RESEARCH ARTICLE

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

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Running title: Application of SNP arrays in oral clefts

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Abstract

Aim and methods: 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 remains mysterious. 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 etiology and candidate genes in patients with congenital oral clefts.

Results: CMA revealed copy number variants **(**CNVs) in every patient, with a range of 2-9 per sample. The size of detected CNVs varied from 100 kb to 3.2 Mb. In 33 patients, we identified 6 clinically significant CNVs. The incidence of clinically significant CNVs was 18.2% (6/33). Three of these 6 CNVs were detected in patients with nonsyndromic clefts, including 1 who presented with isolated cleft lip with cleft palate (CLP) and 2 with cleft palate only (CPO). The remaining 3 CNVs were detected in patients with syndromic clefts. However, no CNV was detected in patients with cleft lip only (CLO). The 6 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, 2 novel candidate genes for oral clefts, KAT6B and MACROD2, were putatively identified. We also found a copy number variant of unknown clinical significance with a detection rate of 3.0% (1/33).

Conclusion: Our results further support the notion that CNVs significantly contribute to the genetic etiology 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.

Introduction

Oral clefts – one of the most common birth defects in the world – range from mild types to complete clefts affecting the lip/alveolus/palate. The estimated occurrence of oral clefts is approximately 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 into 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, approximately 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 etiology 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 genome-wide 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 genome-wide 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 BB 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 non-polymorphic (NP) 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 for 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 locus using a high resolution single nucleotide poly-morphism (SNP) array. In our study, we used the CytoScan^{TM} HD array platform provided by the Affymetrix Corporation to analyze 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 ages of the patients ranged from 3 months old 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; 10 of these patients were CLO, 11 were CLP, and 7 were CPO. Five patients presented with syndromic oral clefts; 3 of these cases were associated with patent foramen ovale (PFO) or an atrial septal defect (ASD), 1 presented with developmental delay (DD), and 1 presented with Van der Woude syndrome (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 blood samples using conventional G-banding techniques at the 300-400 band level.

CytoScan™ HD Array

The Affymetrix CytoScan™ HD array (Affymetrix, California, USA) contains 2,696,550 25~85-mer oligonucleotide probes, including 1,953,246 non-polymorphic

(NP) probes and 743,304 single nucleotide polymorphic (SNP) probes, on an industry standard 1-inch×3-inch glass slide. These oligonucleotide probes cover the whole genome with an average spatial resolution of 1,148 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 CNV

Deletions of a region of at least 100 kb and duplications of a region of at least 200 kb were analyzed (Hanemaaijer et al 2012). The imbalances were further aligned with known copy number variants (CNVs) listed in publically available online databases, such as the database of Chromosomal Imbalance and Phenotype in Humans using ensembl Resources (DECIPHER, http//www.sanger.ac.uk/ PostGenomics/decipher), the Online Mendelian Inheritance in Man database (OMIM, http://www.omim.org), the Database of Genomic Variants (http://www.projects.tcag.ca/variation), and the CHOP database (http://cnv.chop.edu/). In general, a CNV is considered to be clinically significant (Xiang et al 2010) if 1) it involves a region associated with a microdeletion/microduplication syndrome; 2) it is inherited from an affected parent; 3) it involves dosage-sensitive gene(s); or 4) 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 1) it is a CNV in normal populations; 2) it is inherited from a normal parent; or 3) 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: 1) a CNV inherited from a normal parent can be clinically significant due to incomplete

penetrance or variable expressivity; 2) a small deletion or duplication can be e clinically significant when a critical gene is involved; and 3) 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 copy number variations, 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 6 CNVs considered to be clinically significant (Table 1), and the incidence of clinically significant CNVs was 18.2% (6/33). Three of these 6 CNVs were detected in patients with nonsyndromic clefts, including 1 with isolated CLP and 2 with CPO. The remaining 3 were detected in patients with syndromic clefts. No clinically significant CNVs were detected in patients with CLO. The 6 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 approximately 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 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 4 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 5 deletions and duplications of uncertain clinical significance. However, after the analysis of the parental samples by CMA, we found that 4 of them were inherited from a healthy parent and were considered to be benign (showed in the 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 etiologies 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 every 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 aCGH should be recommended as a first-line test prenatally and postnatally. Szczaluba et al. (2015) used a 180K microarray in a group of Polish newborns with cleft lips and palates and detected rearrangements in 8 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. Furthermore, mosaicism was detected in one of the CNVs, which further demonstrated the usefulness of SNP arrays as a great tool for identifying the etiology of oral clefts.

