Craniofacial Microsomia and Variants in Genes Related to Retinoic Acid, Endothelin, and Phenotypically Overlapping Syndromes

Craniofacial microsomia and variants in genes related to retinoic acid, endothelin, and phenotypically overlapping syndromes

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To

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To my dad and mom

To my brother

For their love and support

Abstract

Background: Craniofacial microsomia (CFM) is the second most common facial congenital anomaly. It involves asymmetric underdevelopment of facial skeletal bones and soft tissues, mostly the lower face and ear. Aberration of vascular development and cranial neural crest cell (NCC) migration are believed to be two underlying mechanisms. Etiological factors are heterogeneous and may involve the interplay of environmental and genetic factors. In complex disorders, in addition to an affected child's own genotypes, maternal genotypes can also increase the risk of offspring disease by influencing the *in utero* milieu. Here we studied three groups of candidate genes those relating to retinoic acid, endothelin, and syndromes with phenotypic characteristics overlapping those of CFM. Most of these genes are involved in the development of cranial NCCs or in vasculogenesis,

Methods: We applied tagSNPs (tagged single nucleotide polymorphisms) method to identify 336 SNPs from 14 candidate genes in the retinoic acid pathway, 8 candidate genes in the endothelin pathway, and 8 candidate genes from syndromes with overlapping phenotypes in 98 case-parent sets (83 case-parent trios, 15 case-mother dyads). We used a log-linear approach applied in the software Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling (EMIM) to estimate the relative risk of CFM associated with offspring and maternal genotypes.

Results: After accounting for multiple comparisons, we identified 5 associated child variants and 3 associated maternal variants with the top SNP, rs429738, from *TBX1* [heterozygous and homozygous relative risks (95% CI): 0.4 (0.2, 0.6) and 0.7 (0.3, 1.6) respectively]. The top 5 loci from child genotype effects, harbored by candidate genes of *HOXA1*, *RXRB*, and *TBX1*,

are found to be part of transcriptional regulators involved in NCCs development. For maternal genotype effects, the top 3 loci are harbored by candidate genes of *ALDH1A2* and *CRABP1*, both of which are known to regulate cellular retinoic acid level.

Conclusions: In one of the first studies of genetic risk factors in association to CFM, results provide evidence for the role of genetics as part of CFM pathogenesis. We observed association between genetic variants known to involve in NCC development in both offspring and mothers and the risk of CFM. Follow-up studies with greater power are necessary to validate these results.

Contents **Pages**

PREFACE

This thesis is divided into three chapters according to study's specific aims. Each chapter includes but are not limited to introduction, background and significance, hypothesis, methods, results, and discussion.

CHAPTER I

Craniofacial Microsomia and genetic variations in the retinoic acid pathway in case-parent trios

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Abstract

Craniofacial microsomia (CFM) is the second most common facial congenital anomaly and involves underdevelopment of the lower face and ear, particularly structures derived from the first and second pharyngeal arches. Etiological factors are heterogeneous, and aberration of cranial neural crest cell (NCC) migration is believed to be the underlying mechanism. Retinoic acid (RA) is teratogenic and plays an important role in determining the fate of cranial NCCs. Whole exome sequencing within the RA pathway have suggested a relationship with risk of CFM. We evaluated 126 tagSNPs (single nucleotide polymorphisms) from 14 candidate genes in the RA pathway in 98 case-parent sets. A log-linear approach was fit using the software Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling (EMIM) to estimate relative risk of CFM associated with offspring and maternal genotypes. After accounted for multiple comparisons, child variants in *HOXA1* (rs1801085, rs929249 and rs4722659) and *RXRB* (rs2281390) were associated with CFM risk (heterozygous RR [95% CI]: 2.1 (1.0, 4.1), 2.1 (1.0, 4.1), 2.6 (0.3, 20.3), and 2.02 (0.6, 5.9), respectively). For the maternal genotype effect, variants in *ALDH1A2* (rs7182332 and rs7165127) and *CRABP1* (rs4778810) were associated with CFM risk in offspring [heterozygous RR (95% CI): 5.1 (1.9, 13.8), 5.9 (1.7, 19.7), and 0.3 (0.1,0.7), respectively].

These results support the new insights to genetics role as part of the pathogenesis of CFM. Among the first to provide evidence that variants in the RA pathway are associated with CFM risk, this study suggests some evidence of fetal and/or maternal genetic variations and the risk of CFM. Although a relatively small study, this provides interesting direction to follow up with bench research, to combine with data from other genetic epidemiology studies, or by performing exome sequencing studies.

Introduction

Craniofacial microsomia (CFM) is a developmental disorder involving asymmetric development of structures derived from the first and second pharyngeal arches.[1-4]The CFM phenotype ranges from mild to severe with hallmark characteristics of asymmetric ear anomalies with or without conduction deafness; facial asymmetry, and ocular defects. [1,2,4-6] The extended spectrum may involve malformations of extracranial structures including vertebrae, upper heart, and kidneys.[4,6]

The etiology of CFM is thought to be heterogeneous, however the underlying mechanism remains unclear. The proposed mechanisms are related to the development of structures derived from the first and second pharyngeal arches.[2,3] One possible mechanism involves disturbance of cranial neural crest cell (NCC) fate.[7] For example, maternal exposure to retinoic acid (RA) during early pregnancy has been shown to affect cranial NCCs' fate and has been documented in mothers of infants diagnosed with CFM.[8-12] Segregation analysis and chromosomal anomalies in patients with CFM have also implicated genetic causes.[13-15] The RA role in CFM risk has been further supported by whole-exome sequencing where

mutations in myelin transcription factor 1 (*MYT1*) gene were identified. Functional studies showed that this gene was responsible for craniofacial cartilage development as part of the RA signaling pathway.[16,17]

As long ago as 1956, both hypervitaminosis and vitamin A deficiency were recognized to cause embryopathy.[18-20] It is clear that optimum level of RA is required for normal embryonic development. The level of endogenous RA during vertebrate development is strictly and tightly controlled by the combined action of RA synthesizing and metabolizing enzymes.[21] The cellular mechanism under extreme amount of RA shows disrupted migratory pattern of cranial NCCs. [22-25] Not only endogenous RA level that is important, differential expression of genes encoding the enzymes in RA pathway and other proteins such as HOX and BMPs are also indispensable in embryogenesis.[26-31] The temporally and spatially defined cascades of signaling molecules are what drive normal embryonic development.[32,33] It is natural to hypothesize that offspring genetic variants influencing levels or activity of RA during critical developmental windows could influence risk of CFM.

While the level of endogenous RA is controlled by embryonic machinery, the exogenous RA level is dominated by maternal RA consumption. This is particularly true during the periconceptional period where the rate-limiting RA synthesizing enzyme, retinaldehyde dehydrogenase 2 (RALDH2), in the embryo has yet to express.[34-36] Besides maternal unbalanced RA consumption, improper RA production and reduction in the mother might confer an additional genetic risk factor. In fact, the phenomenon when genetically mediated maternal phenotypes behave as inter-uterine environmental risk factors for offspring disease risk is called maternal indirect genetic effects.[37] Similarly, the well-documented maternal

genetic polymorphisms in folate metabolizing gene, *MTHFR*, have an effect on the risk of neural tube defects in the child.[38,39] Taken together, these observations have suggested that maternal genetic variants influencing RA metabolism during periconceptional period could influence CFM risk in the offspring.

In the present study, we conducted a candidate gene study to investigate the association between CFM risk and genetic variations in the RA pathway in 98 case-parent sets. We hypothesize that that CFM risk could be affected by genetic variations in RA pathway from either the mother or offspring. To estimate relative risks associated with either offspring or maternal genetic effects, we implemented a log-linear approach through multinomial maximum likelihood model.

