RESEARCH ARTICLE

Application of high resolution SNP arrays in patients with congenital oral clefts in south China

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Abstract

Chromosome microarray analysis (CMA) has proven to be a powerful tool in postnatal patients with intellectual disabilities. However, the diagnostic capability of CMA in patients with congenital oral clefts remain mysterious. Here, we present our clinical experience in implementing whole-genome high-resolution SNP arrays to investigate 33 patients with syndromic and nonsyndromic oral clefts in whom standard karyotyping analyses showed normal karyotypes. We aim to identify the genomic aetiology and candidate genes in patients with congenital oral clefts. CMA revealed copy number variants (CNVs) in every patient, which ranged from 2 to 9 per sample. The size of detected CNVs varied from 100 to 3.2 Mb. In 33 patients, we identified six clinically significant CNVs. The incidence of clinically significant CNVs was 18.2% (6/33). Three of these six CNVs were detected in patients with nonsyndromic clefts, including one who presented with isolated cleft lip with cleft palate (CLP) and two with cleft palate only (CPO). The remaining three CNVs were detected in patients with syndromic clefts. However, no CNV was detected in patients with cleft lip only (CLO). The six clinically significant CNVs were as follows: 8p23.1 microduplication (198 kb); 10q22.2-q22.3 microdeletion (1766 kb); 18q12.3 microduplication (638 kb); 20p12.1 microdeletion (184 kb); 6q26 microdeletion (389 kb); and 22q11.21-q11.23 microdeletion (3163 kb). In addition, two novel candidate genes for oral clefts, KAT6B and MACROD2, were putatively identified. We also found a CNV of unknown clinical significance with a detection rate of 3.0% (1/33). Our results further support the notion that CNVs significantly contributed to the genetic aetiology of oral clefts and emphasize the efficacy of whole-genome high-resolution SNP arrays to detect novel candidate genes in patients with syndromic and nonsyndromic clefts.

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Introduction

Oral clefts, one of the most common birth defects worldwide, range from mild types to complete clefts affecting the lip/alveolus/palate. The estimated occurrence of oral clefts is ~1–2 per 1000 births (Shaw *et al.* 1991) based on different ethnic and geographic groups. Both genetic and environmental factors (e.g. smoking and nutrition) are known to contribute to the development of cleft lip and palate (Mitchell *et al.* 2002; Mossey *et al.* 2009), making it difficult to elucidate the causative mechanisms. Oral clefts have been traditionally subdivided into two categories: cleft lip with or without cleft palate (CL ± P); and cleft palate only (CPO) (Mossey *et al.* 2009). However, recent studies have emphasized subdivision of three groups: cleft lip only (CLO), cleft lip with cleft palate (CLP), and CPO, due to differences between these types in terms of embryologic development, prevalence, risk factors and association with other congenital anomalies (Harville *et al.* 2005; Rittler *et al.* 2008; Luijsterburg and Vermeij-Keers 2011). In addition, ~30% of CL ± P and 50% of CP cases are regarded as syndromic, in which the clefts occur with other characteristic features (Stanier and Moore 2004). These syndromes are mostly due to monogenic diseases or chromosomal disorders. By contrast, nonsyndromic clefts consist of isolated, nonspecific malformations that show a multifactorial aetiology due to the interaction between a genetic background of susceptibility and environmental factors. Recently, much effort has been concentrated on identifying the genetic contribution to oral clefts. This has taken the form of direct analysis of candidate genes, association studies with candidate genes, or loci and

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genomewide scans using large collections of families with histories of oral clefts. However, some contributing genes remain to be identified.