In a 7-year-old boy (case 1) with isolated CLP, normal growth development and no other structural abnormalities, we found a 198 kb duplication in 8p23.1 encompassing only one gene, PPP1R3B (OMIM 610541). 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 (approximately 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; Tzschach et al 2010; Reddy et al 2011). The deletion harbors 9 genes: KAT6B, DUPD1, DUSP13, SAMD8, VDAC2, COMTD1, ZNF503, NCRNA00245 and C10orf11 (table 1). KAT6B (OMIM 605880) and C10orf11 (OMIM614537) are OMIM morbid genes and are probably disease-associated. KAT6B is strongly expressed during development in the telencephalic vesicles, trigeminal ganglia, the spinal cord, dorsal root ganglia, the digestive tract, the pancreas, the liver, and the ribs, and after birth in the diaphysis of the long bones, the kidney, and the patella. Szakszon et al. (2013) noted that truncating mutations of the KAT6B gene cause genitopatellar syndrome (GPS, OMIM606170) and Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS, OMIM603736). 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 2009a; 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 2009b). The loss of KAT6B in our female case was characterized by developmental delays and a congenital cleft palate. Therefore, the haploinsufficiency of KAT6B may contribute to these types of abnormalities in cases with 10q22.1q22.3 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 atrial septal defect (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 are both 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 patent foramen ovale (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 approximately 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, 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 harbored 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 5 major phenotypes in the 22q11.2 deletion syndrome: conotruncal facial anomalies, cardiac defects, thymic hypoplasia, velopharyngeal insufficiency with cleft palate, and parathyroid dysfunction with hypocalcemia. Therefore, it is suggested that the deletion of the TBX1 gene probably contributed to the phenotype in

our case. This result also 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, CA) 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 genome-wide 250k SNP arrays on 38 prenatally karyotyped fetuses with ultrasound anomalies and found VOUS in 2 cases; the detection rate was 6%. In those studies, the VOUS were distinguished from pathogenic CNVs by not having been 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 to the 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 counselors, references to parental testing, and comparisons with international data may reduce VOUS.

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Competing Financial Interests

The authors declare no competing financial interests.

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Table 1 Clinical significant CNVs Revealed by CytoScan™ HD Array

Case NO.	Phenotype	CMA Result	Size (kb)	The main genes	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, SAMD8,VDAC2, COMTD1,ZNF503,NCRNA00245,C10orf11	GPS，SBBYSS	de novo
Case 13	CP+ASD	arr18q12.3(41814626-42453303) ×3	638	SETBP1	Patient253569*, Patient259788*, 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+ velopharyngeal insufficiency	arr22q11.21-q11.23(18636749-21800471) ×1	3163	UFD1L,COMT,TBX1	velocardiofacial syndrome, DiGeorge syndrome	de novo

*the patients in the 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

Table2. The sequences of the primers used in real-time qPCR.

Case NO.	Position	F name	F Seq 5' 3' direction	R name	R Seq 5' 3' direction	Product size (bp)	Annealing Temp (°C)
Case 1	chr8:9000187-9000329	PPP1R3B-F	5' ACCCTCACTTTCTGGTAGCC 3'	PPP1R3B-R	5' ACGGGGATGAACCTGGA 3'	143bp	60℃
Case 5	chr10:76789636-76789805	KAT6B-F	5' GCTACGATTCTACTATGGGAGG 3'	KAT6B-R	5' GCTGATGCTGGTTTGCTG 3'	170bp	60℃
Case 13	chr18:42272870-42273014	SETBP1-F	5' AGGTGAAGTAGTCTGGAAATGCT 3'	SETBP1-R	5' GGTTCTCTGACCCTGAAGATACA 3'	145bp	60℃
Case 15	chr20:15203093-15203270	MACROD2-F	5' CAGGTCATAGATTGGTCGTGCT 3'	MACROD2-R	5' CATAATAATGCTGTGCCTTGTCA 3'	178bp	60℃
Case 21	chr6:162370812-162371025	PARK2-F	5' AGCCTGCTGCCTCTGTCCT 3'	PARK2-R	5' TCCCAATCTCCCACCTTCC 3'	214bp	60℃
Case 31	chr22:19747867-19748010	TBX1-F	5' GGGAAGAGCGGGAAACTAAA 3'	TBX1-R	5' TCCTCTCTCTCTCCCAGACG 3'	144bp	60℃

Figure 1. (A) A 1.766 Mb chromosome 10q22.2-q22.3 deletion (chr10: 76652946-78419911) in Case 5 was detected by CytoScanTM 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 Mb 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).