Materials and methods

Case-Parent Trios

This study is part of a larger investigation that included genetic variants in two groups of genes in addition to those in endothelin-related genes and genes causing syndromes affecting structures derived from the first and second pharyngeal arches. CFM probands and their parents had previously participated in a multi-center case-control study of CFM[11,40], for which they contributed buccal swab samples and consented for their future use. Study participants were recruited through craniofacial specialists from 26 centers throughout the United States and two regions in Canada. All CFM cases were classified according to presence of major structural malformations, based on the expanded CFM spectrum. Children with diagnoses of syndromes or chromosomal anomalies were excluded. Mothers were

interviewed within three years of delivery, by telephone, about demographic and reproductive factors, illness, medication use, diet, and other exposures and behaviors. (Table I-IV) This study was approved by Washington State Institute Review Board (WSIRB).

Biospecimen collection

The original interview-based study included 280 cases of which 245 had been diagnosed with CFM and a further 35 with unilateral anotia or microtia without evidence of facial asymmetry. Buccal cytobrushes were mailed to participants' parents, who were asked to obtain buccal cell specimens for themselves and their child, by rotating the cytobrush on the inside of the cheek for 30 seconds. Parents were then instructed to dry the brushes, place them back into the cytobrush containers, and send them back to the coordinating center in Boston through the mail. Buccal cell samples were obtained from approximately 66% of cases and their parents (172 case-parent sets).

Laboratory methods

DNA extraction and whole-genome amplification

DNA was extracted from buccal cytobrushes using QIAamp DNA Mini Kit (QIAGEN Inc., Valencia, CA) according to manufacturer's protocol. After extraction, DNA was quantitated by the Quant-iT PicoGreen technique (Molecular Probes, Eugene, OR). Following PCR, whole-genome-amplified the DNA by using the GenomiPhi DNA Amplification Kit (Amersham Biosciences; Sunnyvale, CA).

SNP discovery by molecular resequencing

We used fluorescence-based DNA resequencing for comprehensive SNP discovery. This sequencing method is an extremely accurate and sensitive method to provide solid basis for direct association studies, because all common variation within a gene is identified and then targeted. European-descended chromosomes and African-descended chromosomes were used as discovery populations to identify common SNPs in the RA pathway. For the endothelin-related genes and syndrome-related genes, the discovery populations additionally included Hispanics, Asians, and Native Americans. SNPs were identified using the PolyPhred program (version 4.26) through pairwise comparison of chromosome peak heights/intensities[41]. Polymorphic sites flagged by PolyPhred were reviewed for genotype accuracy and false positives associated with biochemical artifacts removed. Second-strand confirmation was obtained from a different sequencing primer at 66% polymorphic sites, and third strand confirmation at 33% of all polymorphic sites.

Selection of TagSNPs

We used a linkage disequilibrium (LD)-based approach (r^2) to select an optimal panel of SNPs (tagSNPs). This method reduces the redundancy of information in highly correlated SNPs.[42] We selected tagSNPs by using LDSelect on all known SNPs within each candidate gene with minor allele frequency > 10%. We binned SNPs by grouping all those with pairwise correlations $r^2 \geq 0.8$, and we selected at least one SNP from each bin (the tagSNPs).

Marker genotyping methods

BioMark™ System 96.96 Dynamic Arrays and ABI TaqMan-based genotyping assays were used to conduct high-throughput genotyping of 480 markers in 37 candidate genes of

which 126 SNPs in 14 candidate genes are in the RA pathway and the remaining genes in two other pathways.

Data analysis

Data quality control

For laboratory quality control purposes, 15% (n=78) blind replicates were included, which we assessed for genotyping and sample concordance rate. Low-concordance markers (n=101) and samples (n=2) were removed, and the remaining replicated samples (randomly selected within pairs) were excluded (n=77). For the present analyses, we used markers and samples that passed quality control of 98% and 95% concordance rate respectively. We used PLINK[43] to filter out additional SNPs according to the following quality control criteria: genotypes call rate of ≤91% (n=2), individual genotype call rate of ≤95% (n=41), minor allele frequency <0.02 (n=40), Mendelian error per markers of ≥5 (n=1), Mendelian error per individual of ≥5 (n=102), Hardy-Weinberg equilibrium with a p-value $<1x10^{-6}$ (n=0). Additional samples were excluded due to missing both parents or missing offspring (n=69). After these quality control steps, we have 336 SNPs in 279 samples (83 case-parent triads and 15 case-mother dyads). (Table VI) In this paper, we focus on the analysis of 126 SNPs in 14 candidate genes in the RA pathway in triads and dyads. (Table V)

Statistical analyses

We used the software Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling (EMIM)[44] to estimate relative risks (RRs) of CFM associated with

offspring and maternal genotypes in trios and dyads, with 95% confidence interval (95% CI). This approach allows independent estimation of both offspring and maternal genetic associations with the disease with additional assumptions including Hardy-Weinberg equilibrium, random mating, and known allele frequencies.[44,45] We accounted for multiple comparisons at the gene level by a stepdown approach to control false discovery rates (FDR).[46] We did not account for multiple testing across genes because of the small sample size, which reduced study power. For sensitivity analysis, we performed 10,000 permutations within gene (the option mperm= 10,000). Empirical p-values were generated based on gene-dropping permutation using the max (T) procedure implemented in PLINK.[43] Additionally, we repeated analyses after relaxing Hardy-Weinberg equilibrium assumption and assessment of LD in the genotyped samples. Further, we subgroup-analyzed samples by genders and ethnicity.

Results

From the analyses of DNA data in 98 case-parent sets, greater proportion of probands (58%) were male. (Table II) Most participants were of Caucasian ancestry (53%). From the 126 genotyped SNPs among all samples, 7 SNPs (4 SNPs in child and 3 SNPs in maternal genotypes) reached the FDR threshold within gene (Table IX). For child genotypes, there were associations with CFM risk meeting the FDR threshold for 3 variants in *HOXA1* (rs1801085, rs929249, rs4722659; heterozygous RR and 95% CI: 2.14 (1.09, 4.19), 2.13 (1.09, 4.17), 2.65 (0.35, 20.3) respectively) and 1 variant in *RXRB* (rs2281390; heterozygous RR and 95% CI: 2.02 (0.68, 5.97)) (Table). For maternal genotypes, there were associations with CFM

risk meeting the FDR threshold for two *ALDH1A2* variants (rs7182332 and rs7165127; heterozygous RR and 95% CI: 0.29 (0.14, 0.61) and 0.28 (0.14, 0.57), respectively) and 1 *CRABP1* variant (rs4778810; heterozygous RR and 95% CI: 0.38 (0.18, 0.79)). (Table X, XI) We analyzed further in all top SNPs whether the number of risk alleles of each SNP increased the susceptibility to CFM. We found that homozygous states are associated with higher risk in *RXRB* and *CRABP1* (homozygous RR and 95% CI: 3.94 (1.2, 12.95) and 0.65 (0.27, 1.56) respectively).

Sensitivity analysis

First, permutation procedure was used as a sensitivity analysis. The association with SNP in *RXRB* (rs2281390) remained the top SNP after permutation correction. Second, we relaxed our assumption of Hardy-Weinberg equilibrium and found similar results in both child and maternal genetic effects. Then, we recalculated LD coefficients between genotyped SNPs. We found that among the top variants, 3 SNPs from *HOXA1* and 2 SNPs from *ALDH1A2* are highly correlated (r^2= 0.83-0.99 and r^2= 0.92 respectively). [Unpublished data]

Subgroup analyses

Among subgroup samples, both child and maternal genetic effect appear to be stronger in boys than in girls (increased heterozygous RR). Additionally, more loci appear to be associated with an increased risk of CFM in boys. No apparent trend between white and non-white probands was detected.

