–22 508 432) and a 190 kb deletion of 22q11.23 (25 719 287–25 911 071) as well.

individuals.²⁰ Moreover, *Mn1* acts as a transcriptional activator of *Tbx22* during palatal development in mice.²¹ Variants in the human *TBX22* gene have been associated with X-linked cleft palate and ankyloglossia^{22,23} and with isolated CP.²⁴ Taken together, we assume that haploinsufficiency of *MN1* contributes to the association of cleft palate in humans with 22q12.2 deletions, either resulting from primary defects in palatal growth or from secondary effects of other craniofacial abnormalities (eg, micrognathia). However, consistent with the mouse model, we observe incomplete penetrance for palatal defects. Patients 3, 5 and 6 present with retrognathia, a high arched palate or velopharyngeal insufficiency, but no overt clefting. Moreover, patient 7 features cleft uvula, but the detected 22q12 deletion is located beyond *MN1*. We assume that haploinsufficiency of other genes within the 22q12 region may modify *MN1* expression or may independently affect the genesis of palatal defects.

Patients with loss of *NF2* function through nucleotide changes within or deletions comprising *NF2*, a tumor suppressor gene on 22q12.2, are predisposed to Neurofibromatosis type 2 (OMIM: 101000,²⁵). Constitutional *NF2* mutations in combination with a somatic second hit of *NF2* may lead to the development of schwannoma of the vestibular nerve with progressive hearing loss or tinnitus. In addition, other tumors, including schwannomas of other cranial nerves, peripheral nerve schwannoma, meningioma and ependymoma and non-neoplastic features such as cataracts, retinal hamartomas, epiretinal membranes, meningoangiomas and axonal polyneuropathy may manifest. Truncating *NF2* variants are considered to cause a more severe phenotype, with younger age of onset, higher proportion of meningiomas and spinal tumors and higher number of peripheral nerve tumors, compared with those with an in-frame *NF2* deletion²⁶ or with a large deletion that comprises *NF2* completely.^{13,27} This hypothesis was challenged recently by the report of a severe *NF2*-related phenotype in a patient with a large 22q12 deletion, including *NF2*.⁹ Within our series of 22q12 deletions, two out of three patients with complete loss of *NF2* presented with schwannomas of the vestibulocochlear nerve, one of which was identified before clinical manifestation. The *NF2* phenotype in these two patients is considered severe, given the age at tumor onset, and the presence of peripheral nerve manifestations and spinal tumors in patient 3. The deletions in patient 1 and 4 only partially affect the *NF2* gene. These chromosomal imbalances include the promoter region of *NF2* and therefore we speculate that the tumor risk in these patients will be similar to those lacking a complete allele of *NF2*. Long-term follow-up, including brain and spine MRI, will permit more accurate delineation of the severity of the phenotype in all patients harboring a complete or partial germline loss of the *NF2* gene. Haploinsufficiency of neighboring genes within 22q12 may alleviate or aggravate the patients' phenotype. Co-deletion of *CHEK2*, a DNA repair and tumor suppressor gene on 22q12, in combination with *NF2* may provoke a higher incidence of aggressive meningioma, as enforced *Chk2* knockdown in human meningioma cells decreases DNA repair and increases chromosomal instability.²⁸ To support this hypothesis, regular follow-up by clinical neurologic examinations, audiometry, ophthalmologic examination and imaging of brain and spine by MRI, is recommended in all patients harboring a 22q12.2 deletion, comprising *NF2*. The deletions detected within our cohort do not comprise *SMARCB1* nor *LZTR1*. Constitutional loss-of-function variants in either of these two tumor suppressor genes located on 22q11 were shown to predispose to multiple schwannomatosis. The schwannomas in these individuals show a three-stage four-hit sequence with somatic loss of one copy of 22q together with inactivating somatic variants of *NF2* on the remaining chromosome 22.^{29,30}

Agensis or hypoplasia of the corpus callosum is associated with 22q12 deletions in two of the patients reported here, and in two previously described patients.^{11,12} This region does not contain any known genes involved in corpus callosum development. The association of corpus callosum agenesis, PRS and facial dysmorphism fits the diagnosis of the Toriello–Carey syndrome, a rare presumable autosomal recessive entity with unknown genetic etiology (TCS; OMIM: 217980).³¹ Interestingly, 22q12 deletions were detected before in three patients with clinical diagnosis of TCS,^{12,32} suggesting clinical resemblance between the 22q12 deletion syndrome and TCS. However, in our case series, the facial phenotype does not correspond to the TCS facial gestalt, which includes short palpebral fissures, hypertelorism, a small nose and full cheeks. Nevertheless, chromosomal analysis by means of array CGH is advisory in all patients with a clinical phenotype of TCS.

CONCLUSION

We further delineate a novel contiguous gene deletion syndrome on chromosome 22q12, featuring *NF2*-related schwannoma of the vestibular nerve and palatal defects with or without mandibular hypoplasia. We propose that the patients with a microdeletion comprising *NF2* should be followed by a multidisciplinary *NF2* team in addition to the follow-up for cleft palate and intellectual disability. Combining our results with those provided in the literature,^{9,11–14} eight patients are recorded with palatal defects in association with a 22q12 deletion, involving the *MN1* gene. These observations, together with an increased *MN1* SNP frequency in patients with submucous cleft palate,²⁰ with the mouse expression data and the finding of craniofacial malformations including cleft palate in a *Mn1*-knockout mouse model further suggest that this gene contributes to the cleft palate observed in the reported patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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