Chromosomal microarray analysis (CMA) or molecular karyotyping has been developed as a genomewide screening strategy for detecting DNA copy number imbalances (Kallioniemi *et al*. 1992). It is now widely used for the clinical evaluation of postnatal patients with mental retardation/developmental delays, autism and multiple congenital anomalies. In these studies, the diagnostic yield was much higher than that of standard GTG-banded karyotyping (de Vries *et al*. 2005). Thus, it has been recommended that CMA could be used as a first-line test in the initial postnatal evaluation of individuals with the conditions mentioned above (Manning and Hudgins 2010). Moreover, CMA has been able to simultaneously identify and map microdeletions and microduplications onto the genomic sequence (Pinkel *et al.* 1998) and can be used to narrow down candidate disease gene regions for subsequent gene hunting. CMA has been increasingly used in the populations with syndromic and nonsyndromic cleft lip and palate. Leal *et al.* (2009) reported a case of a woman with mental retardation, tetralogy of Fallot, cleft lip and palate, and other dysmorphic features; this patient carried a 0.8 Mb *de novo* interstitial deletion in 19q13.32 that was detected by a high-resolution array-CGH. The deleted region encompassed 15 genes, including SAE1, a SUMO-1 activating enzyme subunit, which may be related to cleft lip and palate. Kirchhoff *et al.* (2009) applied a 244k Agilent oligonucleotide-based array-CGH to determine the exact breakpoints in 14 patients with partial deletions of chromosome 13q21.1-qter. They were able to refine the smallest deletion region linked to cleft lip/palate (13q31.3–13q33.1). Except for the arrays that measure DNA copy number differences only, SNP arrays, which are covered with nonpolymorphic probes and single-nucleotide polymorphic (SNP) probes are becoming popular. These arrays allow for the detection of allelic imbalances, such as loss of heterozygosity (LOH) and copy number changes, through hybridization signal intensities. Kimani *et al.* (2009) used SNP arrays to scan genomic alterations in a sample of monozygotic twin pairs with discordant cleft lip and/or palate phenotypes. Tan *et al.* (2013) identified a *de novo* 2.3 Mb microdeletion of 1q32.2 involving the Van der Woude syndrome (VWS) locus using a high SNP array. In our study, we used the CytoScan™ HD array platform provided by the Affymetrix Corporation to analyse 33 patients with syndromic and nonsyndromic cleft lip and palate. The aim of this assay was to identify microdeletions and microduplications of candidate gene loci for congenital oral clefts using CMA.

Materials and methods

Patients

A total of 33 patients referred for syndromic and nonsyndromic oral clefts and their parents were recruited from the Department of Stomatology in Guangzhou Women and Children's Hospital between August 2012 and June 2013. Written informed consents were received from all guardians of the patients. The age of the patients ranged from 3 months to 11 years old. All patients were required to have normal karyotypes and uncomplicated perinatal histories, such as no viral infection and no exposure to other teratogens. Twenty-eight of these patients presented with nonsyndromic oral clefts; of which 10 were CLO, 11 were CLP and seven were CPO. Five patients presented with syndromic oral clefts; of which three were associated with patent foramen ovale (PFO) or an atrial septal defect (ASD), one presented with developmental delay (DD), and one with VWS.

Genomic DNA

Genomic DNA was extracted from the peripheral blood of the patients and their parents using QIAamp® DNA Blood mini kits (Qiagen, Dusseldorf, Germany) following the manufacturer's protocol. At least 250 ng of genomic DNA was applied to the CytoScan™ Array.

Cytogenetic studies

Chromosomal analyses were performed on patient's blood samples using conventional G-banding techniques at the 300–400 band level.

CytoScan™ HD array

The Affymetrix CytoScan™ HD array (Affymetrix, Santa Clara, USA) contains 2,696,550 25–85-mer oligonucleotide probes, including 1,953,246 nonpolymorphic probes and 743,304 SNP probes, on an industry standard 1″ × 3″ glass slide. These oligonucleotide probes cover the whole genome with an average spatial resolution of 1148 bp. The University of California Santa Cruz (UCSC) Genome Brower hg19 (NCBI Build37, Feb 2009) was used as the source of the content. Approximately 52.3% of the probes reside in intragenic regions, and the remaining are in intergenic regions. Meanwhile, the probes cover all of the ISCA Constitutional genes, the Sanger cancer genes, the X chromosome OMIM morbid genes, 98% of the OMIM morbid genes, and 96% of the Refseq genes. The samples (250 ng) were run on a CytoScan™ HD Array using the manufacturer's protocol.

Evaluation of copy number variants (CNV)

Deletions of a region of at least 100 kb and duplications of a region of at least 200 kb were analysed (Hanemaaijer *et al*. 2012). The imbalances were further aligned with known CNVs listed in publically available online databases, such as the database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER, https://decipher.sanger.ac.uk/browser), the Online Mendelian Inheritance in Man database (OMIM, http://www.omim.org), the Database of Genomic Variants