Discussion

We performed a CFM candidate gene tagSNP association study covering important genes in RA pathway and downstream genes, such as transcription factors. The use of case-parent trio design helps eliminate undetected ethnic stratification as a cause of false positive results. Although this study has limited power due to small sample size, we used a logical *a priori* hypothesis, as well as the biological plausibility for candidate genes selection to mitigate this limitation. The use of conditional on parental mating types allowed us to estimate child and maternal genotype effect on child relative risks separately. Due to the testing of SNPs in multiple genes, positive results need to be interpret carefully. The stepdown approach may help reduce the FDR. We observed associations between CFM risk and child genetic variants in *RXRB* and *HOXA1* and maternal genetic variants in *ALDH1A2* and *CRABP1*. It is noteworthy that we observed child genetic effect for SNPs related to RA function, while variants from maternal genetic effect are related to RA level.

The two child variants involving in RA function, *RXRB* and *HOXA1*, appear to be associated with CFM risk. The RA receptors, RARs and RXRs, are ligand-activated transcription factors.[47-49] The functional unit, RAR-RXR heterodimer, has been implicated in RA signal transduction, controlling of cell proliferation, differentiation, and apoptosis.[50,51] More recent evidence for the role of RA receptors in modifying CFM risk is from the whole exome sequencing in fifty-seven CFM patients. A *de novo* missense variant was identified in *MYT1*, a gene later determined to regulate all RA receptor genes and RA-mediated transcription.[16,17]

The *Hox* genes are a large group of homeobox genes known to be responsible for positional fate specification of cells during embryogenesis.[52,53] *Hoxa1* plays a crucial role in neural crest specification and specifically for inner ear morphogenesis.[26] It is the earliest

expressed of the *Hox* genes[54] and among the first genes to be induced by retinoids.[26,55] In mice, low dose administration of RA can rescue inner ear structure in *Hoxa1-null* mutant, supporting the evidence for RA role in regulating *Hox* genes.[56] However, it is also plausible that *Hox* signaling may influence the risk of CFM independently of RA activity: human *HOXA1* deficiency is known to disrupt inner and outer ear development, as well as brain, craniofacial and cardiac structures to varying degrees.[30]

When look at maternal genetic polymorphisms, variants in two genes responsible for maintaining optimum intracellular RA level met the FDR threshold. *ALDH1A2* encodes an RA synthesizing enzyme RALDH2, and *CRABP1* encodes cellular retinoic acid binding proteins (CRABP1).[57] Targeted inactivation of mouse *aldh1a2* supports the crucial role of RALDH2 in producing RA for pharyngeal arch development.[58] Although, most mouse studies focused on the effects of *aldh1a2* mutation in the embryos, a study in zebrafish has demonstrated the role of maternal *aldh1a2* in zebrafish embryo.[59] The persistent activity of RA in *aldh1a2*-mutant embryos indicating that the residual *aldh1a2* activity was being passed down from the mother.[59] This finding emphasizes the possible mechanism of maternal *ALDH1A2* and the effect of RA level in the offspring.

In the developing embryo, CRABPs are expressed in tissues that are most susceptible to RA's teratogenic effects including CNS, PNS, and craniofacial mesenchyme. [60] The presence of CRABPs in cells of a given type indicates that those cells require RA at some point during development and cell differentiation.[36] As CRABPs bind to RA to prevent RA from degrading enzymes, thus controlling the level of cellular RA.[61] Although, no studies have looked into activity of *CRABP1* mothers and effect on the offspring, our results show that the

polymorphisms of the mother *CRABP1* may have an indirect genetic effect on the offspring risk of CFM.

Among the subgroup samples, we observed stronger association in boys than girls. To our knowledge, no prior study investigated roles of gender in relation to CFM risk except that CFM is appeared to be more prevalent in boys than girls (ratio of 3:2).[62] In analyses restricted to white probands, no clear trend was observed compared with non-white families or the combined group.

Conclusion

We found that variations in RA pathway in offspring genome and maternal genome may contribute to CFM risk in offspring. We identified four novel loci from two genes (*RXRB* and *HOXA1*) where child genotype may be associated with CFM risk as well as three loci from two genes (*ALDH1A2* and *CRABP1*) in maternal genotype. Our findings highlight the RA functional pathway in the genetic contribution to development of CFM. We considered our result as a preliminary analysis to investigate association of CFM risk and genetic variations in RA pathway. Confirmatory studies are needed to support these results. This study suggests a direction for future experimental models or exome sequencing studies, or for combining with other genetic epidemiologic data.

Table I Associated Major Malformations Among Cases With CFM

Type of Defect	***Craniofacial Microsomia (%) (n = 168)***
Craniofacial Microsomia	139 (82%)
Cardiac	30 (18%)
Cleft Lip/Palate	22 (13%)
Renal	11 (6%)
Limb	11 (6%)
Gastrointestinal	10 (5%)
Questionable Cases	7 (4%)
Central Nervous System	5 (3%)
Anopthalmia	4 (2%)
Limb Reduction Defect	4 (2%)

Table II Case and Mother Characteristics

Characteristics	***CFM (%)***	***Included in analyses (%)***
Case	***(n= 168)***	***(n=98)***
Gender		
Male	95 (56%)	57 (58%)
Female	70 (42%)	39 (40%)
Mother	***(n= 168)***	
Race		
White	101 (60%)	52 (53%)
Hispanic	49 (29%)	
Asian	6 (4%)	
Black	5 (3%)	
Native American	4 (2%)	
Age (y)		
Age < 20	12 (7%)	
Age 20-24	28 (16%)	
Age 25-29	43 (25%)	
Age 30-34	54 (32%)	
Age >34	28 (16%)	
Marital status		
Married	125 (74%)	
Single Living with Partner	31 (18%)	
Single	9 (5%)	

Table IV Maternal Reproductive Characteristics

Characteristics	*CFM (%) (n= 168)*
Body mass index (kg/m^2)	
<19	25 (15%)
19-24	53 (31%)
25-29	26 (15%)
30-34	21 (12%)
36-39	6 (3%)
Parity	
1	51 (30%)
2	114 (68%)
Planned pregnancy	
Yes	65 (38%)
No	100 (59%)

Table V Genes with SNPs Genotyped in This Study

Group	*Subgroup*	*Gene symbol*	*Number of SNPs tested in this study*	*Plausible role in mother*	*Plausible role in child*
RA level	Synthesizing enzyme	*ALDH1A2*	25	X	X
	Metabolizing enzyme	*CYP26A1*	4	X	X
		POR	3	X	X
	RA binding protein	*CRABP1*	3	X	X
		CRABP2	2	X	X
RA action	Transduce cellular signals	*RARA*	6		X
		RARG	6		X
		RXRB	2		X
		RXRG	31		X
	Downstream signaling of	*HOXA1*	3		X
	RA in pharyngeal arches	*HOXA4*	3		X
		HOXB4	1		X
		HOXD4	2		X
		BMP5	35		X

Table VI Quality Control Steps implemented in PLINK[43]

QC steps	*Samples & SNPs removed*	*Samples & SNPs (Total)*
Original		570 samples/480 SNPs
(1) Concordance rate (from 78 duplicated pairs)		
- Concordance rate/samples with duplicates	78-1 pairs	77 pairs (of 568 samples)
- Low concordance rate/SNPs using duplicated samples	480-101 SNPs	379 SNPs
- Low concordance rate among 77 duplicates after remove SNPs with concordance rate <0.98		
(2) Removed duplicated pairs (unique samples)	77 samples	491 samples/379 SNPs
(3) Mendelian error		
- Dummy parents	491+14 dummies	505 samples/379 SNPs
- Mendelian error per sample and SNP > or = 5		389 samples/378 SNPs
(4) Missing rate		
- Missing rate/sample > 5%	55	348 samples/378 SNPs
- Missing rate/locus > 9%	2 SNPs	348 samples/376 SNPs
(5) MAF <0.02 (2%)	40 SNPs	348 samples/336 SNPs
(6) HWE	0	348 samples/336 SNPs
(7) For analyses – triads and dyads	69 (singlets, families with no child)	279 samples/336 SNPs (83 triads 15 dyads)
(8) For analyses of variants in the RA pathway		279 samples/126 SNPs

Table VII Genes investigated in RA pathway and downstream genes, total number of SNPs genotyped per gene, and number of SNPs with child genotypic associations meeting p<0.1 and p<0.05, by allele frequency.

