Maternal Transmission Effect of a PDGF-C SNP on Nonsyndromic Cleft Lip with or without Palate from a Chinese Population

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Abstract

Cleft lip with or without palate (CL/P) is a common congenital anomaly with a high birth prevalence in China. Based on a previous linkage signal of nonsyndromic CL/P (NSCL/P) on the chromosomal region 4q31–q32 from the Chinese populations, we screened the 4q31–q32 region for susceptibility genes in 214 trios of Han Chinese. PDGF-C, an important developmental factor, resides in the region and has been implicated in NSCL/P. However, in our family-based association test (transmission disequilibrium test; TDT), we could not conclude an association between PDGF-C and NSCL/P as previously suggested. Instead, we found strong evidence for parent-of-origin effect at a PDGF-C SNP, rs17035464, by a likelihood ratio test (unadjusted p-value = 0.0018; ln1 = 2.46). The location of rs17035464 is 13 kb downstream of a previously reported, NSCL/P-associated SNP, rs28999109. Furthermore, a patient from our sample trios was observed with a maternal segmental uniparental isodisomy (UPD) in a region containing rs17035464. Our findings support the involvement of PDGF-C in the development of oral clefts; moreover, the UPD case report contributes to the collective knowledge of rare variants in the human genome.

Introduction

Cleft lip with or without palate (CL/P) is one of the most common birth defects, affecting 0.5–1% of newborns worldwide [1,2]. Nonsyndromic CL/P (NSCL/P) accounts for ~70% of cases with cleft lip with or without palate, and is genetically distinct from those with syndromic CL/P [3]. NSCL/P has heterogeneous etiologies including several susceptibility genes and environmental risk factors [4,5,6,7]. With human linkage studies, several susceptibility loci of NSCL/P have been located to the regions of MTHFR, TGFα, 4q28 (D4S175), F13A1, TGFβ3, 17q12 (D17S250) and APOC2. Genes found in the susceptibility loci are involved in a wide range of cellular functions, such as growth promotion, transcriptional regulation, xenobiotic metabolism, nutrient metabolism, and immune response [8].

The first genome-wide linkage scan of NSCL/P in Chinese families found the most significant signal to be at chromosome 4q31–q32 [9]. Other NSCL/P studies reported linkage signals involving 4q31–q32 in Filipino and Syrian families [10,11]. The involvement of chromosome 4q31–q32 in human CL/P was also suggested by both cytogenetic deletion and association studies [12,13,14,15,16]. The rs28999109 SNP found to associate with NSCL/P in Chinese families was mapped to the regulatory region of platelet derived growth factor C (PDGF-C, [MIM:608452]) at 4q32 [17]. The role of PDGF-C in palatogenesis was also supported by a mouse knockout study in which the Pdgfc (-/-) embryos had a phenotype of cleft face [18].

Without considering the parental origin of an allele, association analysis is unable to distinguish the parental preference of transmission on a disease variant. The imprinting process provides a mechanism for a pair of parental alleles to be functionally unequal. A recent association study of type 2 diabetes demonstrated that the parent-of-origin effects had been overlooked by the past large-scale genome-wide association studies [19]. With our family-based study design, the parent-of-origin effects could be taken into consideration.

Uniparental disomy (UPD) is another genetic mechanism that distorts Mendelian transmission. UPD occurs when a child receives both copies of a chromosome or chromosomal region from only one parent, leading to the violation of biparental inheritance [20]. Depending on the genomic location and size of the affected region, UPD can be clinically undetectable. However,
abnormal expression of critical developmental genes due to UPD may lead to harmful phenotypes. Thus far, no studies have suggested UPD as one of the mechanisms in CL/P formation.

In this study, a two-step fine mapping approach was used to locate the susceptibility genes of NSCL/P at the 4q31–q32 region in Han Chinese. We genotyped 10 STR (Short Tandem Repeat) markers in 214 NSCL/P trios and subsequently fine mapped with SNP markers in a subset of our subjects. Later, the parent-of-origin effect of candidate genes was assessed by a parent-of-origin likelihood ratio test (PO-LRT).