(http://dgv.tcag.ca/dgv/app/home), and the CHOP database (http://cnv.chop.edu/). In general, a CNV is considered to be clinically significant (Xiang *et al.* 2010) if (i) it involves a region associated with a microdeletion/microduplication syndrome; (ii) it is inherited from an affected parent; (iii) it involves dosage-sensitive gene(s); or (iv) it is a multigene imbalance that is either *de novo* or inherited from a parent as a product of either segregation of a balanced translocation/insertion or recombination of an inversion. In contrast, a CNV is considered to be benign (Xiang *et al.* 2010) if (i) it is a CNV in normal populations; (ii) it is inherited from a normal parent; or (iii) it does not involve the regions associated with known microdeletion or microduplication syndromes or dosage-sensitive genes. However, there are possible exceptions in some patients, such as the following: (i) a CNV inherited from a normal parent can be clinically significant due to incomplete penetrance or variable expressivity; (ii) a small deletion or duplication can be clinically significant when a critical gene is involved; and (iii) a variant deletion in normal individuals can be clinically significant if it unmasks a recessive gene mutation in a patient (Xiang *et al.* 2010). The term 'variant of uncertain clinical significance' (VOUS) was used when an imbalance involved multiple genes but the significance of the imbalance could not be determined based on available knowledge and family studies (Xiang *et al.* 2010). The DNA from both parents was assessed by CMA to confirm the clinically significant CNVs and whether the VOUS were *de novo* or inherited. All clinically significant CNVs identified by CMA were further confirmed by real-time qPCR according to the standard procedures.

Results

A total of 33 patients were found to have genomic CNVs, with a range of 2–9 per sample. The size of detected CNVs varied from 100 kb to 3.2 Mb. We divided the detected CNVs into three groups. The first group contained six CNVs considered to be clinically significant (table 1), and the incidence of clinically significant CNVs was 18.2% (6/33). Three of these six CNVs were detected in patients with non-syndromic clefts, including one with isolated CLP and two with CPO. The remaining three were detected in patients with syndromic clefts. No clinically significant CNVs were detected in patients with CLO. The six clinically significant CNVs were as follows: a 8p23.1 microduplication (198 kb); a 10q22.2-q22.3 microdeletion (1766 kb); a 18q12.3 microduplication (638 kb); a 20p12.1 microdeletion (184 kb); a 6q26 microdeletion (389 kb); and a 22q11.21-q11.23 microdeletion (3163 kb). Of these, we also found that the 22q11.21-q11.23 microdeletion was a mosaic and the level of mosaicism was ~20% according to the SNP arrays. In addition, the KAT6B and MACROD2 genes from the 10q22.2-q22.3 and 20p12.1 microdeletions were identified as likely causative genes. The copy numbers for the sequences of genes in these regions were determined using real-time qPCR

Table 1. Clinical significant CNVs revealed by CytoScan^{TM} HD array.

Case	Phenotype	CMA result	Size (kb)	Gene	DECIPHER/OMIM	Inheritance
Case 1	CLP	arr8p23.1(8967517-9166356) × 3	198	*PPP1R3B*	Patient 258439*	*De novo*
Case 5	DD+CP	arr10q22.2-q22.3(76652946-78419911) × 1	1766	*KAT6B, DUPD1, DUSP13, S4MD8 VDAC2, COMTD1, ZNF503, NCRNA00245, C10orf11*	GPS, SBBYSS	*De novo*
Case 13	CP+ASD	arr18q12.3(41814626-42453303) × 3	638	*SETBP1*	Patient 253569*, Patient 259788*, Schinzel-Giedion syndrome	*De novo*
Case 15	CPO	arr20p12.1(15179681-15363926) × 1	184	*MACROD2*	Patient 249718*, KS	*De novo*
Case 21	CL+PFO	arr6q26(162326550-162716261) × 1	389	*PARK2*	Patient 255615*	*De novo*
Case 31	CP+velo-pharyngeal insufficiency	arr22q11.21-q11.23(18636749-21800471) × 1	3163	*UFD1L, COMT, TBX1*	Velocardiofacial syndrome, DiGeorge syndrome	*De novo*

*Patients in DECIPHER database; DD, developmental delay; ASD, atrial septal defect; PFO, patent foramen ovale; GPS, genitopatellar syndrome; SBBYSS, Say-Barber-Biesecker-Young-Simpson syndrome; KS, Kabuki syndrome.

to confirm the *de novo* deletions and duplications of the probands' DNA (primers are shown in table 2).

The second group consisted of CNVs considered to be benign in 26 patients. They were either found in the DGV/CHOP database or inherited from a healthy parent. In 22 cases, we could find the identical CNVs in the online DGV/CHOP database; in the other four cases, we identified CNVs inherited from a heathy parent. These inherited CNVs were a 9q31.1 microduplication, a 10p12.33 microdeletion, a 7q31.1 microdeletion and an Xp22.33 microduplication.