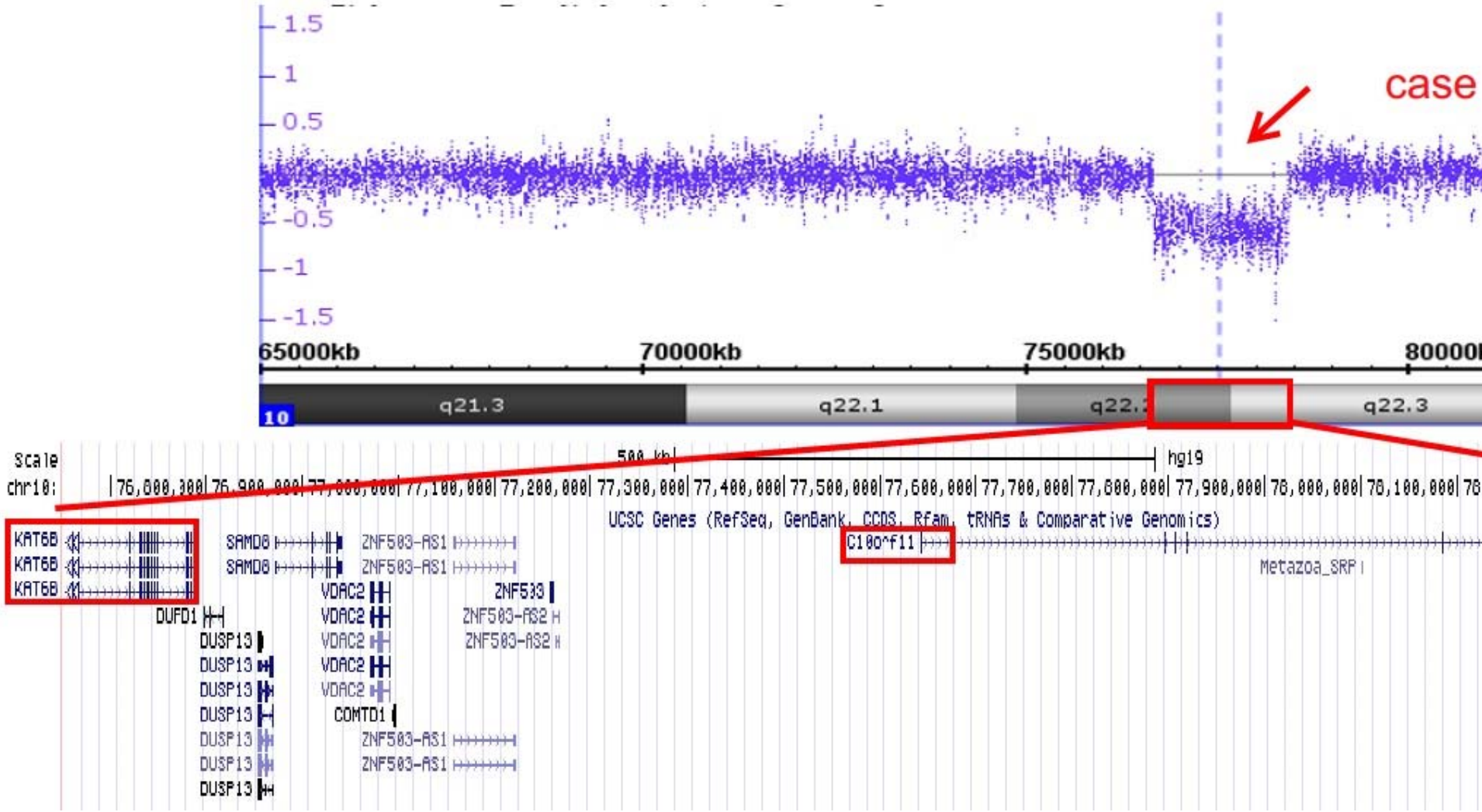

1.5
1
0.5
-0.5
-1
-1.5
case
65000kb
70000kb
75000kb
10
q21.3
q22.1
q22.3
Scale
500 kb
hg19
chr10:
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
KAT6B
SAMD8
ZNF503-AS1
C10orf11
Metazoa_SRP
VDAC2
ZNF533
DUFD1
ZNF503-AS2
DUSP13
COMTD1