Materials and Methods

Study Subjects
We collected 214 case-parent trios through the CL/P Treatment Center, Plastic Surgery Hospital, Chinese Academy of Medical Sciences. All probands underwent clinical genetics evaluations to exclude congenital anomalies other than CL/P and any major developmental delay, and were diagnosed as an isolated, nonsyndromic CL/P. Patients with cleft palate only were not included in this study. The probands consisted of 156 boys and 58 girls with an approximately 3:1 ratio between males and females. In addition, 24 of the 214 trios had positive family history of CL/P. Table 1 is a summary of our sample information. Written informed consents were obtained from all probands' parents. The study was approved by the Ethical Institutional Committee of the Plastic Surgery Hospital, Chinese Academy of Medical Sciences.

Marker Selection
A two-step strategy was used to map the NSCL/P susceptibility gene at the signal, 4q31–132, previously reported in a genome-wide linkage study [21]. First, we genotyped the 4q31–q32 region with 10 STR markers from D4S1644 to D4S2431 (141 Mb–174 Mb, spanning 30 Mb) of the deCODE genetics map [22]. The 10 STR markers were chosen for their high heterozygosity and long PCR product to increase their information content and raise the success rate of genotyping (Table S1). Our complete sample set, which consisted of 214 trios, was genotyped during this step. Second, the target region was shortened to a 500-kb region including D4S413 and the PDGF-C promoter. The location of the 500-kb region was based on two evidences: 1) we observed the lowest p-value at D4S413 in our TDT analysis of the first step; also, 2) Choi et al. [17] reported an association signal to NSCL/P at 4q31–132, previously reported in a genome-wide genotyping study [23].

The 500-kb region with 80 SNP markers (rs894588–rs17036150) (dbSNP version 126). According to the manufacturer's specifications (Illumina, USA), all genomic positions were based upon NCBI Build 36 (hg18). The genotype call rate is 99%. Image data was analyzed using the GenCall scores, according to the manufacturer's specifications (Illumina, USA). GenCall scores <0.25 at any locus were considered “no calls”. All 80 SNPs succeeded, and overall genotype call rate was 98%.

For all genotyped markers and trios, Mendelian consistency was checked within each trio family and inconsistency was found in family 152. To further investigate the issue, a genome-wide genotyping was conducted on the proband of family 152 with the Illumina Human660W-Quad v1 DNA Analysis BeadChip according to the manufacturer's specifications (Illumina, USA). GenCall scores <0.15 at any locus were considered “no calls”. The genotype call rate is 99%. Image data was analyzed using the Chromosome Viewer tool contained in GenomeStudio (Illumina, USA). All genomic positions were based upon NCBI Build 36 (dbSNP version 126).

Statistical Analyses
For the STR markers, the rarest alleles were combined to generate a pseudo-allele until it reached a threshold of 0.1. Hardy-Weinberg equilibrium test was performed by the gap package in R [24]. The allele frequency and Hardy-Weinberg equilibrium test of SNP markers were computed from parents using the Haploviev program [25] with, a cutoff of 0.05. Pairwise linkage disequilibrium (LD) was evaluated with r² for all SNPs using the Haploviev program.

The transmission disequilibrium test (TDT) was performed using the family-based association test (FBAT) program [26]. Haplotype-based TDT analysis of SNP markers in the second step was performed by the Haploviev program. Haplotype blocks were constructed based on the default algorithm [27] in Haploviev. The correction for multiple testing on haplotype-based TDT was conducted with 1000-permutations in Haploviev. Our first step genotyping used only 10 STR markers, and the multiple testing was adjusted with Bonferroni correction. For the second step genotyping, we employed more closely spaced markers with increasing possibility of forming background LD among markers.