Gene	*p>=0.1*	*p<0.1& f<0.1*	*p<0.1& f>=0.1*	*p<0.05& f<0.1*	*p<0.05& f>=0.1*	*Total*
ALDH1A2	19	3	3	3	3	25
BMP5	30	3	2	1	0	35
CRABP1	2	0	1	0	0	3
CRABP2	2	0	0	0	0	2
CYP26A1	2	0	2	0	0	4
HOXA1	0	2	1	2	1	3
HOXA4	3	0	0	0	0	3
HOXB4	1	0	0	0	0	1
HOXD4	2	0	0	0	0	2
POR	2	0	1	0	1	3
RARA	6	0	0	0	0	6
RARG	5	0	1	0	0	6
RXRB	1	0	1	0	1	2
RXRG	26	1	4	0	3	31

Table VIII Genes investigated in RA pathway and downstream genes, total number of SNPs genotyped per gene, and number of SNPs with mother genotypic associations meeting p<0.1 and p<0.05, by allele frequency.

Gene	*p>=0.1*	*p<0.1& f<0.1*	*p<0.1& f>=0.1*	*p<0.05& f<0.1*	*p<0.05& f>=0.1*	*Total*
ALDH1A2	14	4	7	4	6	25
BMP5	33	1	1	0	1	35
CRABP1	2	0	1	0	1	3
CRABP2	1	0	1	0	0	2
CYP26A1	3	0	1	0	0	4
HOXA1	3	0	0	0	0	3
HOXA4	3	0	0	0	0	3
HOXB4	1	0	0	0	0	1
HOXD4	2	0	0	0	0	2
POR	3	0	0	0	0	3
RARA	6	0	0	0	0	6
RARG	6	0	0	0	0	6
RXRB	2	0	0	0	0	2

RXRG	29	0	2	0	1	31

Table IX SNPs that meet FDR threshold for Child and Maternal genotype effect

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous RR (95% CI)*	*Homozygous RR (95% CI)*
Child genotype effect								
rs1801085	7	HOXA1	G	0.089	0.017	0.026	2.14 (1.09,4.19)	Undefined
rs929249	7	HOXA1	C	0.09	0.0176	0.026	2.13 (1.09,4.17)	Undefined
rs4722659	7	HOXA1	T	0.872	0.0345	0.035	2.65 (0.35,20.3)	1.33 (0.15,11.51)
rs2281390	6	RXRB	G	0.665	0.0167	0.033	2.02 (0.68,5.97)	3.94 (1.2,12.95)
Maternal genotype effect								
rs7182332	15	ALDH1A2	A	0.184	0.0005	0.009	0.29 (0.14,0.61)	Undefined
rs7165127	15	ALDH1A2	G	0.205	0.0007	0.009	0.28 (0.14,0.57)	0.18 (0.02,1.45)
rs4778810	15	CRABP1	A	0.717	0.0098	0.029	0.38 (0.18,0.79)	0.65 (0.27,1.56)

Table X Analyses for Child genetic effect in 83 triads 15 dyads

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous RR (95% CI)*	*Homozygous RR (95% CI)*
rs12910118	15	*ALDH1A2*	G	0.18	0.0037	0.061	1.59 (0.89,2.85)	5.38 (2,14.45)
rs7165127	15	*ALDH1A2*	G	0.112	0.0049	0.061	0.97 (0.49,1.95)	5.99 (1.79,20.11)
rs16942386	15	*ALDH1A2*	C	0.198	0.0098	0.082	1.64 (0.93,2.9)	4.56 (1.73,12.02)
rs3912286	15	*ALDH1A2*	C	0.092	0.0156	0.098	2.59 (1.33,5.03)	3.1 (0.54,17.71)
rs7182332	15	*ALDH1A2*	A	0.096	0.0322	0.144	1.13 (0.55,2.31)	5.91 (1.53,22.76)
rs17270062	15	*ALDH1A2*	A	0.091	0.0345	0.144	2.37 (1.21,4.62)	3.04 (0.53,17.35)
rs12899914	15	*ALDH1A2*	T	0.151	0.102	0.317	Undefined	Undefined
rs10468019	15	*ALDH1A2*	G	0.231	0.1111	0.317	1.78 (1.03,3.07)	2.06 (0.73,5.78)
rs1876869	15	*ALDH1A2*	T	0.086	0.1211	0.317	1.79 (0.89,3.62)	4.75 (0.99,22.77)
rs2664180	15	*ALDH1A2*	A	0.119	0.1379	0.317	1.73 (0.92,3.25)	3.41 (0.87,13.28)
rs16942348	15	*ALDH1A2*	C	0.08	0.1617	0.317	1.9 (0.93,3.9)	3.63 (0.61,21.51)

rs1876868	15	*ALDH1A2*	T	0.08	0.1617	0.317	1.9 (0.93,3.9)	3.63 (0.61,21.51)
rs2136557	15	*ALDH1A2*	T	0.21	0.1889	0.317	0.58 (0.26,1.3)	0.39 (0.14,1.09)
rs8040351	15	*ALDH1A2*	A	0.453	0.1903	0.317	0.64 (0.36,1.13)	0.84 (0.38,1.84)
rs10519012	15	*ALDH1A2*	G	0.133	0.1904	0.317	1.46 (0.78,2.74)	3.31 (0.94,11.72)
rs900634	15	*ALDH1A2*	C	0.199	0.3163	0.472	0.67 (0.28,1.64)	0.47 (0.16,1.38)
rs12443115	15	*ALDH1A2*	T	0.156	0.338	0.472	0.79 (0.26,2.4)	0.52 (0.14,1.88)
rs7173862	15	*ALDH1A2*	C	0.088	0.3444	0.472	1.59 (0.78,3.24)	3.04 (0.52,17.72)
rs7495641	15	*ALDH1A2*	T	0.072	0.3585	0.472	1.29 (0.59,2.82)	4.17 (0.69,25.27)
rs338415	15	*ALDH1A2*	A	0.514	0.5393	0.648	0.9 (0.51,1.58)	1.21 (0.54,2.67)
rs1630535	15	*ALDH1A2*	A	0.165	0.5535	0.648	2.42 (0.31,18.58)	2.84 (0.34,23.82)
rs17237199	15	*ALDH1A2*	A	0.061	0.5699	0.648	Undefined	Undefined
rs7181217	15	*ALDH1A2*	G	0.017	0.7625	0.829	1.58 (0.37,6.74)	Undefined
rs6494168	15	*ALDH1A2*	A	0.486	0.9236	0.938	1.13 (0.62,2.03)	1.15 (0.51,2.61)
rs10519017	15	*ALDH1A2*	A	0.118	0.9382	0.938	0.91 (0.46,1.79)	0.72 (0.08,6.29)
rs2475252	6	*BMP5*	T	0.045	0.0338	0.527	2.92 (1.24,6.89)	5.88 (0.52,66.83)
rs9357872	6	*BMP5*	A	0.084	0.066	0.527	0.67 (0.3,1.5)	3.71 (0.79,17.34)
rs2498530	6	*BMP5*	T	0.089	0.066	0.527	0.72 (0.31,1.63)	4.27 (0.89,20.39)
rs7772313	6	*BMP5*	C	0.131	0.0975	0.527	1.88 (1.03,3.46)	2.95 (0.77,11.26)
rs7773142	6	*BMP5*	C	0.127	0.0981	0.527	0.83 (0.23,2.91)	0.42 (0.1,1.83)
rs6459092	6	*BMP5*	A	0.136	0.1016	0.527	1.88 (1.03,3.42)	2.72 (0.72,10.27)
rs228144	6	*BMP5*	G	0.178	0.1362	0.527	0.54 (0.29,1.02)	0.83 (0.21,3.32)
rs6910005	6	*BMP5*	G	0.177	0.1442	0.527	1.44 (0.42,4.94)	0.85 (0.21,3.44)
rs4267958	6	*BMP5*	A	0.256	0.1587	0.527	0.63 (0.31,1.3)	0.41 (0.16,1.05)
rs11537721	6	*BMP5*	A	0.178	0.1783	0.527	1.4 (0.41,4.82)	0.85 (0.21,3.44)
rs3756990	6	*BMP5*	T	0.137	0.1815	0.527	0.9 (0.26,3.16)	0.51 (0.12,2.18)
rs2145898	6	*BMP5*	C	0.128	0.1841	0.527	Undefined	Undefined
rs9475387	6	*BMP5*	G	0.028	0.1958	0.527	2.42 (0.83,7.1)	Undefined
rs4504477	6	*BMP5*	T	0.266	0.2332	0.583	0.66 (0.32,1.36)	0.46 (0.18,1.15)
rs4385313	6	*BMP5*	A	0.269	0.29	0.677	0.67 (0.33,1.38)	0.48 (0.19,1.22)