In the third group, we first classified five deletions and duplications of uncertain clinical significance. However, after the analysis of the parental samples by CMA, we found that four of them were inherited from a healthy parent and were considered to be benign (showed in second group). As a result, the VOUS was a 110-kb microdeletion located on chromosome 5q21.1 (arr5q21.1 (102143617-102253651) × 1), which contained the OMIM gene PAM (MIM 170270). The detection rate of the VOUS was 3.0% (1/33).

Discussion

The genetic aetiologies of congenital oral clefts have extremely high heterogeneity, including multiple-factor inheritance, chromosomal abnormalities and Mendelian single genes. Variation in chromosomal imbalances is one of the most important causes of congenital oral clefts. Deletions or duplications of portions of each chromosome arm, including the X chromosome have been associated with clefts and five regions are considered to be highly associated with clefts: 4p16-14, 4q31-35, 1q25, 3p26-21 and 10p15-11 (Brewer *et al.* 1998, 1999). Maarse *et al.* (2012) who reviewed 20 studies of congenital oral clefts with or without other anomalies, concluded that array-CGH should be recommended as a first-line test prenatally and postnatally. Szczaluba *et al.* (2015) used a 180 K microarray in a group of Polish newborns with cleft lips and palates, and detected rearrangements in eight of 52 patients (15%). In our study, we used SNP arrays to investigate 33 patients with nonsyndromic and syndromic clefts and normal karyotypes in south China. Six clinically significant CNVs were identified at a detection rate of 18.2% (6/33). The size of the detected CNVs varied from 100 kb to 3.2 Mb, which will certainly be missed by conventional G-banding techniques (>5–10 Mb). In addition, four of these CNVs were smaller than 1 Mb and would thus be undetectable by low resolution arrays such as BAC arrays (>1 Mb). Therefore, our results further demonstrated the value of whole-genome and high-resolution SNP arrays in patients with congenital clefts.

We selected the first group of clinically significant CNVs for the detailed description. Two of them probably contained candidate genes that may have contributed to the phenotypes observed in our patients. Further, mosaicism was detected in one of the CNVs, which further demonstrated the usefulness

Table 2. Sequences of the primers used in real-time qPCR.

Case	Position	F name	Forward sequence 5′–3′ direction	R name	Reverse sequence 5′–3′ direction	Product size (bp)	Annealing temp. (°C)
Case 1	Chr8:9000187-9000329	PPP1R3B-F	5′-ACCCTCACTTTCTGGTAGCC-3′	PPP1R3B-R	5′-ACGGGGATGAACCTGGA-3′	143	60
Case 5	Chr10:76789636-76789805	KAT6B-F	5′-GCTACGATTCTACTATGGGAGG-3′	KAT6B-R	5′-GCTGATGCTGGTTTGCTG-3′	170	60
Case 13	Chr18:42272870-42273014	SETBP1-F	5′-AGGTGAAGTAGTCTGGAAATGCT-3′	SETBP1-R	5′-GGTTCTCTGACCCTGAAGATACA-3′	145	60
Case 15	Chr20:15203093-15203270	MACROD2-F	5′-CAGGTCATAGATTGGTGGTGCT-3′	MACROD2-R	5′-CATAATAATGCTGTGCCTTGTCA-3′	178	60
Case 21	Chr6:162370812-162371025	PARK2-F	5′-AGCCTGCTGCCTCTGTCCT-3′	PARK2-R	5′-TCCCAATCTCCCACCTTCC-3′	214	60
Case 31	Chr22:19747867-19748010	TBX1-F	5′-GGGAAGAGGCGGAAAGTAAA-3′	TBX1-R	5′-TCCTCTCTCTCTCCCAGACG-3′	144	60

of SNP arrays as a great tool for identifying the aetiology of oral clefts.