Table 1. Description of sample in two-step genotyping.

| Number of trios | Step 1 | Step 2 |
|-----------------|--------|--------|
| Total           | 214    | 160    |
| Boys vs. girls in probands | 156:58 | 115:45 |
| Triads vs. Duos | 205:9  | 160:0  |
| Trios have family history or not | 22:192 | 14:146 |

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Genotyping
All DNA samples were extracted from whole blood using the QiAamp DNA Mini Kit (Qiagen, USA). For STR genotyping, one of each primer pairs was 5’ FAM-labeled (Invitrogen, Shanghai, China), and PCRs were performed on the GeneAmp PCR system 9700 (Applied Biosystems, USA). Electrophoresis of the amplified products was conducted on an ABI 3730 XL DNA Analyzer (Applied Biosystems, USA). A size-call panel was trained for each marker, using the software GeneMarker (SoftGenetics, USA), with data from a random successfully typed 96-well plate. Fragment sizes of a reaction were automatically determined with established panels and manually double-checked by two readers. Unsuccessful reactions were retried until either success or three failures.

For all genotyped markers and trios, Mendelian consistency was checked within each trio family and inconsistency was found in family 152. To further investigate the issue, a genome-wide genotyping was conducted on the proband of family 152 with the Illumina Human660W-Quad v1 DNA Analysis BeadChip according to the manufacturer's specifications (Illumina, USA). GenCall scores <0.15 at any locus were considered “no calls”. The genotype call rate is 99%. Image data was analyzed using the Chromosome Viewer tool contained in GenomeStudio (Illumina, USA). All genomic positions were based upon NCBI Build 36 (dbSNP version 126).

The SNP genotyping was performed using Illumina VeraCode® technology on the BeadXpress® system (Illumina, USA). Genotypes were generated on its accompanying GenomeStudio software suite (version 1.0). GenCall scores <0.25 at any locus were considered “no calls”. All 80 SNPs succeeded, and overall genotype call rate was 98%.

Family-Based Association Study on NSCL/P

Statistical Analyses
For the STR markers, the rarest alleles were combined to generate a pseudo-allele until it reached a threshold of 0.1. Hardy-Weinberg equilibrium test was performed by the gap package in R [24]. The allele frequency and Hardy-Weinberg equilibrium test of SNP markers were computed from parents using the Haploviev program [25] with, a cutoff of 0.05. Pairwise linkage disequilibrium (LD) was evaluated with r² for all SNPs using the Haploviev program.

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To test the parent-of-origin effect, a parent-of-origin likelihood ratio test (PO-LRT), implemented in the LEM software, was used to estimate the relative risks for heterozygotes and homozygotes of overtransmitted alleles. The PO-LRT model considers the inherited number of copies of the overtransmitted allele as maternal genotypic effects on the phenotype of a fetus [30]. The estimated risk ratios \( I_{(m)} \) for an imprinting effect suggest that there is an excess maternal transmission compared to paternal transmission in this region when \( I_{(m)}>1 \), and vice versa.

### Results

#### TDT and Parent-of-Origin Effects

Our first step genotyping with 10 STR markers was on a 30-Mb region encompassing the linkage peak reported by Marazita at 4q31-q32 [9]. The heterozygosity of the STR markers estimated from the parents is displayed in Table S1. All of the markers passed the Hardy-Weinberg equilibrium test. With our complete NSCL/P sample set of 214 trios, we found most-significant association between the D4S141 marker and the disease by TDT (unadjusted \( p\)-value = 0.01974). However, after stringent adjustment for multiple testing with the Bonferroni correction, the \( p\)-value of this association did not reach the significance level of 0.05. In addition, we observed a non-Mendelian transmission of D4S1498 in family 152, for the patient received both copies of D4S1498 only from the mother.