rs2475251	6	*BMP5*	C	0.156	0.3754	0.715	0.66 (0.35,1.26)	0.38 (0.04,3.18)
rs2475249	6	*BMP5*	T	0.156	0.3754	0.715	0.66 (0.35,1.26)	0.38 (0.04,3.18)
rs9382529	6	*BMP5*	G	0.084	0.3881	0.715	Undefined	Undefined
rs2145900	6	*BMP5*	C	0.084	0.3881	0.715	Undefined	Undefined
rs12662388	6	*BMP5*	A	0.467	0.4136	0.724	0.87 (0.48,1.6)	1.24 (0.55,2.78)
rs9367660	6	*BMP5*	G	0.288	0.4892	0.815	1.46 (0.54,3.92)	1.87 (0.61,5.73)
rs3798848	6	*BMP5*	G	0.478	0.5144	0.818	0.96 (0.53,1.74)	1.31 (0.59,2.93)
rs228143	6	*BMP5*	T	0.438	0.6087	0.926	1.2 (0.62,2.3)	1.5 (0.65,3.49)
rs2397190	6	*BMP5*	C	0.475	0.6597	0.946	0.97 (0.53,1.76)	1.24 (0.55,2.79)
rs228141	6	*BMP5*	C	0.492	0.6963	0.946	0.8 (0.46,1.39)	0.89 (0.4,1.97)
rs3798846	6	*BMP5*	T	0.184	0.7359	0.946	1.16 (0.66,2.05)	1.6 (0.49,5.24)
rs2498529	6	*BMP5*	A	0.402	0.7437	0.946	0.84 (0.45,1.55)	0.72 (0.31,1.66)
rs11758071	6	*BMP5*	A	0.039	0.7606	0.946	Undefined	Undefined
rs9475382	6	*BMP5*	C	0.19	0.8185	0.946	1.07 (0.6,1.89)	1.47 (0.45,4.76)
rs9382522	6	*BMP5*	C	0.249	0.8298	0.946	0.9 (0.52,1.55)	1.12 (0.4,3.17)
rs6927049	6	*BMP5*	C	0.171	0.8503	0.946	1.11 (0.61,2)	1.45 (0.4,5.23)
rs2208606	6	*BMP5*	G	0.073	0.8652	0.946	1.08 (0.48,2.4)	1.94 (0.2,18.83)
rs13216987	6	*BMP5*	A	0.184	0.9055	0.954	1.02 (0.57,1.82)	1.31 (0.37,4.71)
rs13217868	6	*BMP5*	C	0.157	0.9266	0.954	0.9 (0.49,1.65)	0.8 (0.16,4.06)
rs9382544	6	*BMP5*	C	0.339	0.9644	0.964	0.96 (0.47,1.96)	0.9 (0.37,2.21)
rs4423382	15	*CRABP1*	C	0.202	0.0667	0.2	2.7 (0.62,11.68)	1.68 (0.34,8.29)
rs4074666	15	*CRABP1*	T	0.397	0.4024	0.573	0.88 (0.45,1.7)	1.24 (0.54,2.87)
rs4778810	15	*CRABP1*	G	0.271	0.573	0.573	1.36 (0.55,3.41)	1.11 (0.38,3.25)
rs7519343	1	*CRABP2*	T	0.011	0.373	0.469	2.87 (2.87,2.87)	Undefined
rs12119297	1	*CRABP2*	T	0.398	0.4694	0.469	0.95 (0.52,1.76)	0.68 (0.29,1.58)
rs6583854	10	*CYP26A1*	T	0.345	0.0618	0.166	2.63 (0.9,7.7)	3.74 (1.13,12.32)
rs12359135	10	*CYP26A1*	T	0.537	0.0831	0.166	1.92 (1.02,3.62)	2.44 (1.04,5.72)
rs913423	10	*CYP26A1*	G	0.46	0.2714	0.337	1.71 (0.87,3.35)	1.85 (0.77,4.42)
rs10786069	10	*CYP26A1*	C	0.469	0.3371	0.337	1.58 (0.81,3.06)	1.84 (0.77,4.36)

rs1801085	7	*HOXA1*	G	0.089	0.017	0.026	2.14 (1.09,4.19)	Undefined
rs929249	7	*HOXA1*	C	0.09	0.0176	0.026	2.13 (1.09,4.17)	Undefined
rs4722659	7	*HOXA1*	C	0.128	0.0345	0.035	2.65 (0.35,20.3)	1.33 (0.15,11.51)
rs10259620	7	*HOXA4*	A	0.198	0.1177	0.318	0.88 (0.35,2.24)	0.5 (0.16,1.53)
rs7810502	7	*HOXA4*	G	0.273	0.2842	0.318	0.78 (0.38,1.64)	0.52 (0.2,1.34)
rs875898	7	*HOXA4*	A	0.181	0.3179	0.318	1.05 (0.35,3.15)	0.68 (0.19,2.43)
rs1860574	17	*HOXB4*	C	0.446	0.1187	0.119	2.1 (1,4.4)	2.14 (0.85,5.42)
rs6753677	2	*HOXD4*	A	0.403	0.7679	0.913	0.86 (0.46,1.63)	1.01 (0.44,2.31)
rs6433589	2	*HOXD4*	A	0.382	0.9125	0.913	0.87 (0.46,1.66)	0.88 (0.38,2.05)
rs6467995	7	*POR*	A	0.106	0.0412	0.124	0.38 (0.16,0.89)	Undefined
rs10952840	7	*POR*	T	0.404	0.3667	0.55	0.68 (0.41,1.16)	0.71 (0.3,1.65)
rs6953665	7	*POR*	T	0.374	0.5765	0.577	0.76 (0.45,1.3)	0.86 (0.36,2.07)
rs3950989	17	*RARA*	A	0.259	0.116	0.42	1.07 (0.47,2.43)	0.61 (0.22,1.7)
rs799916	17	*RARA*	G	0.303	0.2189	0.42	0.75 (0.38,1.47)	0.49 (0.2,1.19)
rs3737559	17	*RARA*	T	0.063	0.2303	0.42	1.11 (0.47,2.63)	5.68 (0.88,36.6)
rs3092994	17	*RARA*	T	0.249	0.346	0.42	0.81 (0.37,1.79)	0.55 (0.21,1.49)
rs8176265	17	*RARA*	T	0.244	0.3503	0.42	0.89 (0.39,2.04)	0.6 (0.22,1.66)
rs4986850	17	*RARA*	T	0.047	0.8311	0.831	0.97 (0.35,2.67)	Undefined
rs12369902	12	*RARG*	C	0.402	0.0551	0.331	0.53 (0.31,0.9)	0.63 (0.27,1.47)
rs12303393	12	*RARG*	A	0.173	0.1175	0.353	0.36 (0.14,0.91)	0.42 (0.14,1.26)
rs10783696	12	*RARG*	C	0.381	0.3676	0.591	0.75 (0.39,1.44)	1.02 (0.44,2.34)
rs7303807	12	*RARG*	G	0.275	0.4093	0.591	1.04 (0.61,1.78)	1.73 (0.7,4.32)
rs4759123	12	*RARG*	G	0.426	0.4922	0.591	0.78 (0.42,1.44)	0.99 (0.44,2.23)
rs11614470	12	*RARG*	A	0.134	0.9219	0.922	0.97 (0.22,4.35)	0.85 (0.16,4.42)
rs2281390	6	*RXRB*	T	0.335	0.0167	0.033	2.02 (0.68,5.97)	3.94 (1.2,12.95)
rs2068204	6	*RXRB*	A	0.05	0.7696	0.77	0.84 (0.32,2.26)	Undefined
rs4657637	1	*RXRG*	G	0.108	0.004	0.124	0.13 (0.04,0.42)	0.23 (0.06,0.85)
rs1032608	1	*RXRG*	T	0.161	0.0101	0.157	1.59 (0.89,2.82)	Undefined
rs11591049	1	*RXRG*	T	0.169	0.0184	0.19	0.44 (0.22,0.87)	1.23 (0.34,4.41)