In a 7-year-old boy (case 1), we found a 198-kb duplication in 8p23.1 encompassing only one gene, *PPP1R3B* (OMIM 610541), with isolated CLP, normal growth development and no other structural abnormalities were found. The product of the *PPP1R3B* gene is the targeting subunit of PP1-a serine/threonine phosphatase involved in the modulation of glycogen synthesis in the liver and skeletal muscles. The *PPP1R3B* gene is considered to be a candidate gene for type 2 diabetes and maturity-onset diabetes of the young (MODY) (Dunn *et al.* 2006), and there has been no previous evidence that the gene is related to CLP. However, the 8p23.1 microduplication region is close to (~1 Mb) the common cleft lip and palate candidate locus 8p11-23, which encompasses the gene *SOX7* (OMIM 612202) (Riley *et al.* 2007). *SOX7* is a member of the *SOX* gene family, and SOX proteins are transcription factors with critical roles in the regulation of diverse developmental processes (Takash *et al.* 2001). Murakami *et al*. (2004) found that SOX7 is a potent activator of Fgf3 transcription, and Fgf3 expression was virtually abolished when *SOX7* expression was suppressed by RNA interference in mouse embryonic stem cell cultures. Fgf3, a member of the fibroblast growth factor (Fgf) family is described as a candidate gene for cleft lip and palate (Riley *et al.* 2007). Therefore, we speculated that the 8p23.1 microduplication affected the function of the *SOX7* gene thereby causing the clefts.

In a girl (case 5) with delayed development and congenital cleft palate, we detected a 1.766 Mb *de novo* deletion at 10q22.2-q22.3 (figure 1). To the best of our knowledge, only five other cases with deletions spanning from 10q22.1 to q22.3 have been reported (Cook *et al.* 1999; Tzschach *et al.* 2006, 2010; Reddy *et al.* 2011). The deletion harbours nine genes: *KAT6B*, *DUPD1*, *DUSP13*, *SAMD8*, *VDAC2*, *COMTD1*, *ZNF503*, *NCRNA00245* and *C10orf11* (table 1). *KAT6B* (OMIM 605880) and *C10orf11* (OMIM 614537) are OMIM morbid genes and are probably disease-associated. *KAT6B* is strongly expressed during development in telencephalic vesicles, trigeminal ganglia, spinal cord, dorsal root ganglia, digestive tract, pancreas, liver and ribs, and after birth in the diaphysis of the long bones, kidney and patella. Szakszon *et al.* (2013) noted that truncating mutations of the *KAT6B* gene cause genitopatellar syndrome (GPS, OMIM 606170) and Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS, OMIM 603736). The phenotypes of these syndromes are characterized by mental retardation, skeletal problems and craniofacial dysmorphism; congenital cleft lip and palate, and dental anomalies are especially common in SBBYSS (Penttinen *et al.* 2009; Clayton-Smith *et al.* 2011). Clayton-Smith *et al.* (2011) showed that mice with a hypomorphic KAT6B gt/gt mutation are of normal size at birth but fail to thrive and have brain developmental defects as well as craniofacial defects. Their study also found that mutations in the domains that facilitate protein–protein interactions result in a more complex phenotype compared to cases of simple *KAT6B* haploinsufficiency. However, the deletion of KAT6B has not been reported as a cause of GPS, SBBYSS or any other disorders (Penttinen *et al*. 2009). The loss of *KAT6B* in our female case was characterized by developmental delays and a congenital cleft palate. Therefore, the haploinsufficiency of *KAT6B* may