In the second step genotyping, the minor allele frequencies of the SNP markers estimated from the parents are displayed in Table S2. All of the markers passed the Hardy-Weinberg equilibrium test. Figure 1 shows that the LD pattern among the 80 SNPs. Individually testing each SNP by TDT analysis, we found the lowest unadjusted \( p\)-value of 0.0066 at SNP rs10517665. To correct for the presence of LD, we calculated the effective number of the 80 SNPs employing Li’s method [28] and used the value, 24, for correction of multiple testing. However, no SNPs reached the significant level after correction with the effective number for multiple testing. The result is summarized in Table S3. Ten haplotype blocks were identified based on LD. We observed the lowest \( p\)-value (0.0043; 0.06 after correction) on the haplotype GTGAAGCAAAGA, consisting of 12 SNPs from rs4691396 to rs6843489. No haplotypes are statistically significant after 1000-permutations. The result of haplotype-based TDT analysis is presented in Table S5.

Considering the parent-of-origin effects, we found an excess of maternal transmission and a deficit of paternal transmission of the allele A of rs17035464 (unadjusted \( p\)-value = 0.0018, \( I_{(m)} = 2.46 \)) in PDGF-C (Figure 2). After correction with the effective number of our SNPs, the adjusted \( p\)-value (0.0432) remained significant. Table S3 shows the result of the PO-LRT test.

#### Evidence of UPD

After genotyping, an aberrant transmission of D4S1498 was found in family 152. Proband 152 obtained both alleles of D4S1498 from the mother, but none from the father (Table S4). We then examined the markers surrounding D4S1498 for Mendelian inconstancy. Two SNPs, rs17035464 and rs6843522, that are about 10 kb away from D4S1498 (Figure 3) transmitted in the same pattern as D4S1498. All genotypes of the other STR and SNP markers followed the Mendelian transmission. Whole genome genotyping and CNV analysis were performed on proband 152 and indicated that the region containing rs17035464, rs6843522, and D4S1498 was diploid in content, which means no deletion at the region (Figure S1). Taken together, the abnormal transmission of a minimal region of 14 kb, from rs17035464 to D4S1498, is likely to be a result of segmental maternal uniparental isodisomy (UPD), and the region is mapped to the PDGF-C promoter.

Proband 152 was a 1-year-old girl with bilateral cleft lip and palate, and no other noticeable abnormalities were found after a comprehensive postnatal physical examination (Figure 3). The proband is the first child from a healthy, non-consanguineous pair of parents. No history of any other family members with a cleft lip or other congenital defects was reported. Furthermore, neither passive smoking nor folic acid supplementation during the first trimester was reported.

#### Discussion

In this family-based association study, we aimed to fine-map the susceptibility genes of NSCL/P on 4q31–q32 with 214 Chinese trios. Our selection of the 4q31–q32 region was based on a previous linkage signal from a genome-wide study with Chinese families by Marazita [9]. Their highest multipoint linkage peak fell at the STR marker, D4S1629, on 4q31–q32. Although we genotyped the region with 10 STRs and 80 SNPs in a two-step approach, without considering the parent-of-origin effects, no markers reached the significant level after correction for multiple testing.

During the first step of the approach, we genotyped the 4q31–q32 region with 10 STR markers and found the lowest \( p\)-value at D4S413, which is in strong LD with the previously reported D4S1629. Both of the STR markers were located upstream on the GRLA2 gene. During the second step of genotyping, we found the lowest \( p\)-value of our individual SNP tests at rs10517665 in GRLA2. A haplotype analysis of TDT showed that the GTGAAGCAAAAGA haplotype with the lowest \( p\)-value is also located upstream in GRLA2. The GTGAAGCAAAAGA haplotype contains several SNPs from rs4691396 (Chr4:158525561) to rs6843489 (Chr4:158538880). Interestingly, this region contains the D4S143 and D4S1629 markers. Together, these lines of evidence indicate an involvement of GRLA2 in NSCL/P. The gene product of GRLA2 is a member of the glutamate receptor family. This family is sensitive to alpha-amin-o-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and functions as ligand-activated cation channels. Glutamate receptors play an important role in excitatory neurotransmission, with no known biological functions related to embryonic development [31].