rs16858531	1	*RXRG*	G	0.061	0.0531	0.412	1.7 (0.76,3.79)	9.18 (1.71,49.2)
rs10800287	1	*RXRG*	C	0.112	0.0775	0.481	2.02 (1.07,3.79)	3.01 (0.68,13.29)
rs11587291	1	*RXRG*	C	0.167	0.1377	0.533	0.51 (0.26,1)	0.63 (0.13,3.15)
rs12091071	1	*RXRG*	A	0.034	0.1507	0.533	2.38 (0.88,6.43)	Undefined
rs6691080	1	*RXRG*	G	0.455	0.1515	0.533	0.6 (0.34,1.05)	0.74 (0.34,1.63)
rs748800	1	*RXRG*	G	0.039	0.1547	0.533	2.23 (0.88,5.69)	Undefined
rs12143783	1	*RXRG*	T	0.189	0.2222	0.593	1.16 (0.66,2.03)	0.29 (0.04,2.42)
rs12077809	1	*RXRG*	A	0.1	0.2251	0.593	1.74 (0.9,3.35)	1.16 (0.13,10.45)
rs11590513	1	*RXRG*	T	0.121	0.2481	0.593	1.03 (0.53,2.01)	Undefined
rs11806848	1	*RXRG*	A	0.249	0.2552	0.593	0.76 (0.29,1.95)	1.19 (0.4,3.49)
rs6679415	1	*RXRG*	A	0.118	0.2677	0.593	1.02 (0.53,1.98)	Undefined
rs4657643	1	*RXRG*	C	0.039	0.3131	0.647	1.88 (0.72,4.88)	Undefined
rs12129059	1	*RXRG*	A	0.124	0.3828	0.74	0.53 (0.11,2.47)	0.81 (0.15,4.25)
rs16858608	1	*RXRG*	A	0.251	0.4059	0.74	0.69 (0.39,1.22)	0.55 (0.16,1.88)
rs869714	1	*RXRG*	C	0.471	0.5141	0.764	1.36 (0.72,2.58)	1.63 (0.7,3.81)
rs1027495	1	*RXRG*	G	0.343	0.5154	0.764	0.83 (0.4,1.69)	1.09 (0.45,2.63)
rs10918635	1	*RXRG*	T	0.333	0.5286	0.764	0.74 (0.44,1.27)	0.64 (0.24,1.73)
rs6427069	1	*RXRG*	C	0.346	0.5472	0.764	0.92 (0.44,1.93)	1.23 (0.5,3.05)
rs7527143	1	*RXRG*	G	0.469	0.5588	0.764	1.34 (0.71,2.53)	1.59 (0.68,3.71)
rs988778	1	*RXRG*	G	0.455	0.5667	0.764	1.36 (0.76,2.41)	1.44 (0.63,3.3)
rs11808527	1	*RXRG*	T	0.349	0.605	0.781	1.31 (0.77,2.23)	1.4 (0.58,3.36)
rs4657638	1	*RXRG*	T	0.127	0.6657	0.807	0.72 (0.36,1.47)	0.72 (0.08,6.35)
rs7540969	1	*RXRG*	C	0.337	0.6811	0.807	0.8 (0.48,1.35)	0.71 (0.28,1.83)
rs12029632	1	*RXRG*	T	0.287	0.7046	0.807	1.11 (0.65,1.89)	1.48 (0.59,3.74)
rs1532482	1	*RXRG*	T	0.36	0.7287	0.807	1.22 (0.72,2.08)	1.36 (0.58,3.22)
rs904222	1	*RXRG*	T	0.1	0.9446	0.991	0.89 (0.43,1.83)	1 (0.11,9.02)
rs10918653	1	*RXRG*	A	0.352	0.9708	0.991	0.99 (0.58,1.67)	1.07 (0.45,2.58)
rs12734731	1	*RXRG*	A	0.25	0.9913	0.991	1 (0.39,2.53)	1.04 (0.35,3.07)

Table XI Analyses for Maternal genetic effect in 83 triads 15 dyads

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous RR (95% CI)*	*Homozygous RR (95% CI)*
rs7182332	15	*ALDH1A2*	A	0.184	0.0005	0.009	0.29 (0.14,0.61)	Undefined
rs7165127	15	*ALDH1A2*	G	0.205	0.0007	0.009	0.28 (0.14,0.57)	0.18 (0.02,1.45)
rs16942348	15	*ALDH1A2*	C	0.061	0.0066	0.055	2.88 (1.34,6.17)	10.32 (1.92,55.43)
rs1876869	15	*ALDH1A2*	T	0.072	0.0121	0.068	2.82 (1.38,5.8)	5.05 (0.83,30.68)
rs12899914	15	*ALDH1A2*	T	0.092	0.0159	0.068	Undefined	Undefined
rs1876868	15	*ALDH1A2*	T	0.066	0.0164	0.068	2.77 (1.32,5.81)	5.82 (0.93,36.27)
rs7173862	15	*ALDH1A2*	C	0.067	0.0193	0.069	2.72 (1.29,5.7)	5.68 (0.91,35.4)
rs10468019	15	*ALDH1A2*	G	0.243	0.0234	0.073	0.96 (0.54,1.69)	2.74 (1.11,6.75)
rs338415	15	*ALDH1A2*	A	0.557	0.0348	0.097	2.21 (1.17,4.21)	2.62 (1.11,6.2)
rs8040351	15	*ALDH1A2*	A	0.4	0.0397	0.099	0.74 (0.42,1.31)	0.37 (0.16,0.85)
rs6494168	15	*ALDH1A2*	A	0.53	0.0663	0.151	2.04 (1.06,3.94)	2.51 (1.06,5.94)
rs17270062	15	*ALDH1A2*	A	0.104	0.1291	0.263	1.55 (0.8,3.03)	4.36 (1.09,17.44)
rs1630535	15	*ALDH1A2*	A	0.126	0.1368	0.263	2.28 (0.3,17.54)	1.33 (0.15,11.5)
rs3912286	15	*ALDH1A2*	C	0.11	0.1477	0.264	1.56 (0.81,3.01)	3.99 (1.01,15.75)
rs7495641	15	*ALDH1A2*	T	0.081	0.2991	0.486	0.95 (0.43,2.1)	4.04 (0.72,22.57)
rs900634	15	*ALDH1A2*	C	0.201	0.3108	0.486	0.81 (0.32,2.06)	0.53 (0.18,1.62)
rs17237199	15	*ALDH1A2*	A	0.038	0.3512	0.516	Undefined	Undefined
rs2136557	15	*ALDH1A2*	T	0.228	0.4518	0.628	1 (0.4,2.52)	0.71 (0.24,2.11)
rs2664180	15	*ALDH1A2*	A	0.137	0.5179	0.681	1.4 (0.76,2.59)	1.86 (0.44,7.79)
rs16942386	15	*ALDH1A2*	C	0.289	0.5858	0.728	0.84 (0.49,1.43)	0.57 (0.18,1.74)
rs12910118	15	*ALDH1A2*	G	0.265	0.6118	0.728	0.94 (0.55,1.61)	0.57 (0.17,1.9)
rs7181217	15	*ALDH1A2*	G	0.027	0.7017	0.797	0.58 (0.14,2.49)	Undefined
rs12443115	15	*ALDH1A2*	T	0.176	0.8682	0.944	0.98 (0.28,3.4)	0.84 (0.21,3.33)
rs10519017	15	*ALDH1A2*	A	0.109	0.9382	0.95	1.1 (0.56,2.16)	0.87 (0.1,7.69)
rs10519012	15	*ALDH1A2*	G	0.163	0.9504	0.95	1.03 (0.56,1.88)	1.26 (0.31,5.05)