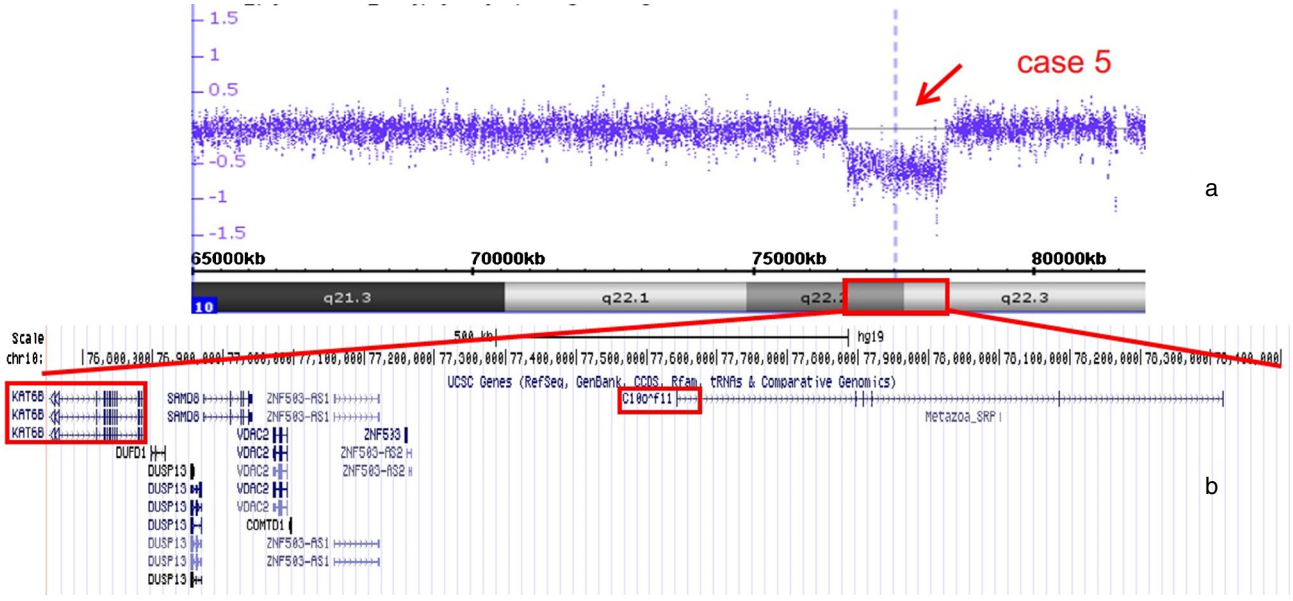

Figure 1. (a) A 1.766 Mb chromosome 10q22.2-q22.3 deletion (chr10: 76652946-78419911) in case 5 was detected by CytoScan^{TM} HD array. The log 2-based test/reference intensity ratios of DNA clones located on chromosome 10 were below −0.5 (red arrow), the threshold indicating chromosomal deletion. (b) Schematic from the UCSC genome browser showing the position of the 10q22.2-q22.3 deletion from 76 to 78 Mb. The region overlaps with KAT6B, DUPD1, DUSP13, SAMD8, VDAC2, COMTD1, ZNF503, NCRNA00245 and C10orf11. KAT6B and C10orf11 are OMIM morbid genes (red box).

contribute to these types of abnormalities in cases with 10q22.1-q22.3 deletions. Additional patients with deletions of *KAT6B* will need to be assessed to confirm this hypothesis. C10orf11 is located on chromosome 10q22.3 (genomic coordinates (GRCh37):10:77191216-78317132). Mutations in C10orf11 are associated with autosomal-recessive albinism (Gronskov *et al.* 2013), and Tzschach *et al.* (2010) reported that C10ORF11 haploinsufficiency contributes to cognitive defects. In our study, the patient with the deletion in 10q22.2-q22.3 was characterized with developmental delays, which further indicates that C10orf11 is associated with cognitive development. However, more investigations are required to determine the associated pathogenesis.

In a 7-month-old girl (case 13) with bilateral cleft palates and an ASD, we detected a 638 kb *de novo* duplication at 18q12.3 (genomic coordinates (GRCh37):18:41814626-42453303), which partly overlapped with the gene *SETBP1* (OMIM 611060, genomic coordinates (GRCh37):18:42260137-42648474). In a study by Hoischen *et al.* (2010), heterozygous mutations of the *SETBP1* gene were identified in individuals with Schinzel–Giedion syndrome. This syndrome is highly recognizable and is characterized by severe mental retardation, distinctive facial features and multiple congenital malformations that include skeletal abnormalities, genitourinary and renal malformations, and cardiac defects. These phenotypes partly overlapped with the case in our study. To our knowledge, this is the first report of a patient in whom a cleft palate and ASD both are associated with the duplication of *SETBP1*. However, further investigation is required to support our finding.

In an 11-month-old girl (case 15) with CPO and no other structural anomalies, we found a 184 kb deletion in 20p12.1 (genomic coordinates (GRCh37):20:15179681-15363926) located in the gene MACROD2 (genomic coordinates (GRCh37):20:13976145-16033841) (figure 2). *MACROD2* (MIM 611567) is a candidate gene for Kabuki syndrome (KS, OMIM 147920) which is typically characterized by postnatal growth retardation, craniofacial dysmorphism with long palpebral fissures, eversion of the lateral third of the lower eyelids, cleft anomalies and cardiac defects. Maas *et al*. (2007) identified a heterozygous *de novo* 250 kb deletion in the *MACROD2* gene from a patient with KS; in the mouse embryo, *MACROD2* is expressed in several craniofacial regions, which, given the specific facial characteristics of patients with KS can be expected. Therefore, our results further support the hypothesis that *MACROD2* is part of a novel class of dose-sensitive genes that may cause craniofacial anomalies. In patients, the phenotypes can vary depending on the types of aberrations. Further investigation is required to support the finding that the 20p12.1 microdeletion is in the exact region that confers functional haploinsufficiency to the *MACROD2* gene.