From a subset of Chinese families used in Marazita’s study of NSCL/P, Choi reported an association between NSCL/P and the PDGF-C SNP rs28999109 [17]. This NSCL/P-associated SNP is located in the promoter region of PDGF-C. Without considering the parent-of-origin effects, our study did not yield any significant association between NSCL/P and PDGF-C. However, we observed low \( p\)-values from our association test in GRLA2. The GRLA2 gene is located next to PDGF-C with a physical distance of 240 kb, and their transcriptional units face away from each other. This 240-kb intergenic region of GRLA2 and PDGF-C contains a number of highly conserved segments, which may influence the simultaneous expression of both genes. Furthermore, in a genome-wide study, Pickrell et al. reported signals of positive selection on GRLA2 and another nearby gene, GLRB, in an Asian-specific manner [32]. Although based on limited observation, it is possible that the weak association that we noticed between GRLA2 and NSCL/P may be due to the nearby PDGF-C gene.
PDGF-C in palate formation. The Pdgfc knockout mice have a cleft face phenotype [33,34], indicating an important role of PDGF-C in the fusion of palate shelf during embryonic growth. Also, a zebrafish study demonstrated that Pdgf signaling was negatively regulated by binding of microRNA Mirn140 to Pdgfra 3’ UTR during palatal development [35]. Other animal studies reported that both Pdgfa and Pdgfc can regulate the development of palate via binding to Pdgfr-α.

The PDGF-C gene is a newly found member of the PDGF family. The encoded product, PDGF-CC, binds to the PDGF receptors (PDGFR-αα and PDGFR-αβ) and subsequently activates the extracellular signal-regulated protein kinase/mitogen-

Figure 1. Linkage disequilibrium (LD) pattern of the 80 SNPs in step 2. Linkage disequilibrium derived from the parents of our sample set as measured in $r^2$. White, $r^2 = 0$; Shades of gray, $0 < r^2 < 1$; Black, $r^2 = 1$.

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Figure 2. Scatter diagram of the p-values of FBAT and PLORT tests. Scatter diagram of the p-values ($-\log_{10}$ (p-value)) of FBAT and POLRT tests. The p-value of rs17035464 exceeds the corrected threshold of multiple testing.

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activated protein kinase (Erk/MAPK) and Akt/PKB pathways by phosphorylation [36,37,38]. Through these two signaling pathways, PDGF-C regulates the processes of cell proliferation, survival, and migration, as well as deposition/maintenance of extracellular matrix [39]. Parent-of-origin effects have been previously reported for several CL/P candidate genes, namely MTHFR, TGFA, and RUNX2 [40,41,42]. The mechanism of parent-of-origin effects remains elusive. One potential mechanism is mediated through genomic imprinting. To our knowledge, PDGF-C has not been reported as an imprinted gene. However, a CpG island (Chr4: 158112136–158112736) can be found adjacent to the PDGF-C SNP rs17035464 and also resides within the segmental UPD region of proband 152 (Figure 3). Despite any biological evidence here, it is still possible that failed imprinting process at the region may disturb embryonic and neonatal growth, such as found in Prader-Willi syndrome. A direct relationship between CL/P and imprinting has not been reported, and the imprinting study of PDGF-C genomic region may provide clues to the causal effect of the gene on NSCL/P.