rs13216987	6	*BMP5*	A	0.243	0.0281	0.91	0.47 (0.26,0.84)	0.31 (0.07,1.46)
rs2208606	6	*BMP5*	G	0.055	0.0858	0.91	2.17 (0.96,4.89)	Undefined
rs6927049	6	*BMP5*	C	0.158	0.186	0.91	1.63 (0.91,2.9)	0.97 (0.19,4.87)
rs2475251	6	*BMP5*	C	0.108	0.2519	0.91	1.66 (0.87,3.16)	0.98 (0.11,8.72)
rs2475249	6	*BMP5*	T	0.108	0.2519	0.91	1.66 (0.87,3.16)	0.98 (0.11,8.72)
rs9382522	6	*BMP5*	C	0.28	0.2655	0.91	0.64 (0.37,1.1)	0.62 (0.21,1.88)
rs9382529	6	*BMP5*	G	0.076	0.3116	0.91	Undefined	Undefined
rs2145900	6	*BMP5*	C	0.076	0.3116	0.91	Undefined	Undefined
rs12662388	6	*BMP5*	A	0.451	0.3328	0.91	1.45 (0.76,2.74)	1.12 (0.47,2.64)
rs2498530	6	*BMP5*	A	0.07	0.3369	0.91	1.27 (0.58,2.81)	4.39 (0.73,26.58)
rs9475382	6	*BMP5*	T	0.223	0.4027	0.91	0.83 (0.48,1.45)	0.37 (0.08,1.8)
rs3798846	6	*BMP5*	C	0.223	0.4027	0.91	0.83 (0.48,1.45)	0.37 (0.08,1.8)
rs9357872	6	*BMP5*	T	0.076	0.4062	0.91	1.18 (0.54,2.56)	3.75 (0.63,22.14)
rs9382544	6	*BMP5*	C	0.317	0.4192	0.91	0.94 (0.45,1.93)	0.66 (0.26,1.64)
rs228143	6	*BMP5*	T	0.429	0.4233	0.91	1.58 (0.78,3.23)	1.52 (0.62,3.71)
rs9367660	6	*BMP5*	G	0.231	0.4478	0.91	0.78 (0.33,1.87)	0.57 (0.2,1.61)
rs4504477	6	*BMP5*	T	0.284	0.4522	0.91	1.02 (0.47,2.24)	0.73 (0.28,1.91)
rs4385313	6	*BMP5*	A	0.286	0.4678	0.91	1.03 (0.47,2.26)	0.74 (0.28,1.94)
rs4267958	6	*BMP5*	A	0.28	0.5157	0.95	0.98 (0.45,2.15)	0.72 (0.27,1.89)
rs13217868	6	*BMP5*	C	0.168	0.583	0.952	0.72 (0.39,1.34)	0.66 (0.13,3.29)
rs3798848	6	*BMP5*	C	0.459	0.5981	0.952	1.26 (0.68,2.33)	1.03 (0.44,2.39)
rs2397190	6	*BMP5*	G	0.457	0.5981	0.952	1.27 (0.68,2.36)	1.05 (0.45,2.44)
rs7773142	6	*BMP5*	C	0.156	0.6936	0.967	1.24 (0.28,5.46)	0.97 (0.19,4.85)
rs11758071	6	*BMP5*	A	0.038	0.7216	0.967	Undefined	Undefined
rs228141	6	*BMP5*	C	0.497	0.7425	0.967	0.99 (0.56,1.74)	0.79 (0.34,1.79)
rs2145898	6	*BMP5*	C	0.12	0.7549	0.967	0.54 (0.12,2.45)	0.53 (0.1,2.71)
rs9475387	6	*BMP5*	G	0.043	0.7842	0.967	1.15 (0.43,3.07)	Undefined
rs2475252	6	*BMP5*	T	0.087	0.8387	0.967	0.84 (0.38,1.83)	1.32 (0.14,12.15)
rs3756990	6	*BMP5*	T	0.166	0.8499	0.967	1.24 (0.28,5.47)	1.07 (0.22,5.27)

rs228144	6	*BMP5*	G	0.141	0.8566	0.967	1.18 (0.64,2.19)	1.08 (0.21,5.53)
rs2498529	6	*BMP5*	A	0.432	0.9042	0.967	1.01 (0.54,1.89)	1.14 (0.5,2.6)
rs7772313	6	*BMP5*	A	0.204	0.9327	0.967	0.89 (0.49,1.61)	0.91 (0.23,3.61)
rs6459092	6	*BMP5*	C	0.181	0.9459	0.967	0.91 (0.51,1.63)	0.86 (0.22,3.4)
rs6910005	6	*BMP5*	A	0.187	0.967	0.967	0.86 (0.29,2.6)	0.86 (0.25,2.93)
rs11537721	6	*BMP5*	G	0.204	0.967	0.967	0.86 (0.29,2.6)	0.86 (0.25,2.93)
rs4778810	15	*CRABP1*	G	0.283	0.0098	0.029	0.38 (0.18,0.79)	0.65 (0.27,1.56)
rs4423382	15	*CRABP1*	C	0.214	0.1773	0.266	0.44 (0.18,1.04)	0.53 (0.19,1.47)
rs4074666	15	*CRABP1*	T	0.389	0.319	0.319	0.76 (0.4,1.47)	1.09 (0.48,2.48)
rs12119297	1	*CRABP2*	T	0.386	0.0514	0.103	0.47 (0.26,0.86)	0.5 (0.23,1.11)
rs7519343	1	*CRABP2*	T	0.016	0.7479	0.748	1.67 (1.67,1.67)	Undefined
rs6583854	10	*CYP26A1*	T	0.25	0.0639	0.256	1.15 (0.49,2.72)	0.61 (0.21,1.75)
rs913423	10	*CYP26A1*	G	0.412	0.575	0.814	1.09 (0.58,2.05)	0.82 (0.35,1.92)
rs10786069	10	*CYP26A1*	C	0.425	0.6474	0.814	1.15 (0.61,2.17)	0.92 (0.39,2.14)
rs12359135	10	*CYP26A1*	T	0.492	0.8137	0.814	1.21 (0.67,2.2)	1.17 (0.51,2.69)
rs929249	7	*HOXA1*	C	0.14	0.5325	0.91	0.69 (0.35,1.36)	0.5 (0.06,4.27)
rs1801085	7	*HOXA1*	G	0.136	0.6493	0.91	0.74 (0.38,1.44)	0.57 (0.07,4.82)
rs4722659	7	*HOXA1*	C	0.163	0.9096	0.91	0.77 (0.22,2.73)	0.82 (0.2,3.3)
rs7810502	7	*HOXA4*	G	0.331	0.6652	0.969	1.4 (0.59,3.3)	1.57 (0.58,4.28)
rs10259620	7	*HOXA4*	A	0.258	0.7199	0.969	1.4 (0.47,4.14)	1.61 (0.48,5.35)
rs875898	7	*HOXA4*	A	0.214	0.9689	0.969	1.02 (0.34,3.07)	1.1 (0.32,3.76)
rs1860574	17	*HOXB4*	C	0.4	0.2755	0.276	0.63 (0.34,1.17)	0.77 (0.34,1.72)
rs6753677	2	*HOXD4*	A	0.389	0.839	0.934	0.82 (0.44,1.55)	0.83 (0.36,1.89)
rs6433589	2	*HOXD4*	A	0.383	0.9336	0.934	1.04 (0.53,2.03)	0.94 (0.4,2.23)
rs6467995	7	*POR*	A	0.071	0.5635	0.984	1.13 (0.51,2.51)	Undefined
rs10952840	7	*POR*	T	0.388	0.8988	0.984	0.9 (0.53,1.52)	0.83 (0.35,1.99)
rs6953665	7	*POR*	T	0.358	0.984	0.984	0.97 (0.57,1.66)	1.03 (0.42,2.5)
rs3737559	17	*RARA*	T	0.075	0.6132	0.979	1.06 (0.48,2.32)	Undefined
rs4986850	17	*RARA*	T	0.044	0.7842	0.979	1.15 (0.43,3.07)	Undefined