In a 7-month-old boy (case 21) with cleft lip and PFO, we found a 389 kb deletion located at 6q26 that encompassed only one gene, *PARK2* (OMIM 602544). The product of the gene *PARK2* is a RING domain-containing E3 ubiquitin ligase involved in the proteasome-dependent degradation of proteins; this protein is also important for mitochondrial quality control, as it facilitates the lysosome-dependent degradation of damaged mitochondria through either autophagy or mitophagy (Yoshii *et al.* 2011). *PARK2* is expressed in neuronal processes and the cell bodies of neurons in the midbrain, basal ganglia, cerebral cortex, and cerebellum (Huynh *et al.* 2000). Currently, studies have shown that mutations in the *PARK2* gene lead to the onset of Parkinson disease (Hayashi *et al.* 2000; Hedrich *et al.* 2001; Foroud *et al.* 2003). Further investigation is required to explore the relationship between the gene *PARK2* and oral clefts.

In a 3-year-old boy (case 31) with CP and velopharyngeal insufficiency, we detected a mosaic 3163 kb deletion at 22q11.21-q11.23, with a level of mosaicism of ~20% according to the SNP arrays (figure 3). The 22q11.21-q11.23 deletion partially overlapped with a common-sized ~3 Mb deletion found in patients with DiGeorge syndrome (DGS,

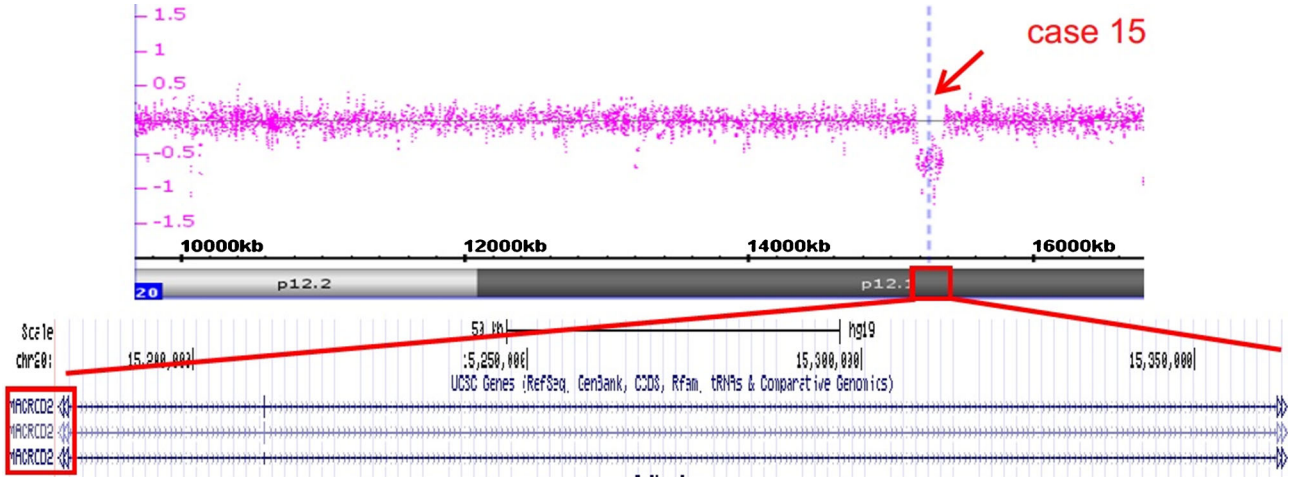

Figure 2. (a) CMA image showing a 184 kb deletion at 20p12.1 (chr20: 15179681-15363926) from case 15. (b) Schematic from UCSC genome browser showing the position of the 20p12.1 deletion within the *MACROD2* gene (red box).