In addition to the association analysis, we used genotyping information of a trio family for alleles violating Mendelian transmission. Our proband 152, a 1-year-old girl with bilateral cleft lip and palate, was found to contain a segmental maternal uniparental isodisomy on 4q31–q32 [25,43,44]. All our marker information on 4q31–q32 were used to validate the aberrant transmission on D4S1498 in family 152. Three contiguous markers, rs17035464, rs6845322, and D4S1498, were found with both alleles of the proband exclusively derived from the mother. The three markers constitute a minimal region of 14 kb in question (Figure 3). The rest of the markers were either transmitted in the Mendelian mode or uninformative. To define the largest region, two informative SNPs, rs8945388 (Chr4: 158036393) and rs7674099 (Chr4: 158131475), marked the boundaries for the Mendelian transmission, were identified to be 94 kb apart. As a result, the UPD region is between 14 to 94 kb in length and maps to the promoter region of PDGF-C. This minimal 14 kb region also contains a known SNP, rs28999109, which was reported to affect the transcriptional activity from the PDGF-C promoter [17]. Our PO-LRT signal SNP rs17035464 was one of the uniparental transmitted SNPs. Still, there is no direct evidence indicating that this UPD can directly mediate the formation of CL/P. The most relevant report came from a Malay group, who observed a segmental UPD on chromosome 6 from a NSCL/P boy [45]. Without further investigation, the report did not conclude that UPD was a mechanism of NSCL/P. In the future, more cases of normal karyotype with a segmental UPD should be found with the use of a high resolution SNP genotyping microarray, and then we will obtain a better picture of how UPD influences gene expression and leads to the disruption of embryonic development.

In summary, our study of sporadic NSCL/P case-parent trios from Han Chinese demonstrates an association between PDGF-C and NSCL/P only when considering parent-of-origin effects. Additionally, an abnormal transmission pattern found in the promoter region of PDGF-C of proband 152 suggests that study of small-sized UPD may become a fruitful area for gene mapping of diseases.

**Supporting Information**

- Table S1 STR markers in genotyping Step 1. (DOC)
- Table S2 SNP markers in genotyping Step 2. (DOC)
- Table S3 TDT and PO-LRT analysis. (DOC)
- Table S4 Genotypes of family 152. (DOC)
- Table S5 Haplotype TDT Analysis. (DOC)

**Figure S1 CNV analysis of proband 152 on 4q32.** The whole genome genotyping and CNV analysis was performed by Illumina Human 660W-Quad v1 DNA Analysis BeadChip. (TIF)
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Author Contributions
Conceived and designed the experiments: ZZ DMW JY. Performed the
experiments: DW MW FZ. Analyzed the data: DW MW YZ ZY. Contributed reagents/materials/analysis tools: XW NY TS HL. Wrote the
paper: MW DW DMW.