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

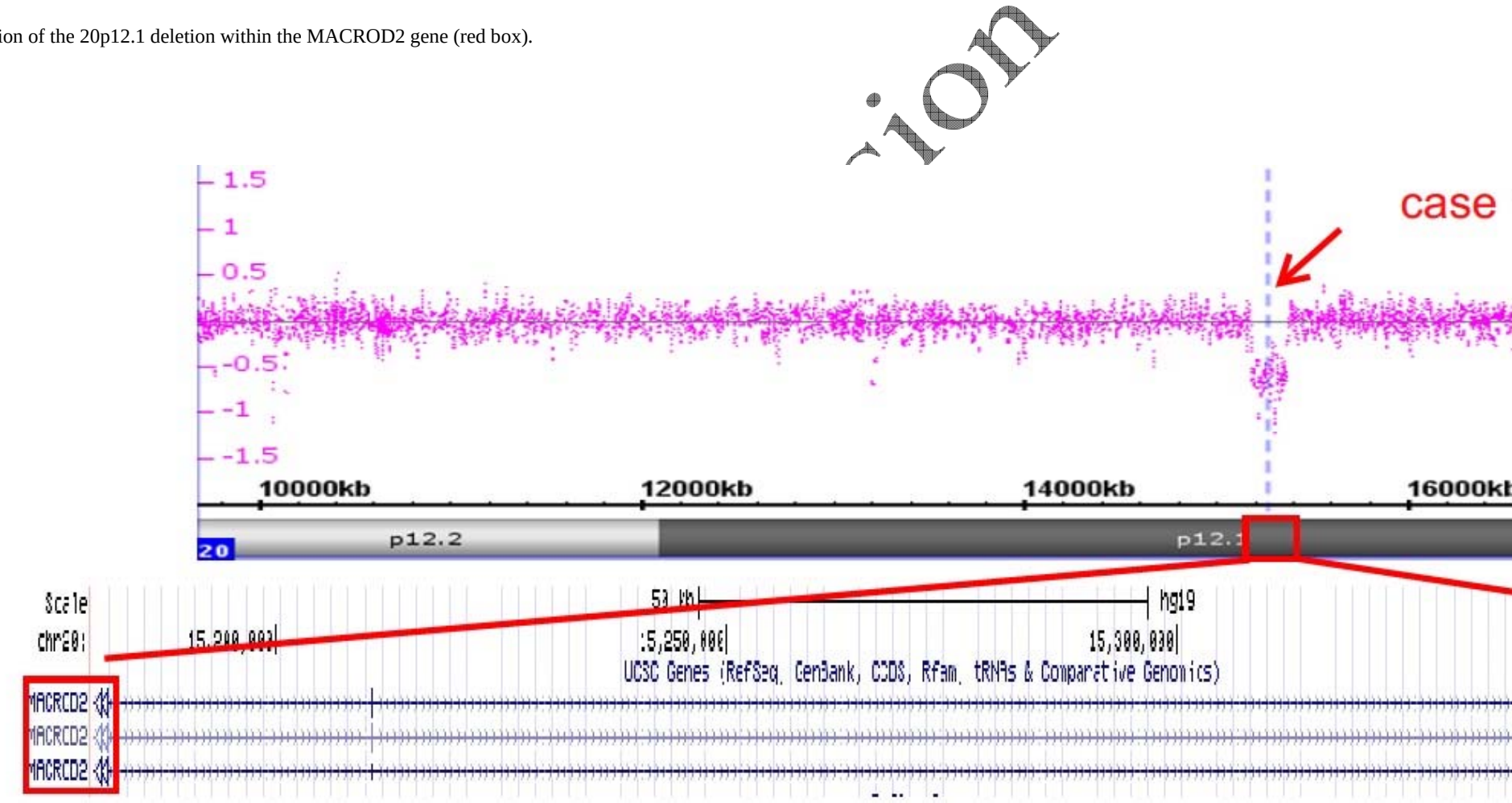
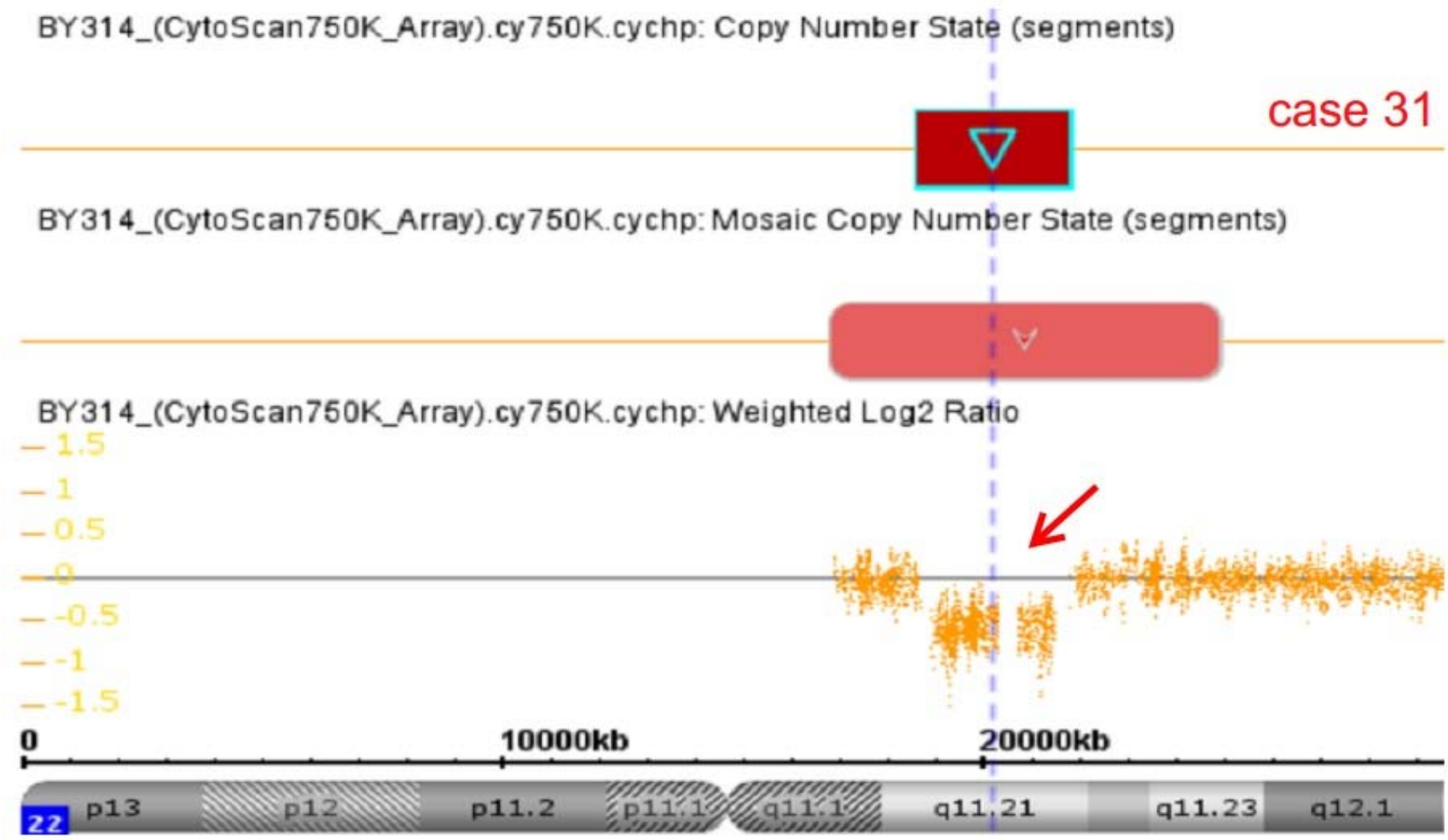


Figure 3. CMA image showing a 3163 kb deletion at 22q11.21-q11.21 (chr22:18636749-21800471) from Case 31. The level of mosaicism was approximately 20% according to the SNP arrays.


BY314_(CytoScan750K_Array).cy750K.cychp: Copy Number State (segments)
case 31
BY314_(CytoScan750K_Array).cy750K.cychp: Mosaic Copy Number State (segments)
BY314_(CytoScan750K_Array).cy750K.cychp: Weighted Log2 Ratio
1.5
1
0.5
0
-0.5
-1
-1.5
0
10000kb
20000kb
22
p13
p12
p11.2
p11.1
q11.1
q11.21
q11.23
q12.1