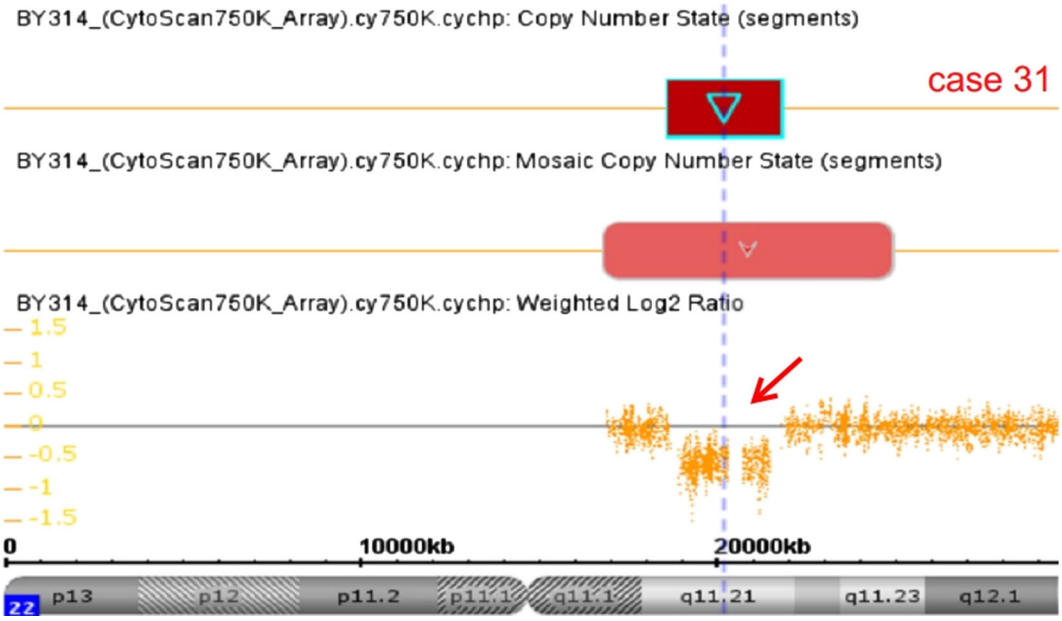

Figure 3. CMA image showing a 3163 kb deletion at 22q11.21-q11.21 (chr22:18636749-21800471) from case 31. The level of mosaicism was ~20% according to the SNP arrays.

OMIM 188400), which is characterized by outflow tract heart defects, immune deficiency, transient neonatal hypocalcemia, velopharyngeal insufficiency, cleft palate and a distinctive facial appearance. Bassett *et al.* (2005) described the phenotypic features of 78 adults with 22q11 deletion syndrome and identified 42% patients with palatal anomalies. The deletion identified in our patient harboured many genes, including *UFD1L*, *COMT*, *TBX1* and so on. Haploinsufficiency of the *TBX1* gene in particular is responsible for most of the physical malformations. A study by Yagi *et al.* (2003) indicated that TBX1 mutations are responsible for five major phenotypes in the 22q11.2 deletion syndrome: conotruncal facial anomalies, cardiac defects, thymic hypoplasia, velopharyngeal insufficiency with cleft palate and parathyroid dysfunction with hypocalcaemia. Therefore, it is suggested that the deletion of the *TBX1* gene probably contributed to the phenotype in our case. Also this result further demonstrated that SNP arrays enable the detection and estimation of the low-level mosaicism that may remain undetected by conventional cytogenetic methods (Cheung *et al.* 2007).

CMA also detects many CNVs of uncertain clinical significance (VOUS), which can be difficult to interpret. In our study, we identified one case with VOUS at a detection rate of 3.0%. Bartnik *et al.* (2014) used a custom-designed exon-targeted clinical array CGH manufactured by Agilent Technologies (Santa Clara, USA) on 256 patients with developmental delays or intellectual disabilities and found VOUS in 28 cases; the detection rate was 10.9%. Faas *et al.* (2010) used genomewide 250k SNP arrays on 38 prenatally karyotyped foetuses with ultrasound anomalies and found VOUS in two cases; the detection rate was 6%. In those studies, the VOUS were distinguished from pathogenic CNVs by not having previously reported in the peer review literature. We could not find the VOUS identified in our study in the DGV/CHOP database. We performed CMA on the parental samples to determine whether the CNVs were *de novo* or inherited. Parental testing revealed that the CNV in this patient was *de novo*, so the CNV was defined as a VOUS. The interpretation of the VOUS finding is a limitation for this study. For the purposes of the analysis, cases of VOUS were always taken as pathogenic unless they could be proven to be benign (Dhillon *et al.* 2014). Therefore, the significance of the microarray detecting clinically and pathogenically significant abnormalities over karyotyping has to be interpreted with a degree of caution.

In summary, our results further confirm the usefulness of SNP arrays in the detection of clinically significant CNVs in patients with oral clefts at a detection rate of 18.2%. We also have shown that the SNP array-based analysis of DNA samples derived from the clefts is an efficient and productive method of identifying candidate genes. Sufficient communication between technicians and genetic counsellors, references to parental testing and comparisons with international data may reduce VOUS.

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