References
1. Murray JC (2002) Gene/environment causes of cleft lip and/or palate. Clinical
Genetics 61: 240–256.
2. Cooper ME, Stone RA, Liu Y-E, Hu D-N, Melnick M, et al. (2000) Descriptive
Epidemiology of Non-syndromic Cleft Lip with or without Cleft Palate in
Shanghai, China, from 1980 to 1989. Cleft Palate–Craniofacial Journal 37: 274–
280.
3. Harville E, Wållmark A, Lie R, Åbyholm F, Vindenes H (2007) Epidemiology of
cleft palate alone and cleft palate with accompanying defects. European Journal
of Epidemiology 22: 389–395.
4. Judgesar A, Murray JC (2003) Orofacial clefting: recent insights into a complex
trait. Curr Opin Genet Dev 15: 270–278.
5. Juriloff DM, Harris MJ, Brown CJ (2001) Unravelling the complex genetics of
cleft lip in the mouse model. Mamm Genome 12: 426–433.
6. Murray JC, Schuette BC (2004) Cleft palate: players, pathways, and pursuits. The
Journal of Clinical Investigation 113: 1676–1670.
7. Vieira AR, Arla JD, Daak-Hirsch S, Dragan E, Fexi TM, et al. (2005) Medical
Sequencing of Candidate Genes for Nonsyndromic Cleft Lip and Palate. PLoS Genet 1: e64.
8. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC (2009) Cleft lip and palate.
Lancet 374: 1773–1785.
9. Marazita ML, Field LL, Cooper ME, Tobias R, Maher BS, et al. (2002) Genome Scan for Loci Involved in Cleft Lip With or Without Cleft Palate, in
Chinese Multiplex Families. Am J Hum Genet 71: 349–364.
10. Wyszynski DF, Albach-Hejazi H, Eldrani M, Hammoud M, Shkair H, et al. (2003) A genome-wide scan for loci predisposing to non-syndromic cleft lip with or without cleft palate in two large Syrian families. Am J Med Genet A 125A:
140–147.
11. Schultz RE, Cooper ME, Daack-Hirsch S, Shi M, Nepomuceno B, et al. (2004) Targeted scan of fifteen regions for nonsyndromic cleft lip and palate in Filipino
families. Am J Med Genet A 125A: 17–22.
12. Paredes M, Carreno H, Sola JA, Segu J, Palomino H, et al. (1999) [Association between nonsyndromic cleft lip/palate and microsatellite markers located in 4q]. Rev Med Chil 127: 1431–1438.
13. Breuer C, Holloway S, Zawalnyski P, Schinzel A, FizPatrick D (1998) A
chromosomal deletion map of human malformations. Am J Hum Genet 63:
1153–1159.
14. Mitchell LE, Healey SC, Chenevix-Trench G (1995) Evidence for an association
between nonsyndromic cleft lip with or without cleft palate and a gene located
on the long arm of chromosome 4. Am J Hum Genet 57: 1130–1136.
15. Reigstad LJ, Varhaug JE (2005) Structural and functional specificities of PDGF-
A regulatory region SNP rs28999109 decreases promoter transcriptional activity
and is associated with CL/P. Eur J Hum Genet.
16. Ding H, Wu X, Bostrom H, Kim I, Wong N, et al. (2004) A specific requirement for PDGF-C in palate formation and PDGFR-a signaling. NATURE
GENETICS 36: 1111–1116.
17. Wu X, Ding H (2007) Generation of Conditional Knockout Alleles for PDGF-C:
genesis 45: 653–657.
18. Eberhart JK (2007) MicroRNA Mir140 modulates Pdgf signaling during palatogenesis. NATURE GENETICS.
19. Reisgud LJ, Sunde HM, Fluge O, Buland O, Muga A, et al. (2005) Platelet-
derived growth factor (PDGF)-C, a PDGF family member with a vascular
endothelial growth factor-like structure. J Biol Chem 276: 17114–17120.
20. Ustach CV, Taube ME, Hurst NJ, Jr., Bhagat S, Bonfil RD, et al. (2004) A potential oncogenic activity of platelet-derived growth factor d in prostate cancer progression. Cancer Res 64: 1722–1729.
21. O’Connell RM, Altshuler D, Goldfine J, Dameron B, Daly MJ, et al. (2004) Analysis of Epidemiology 22: 389–395.
22. Prescott NJ, Winter RM, Malcolm S (2002) Maternal MTHFR genotype
contributes to the risk of non-syndromic cleft lip and palate. J Med Genet 39:
361–369.
23. Sull J, Liang K-Y, Hettmanski J, Wu T, Fallon M, et al. (2009) Evidence that
TGFA influences risk to cleft lip with/without cleft palate through unconven-
tional genetic mechanisms. Human Genetics.
24. Sull JW, Liang K-Y, Hettmanski JB, Fallon MD, Ingessan RG, et al. (2008)
Differential parental transmission of markers in RUNX2 among cleft case-
parent trios from four populations. Genetic Epidemiology 32: 505–512.
25. Magolda E, Reuter H, Birnbaum S, Waller M, Matthiesen M, et al. (2009)
Genome-wide linkage scan of nonsyndromic orofacial clefting in 91 families of
central European origin. Am J Med Genet A 149A: 2609–2694.
26. Sowpati DT, Thiagarajan D, Sharma S, Sultana H, John R, et al. (2008) An
intronic DNA sequence within the mouse Neuronatin gene exhibits biochemical
characteristics of an ICR and acts as a transcriptional activator in Drosophila.
Mech Dev 125: 963–973.
27. Salalhashourif A, Halmi AS, Sulaiman WA, Zilfali BA (2010) Maternal
uniparental heterodisomy of chromosome 6 in a boy with an isolated cleft lip and palate. Am J Med Genet A 152A: 1818–1821.