rs3092994	17	*RARA*	T	0.295	0.8906	0.979	1.05 (0.44,2.5)	1.19 (0.43,3.26)
rs3950989	17	*RARA*	A	0.301	0.8946	0.979	1.2 (0.51,2.86)	1.14 (0.41,3.17)
rs799916	17	*RARA*	G	0.35	0.9501	0.979	1.12 (0.53,2.37)	1.09 (0.44,2.72)
rs8176265	17	*RARA*	T	0.28	0.9793	0.979	0.98 (0.41,2.33)	1.03 (0.37,2.84)
rs12303393	12	*RARG*	A	0.206	0.121	0.726	0.51 (0.18,1.44)	0.86 (0.27,2.73)
rs11614470	12	*RARG*	A	0.119	0.4257	0.865	0.53 (0.15,1.91)	0.39 (0.09,1.67)
rs7303807	12	*RARG*	G	0.293	0.5528	0.865	1.2 (0.71,2.03)	0.82 (0.29,2.37)
rs10783696	12	*RARG*	C	0.348	0.5769	0.865	0.72 (0.37,1.37)	0.65 (0.28,1.52)
rs4759123	12	*RARG*	G	0.426	0.8354	0.979	1.2 (0.62,2.29)	1.12 (0.48,2.61)
rs12369902	12	*RARG*	C	0.362	0.9785	0.979	1.01 (0.6,1.72)	0.94 (0.39,2.28)
rs2068204	6	*RXRB*	A	0.059	0.3803	0.761	0.53 (0.19,1.48)	Undefined
rs2281390	6	*RXRB*	T	0.273	0.9494	0.949	1.12 (0.45,2.82)	1.19 (0.41,3.44)
rs4657638	1	*RXRG*	T	0.114	0.0467	0.85	0.65 (0.31,1.36)	3.02 (0.78,11.72)
rs988778	1	*RXRG*	G	0.536	0.0896	0.85	0.68 (0.4,1.17)	0.39 (0.17,0.92)
rs11806848	1	*RXRG*	A	0.231	0.1262	0.85	0.48 (0.2,1.14)	0.71 (0.26,1.93)
rs12143783	1	*RXRG*	T	0.172	0.1316	0.85	0.91 (0.5,1.66)	2.48 (0.83,7.38)
rs12077809	1	*RXRG*	A	0.119	0.1671	0.85	0.85 (0.43,1.69)	2.87 (0.75,11)
rs4657637	1	*RXRG*	G	0.098	0.1962	0.85	0.25 (0.07,0.94)	0.25 (0.06,1.12)
rs10918635	1	*RXRG*	T	0.264	0.2435	0.85	1.41 (0.81,2.43)	2.2 (0.87,5.56)
rs12129059	1	*RXRG*	A	0.108	0.2523	0.85	0.31 (0.08,1.16)	0.38 (0.09,1.67)
rs11587291	1	*RXRG*	C	0.148	0.2899	0.85	0.65 (0.34,1.27)	1.29 (0.31,5.31)
rs11808527	1	*RXRG*	T	0.409	0.3287	0.85	0.68 (0.4,1.15)	0.58 (0.24,1.41)
rs1027495	1	*RXRG*	G	0.31	0.3616	0.85	0.6 (0.3,1.19)	0.62 (0.26,1.46)
rs904222	1	*RXRG*	T	0.097	0.3799	0.85	1.09 (0.54,2.22)	Undefined
rs7540969	1	*RXRG*	C	0.282	0.3949	0.85	1.34 (0.78,2.31)	1.86 (0.75,4.63)
rs16858531	1	*RXRG*	G	0.086	0.3991	0.85	0.91 (0.42,1.97)	3.06 (0.55,17.14)
rs1032608	1	*RXRG*	T	0.156	0.4314	0.85	1.17 (0.64,2.14)	2.29 (0.68,7.73)
rs1532482	1	*RXRG*	T	0.41	0.4513	0.85	0.72 (0.43,1.22)	0.64 (0.27,1.53)
rs6679415	1	*RXRG*	A	0.092	0.466	0.85	1.44 (0.72,2.89)	2.63 (0.47,14.71)

rs10800287	1	*RXRG*	C	0.141	0.5164	0.864	1.1 (0.59,2.06)	2.19 (0.59,8.07)
rs6427069	1	*RXRG*	C	0.31	0.5296	0.864	0.66 (0.33,1.34)	0.66 (0.28,1.59)
rs11590513	1	*RXRG*	T	0.098	0.5794	0.864	1.34 (0.67,2.67)	2.29 (0.41,12.63)
rs748800	1	*RXRG*	G	0.054	0.6191	0.864	1.26 (0.52,3.04)	Undefined
rs11591049	1	*RXRG*	T	0.142	0.6357	0.864	0.85 (0.44,1.62)	1.49 (0.36,6.18)
rs4657643	1	*RXRG*	C	0.06	0.6855	0.864	0.82 (0.33,2.05)	Undefined
rs6691080	1	*RXRG*	G	0.451	0.6911	0.864	0.86 (0.48,1.52)	0.7 (0.31,1.59)
rs12091071	1	*RXRG*	A	0.059	0.697	0.864	0.83 (0.33,2.09)	Undefined
rs7527143	1	*RXRG*	G	0.426	0.8609	0.938	0.91 (0.49,1.69)	0.8 (0.35,1.84)
rs10918653	1	*RXRG*	A	0.362	0.8627	0.938	0.87 (0.51,1.48)	0.93 (0.39,2.24)
rs869714	1	*RXRG*	C	0.428	0.8891	0.938	0.88 (0.48,1.62)	0.82 (0.36,1.86)
rs16858608	1	*RXRG*	A	0.22	0.9053	0.938	1.07 (0.61,1.85)	0.87 (0.25,2.98)
rs12734731	1	*RXRG*	A	0.255	0.9082	0.938	1.22 (0.45,3.29)	1.28 (0.41,3.97)
rs12029632	1	*RXRG*	T	0.308	0.9584	0.958	1.01 (0.59,1.71)	0.89 (0.34,2.37)

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CHAPTER II

Craniofacial Microsomia and variants in genes related to endothelin

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Abstract

Craniofacial microsomia (CFM) involves asymmetric ear and jaw hypoplasia. Faulty cranial neural crest cell (NCC) migration is believed to be the underlying mechanism. Endothelin signaling plays an important role in determining the fate of cranial NCCs. Whether genetic variation in the endothelin pathway is associated with CFM is not known. We evaluated 63 tagSNPs (single nucleotide polymorphisms) from 8 candidate genes in the endothelin pathway in 98 case-parent sets. We used the software Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling (EMIM) to estimate relative risk of CFM associated with offspring and maternal genotypes in trios and dyads. Maternal genotypes associated with CFM risk with $p<0.05$ included three variants in *NOS3* (rs6979482; heterozygous relative risk and 95% CI: 0.38 (0.19,0.76), $P = 0.018$), *ECE1* (rs4614227; 0.013 (0.15,0.97), $P = 0.022$), and *EDNRB* (rs7334914; 0.64 (0.36,1.15), $P = 0.025$). For children's genotypes there was just one such variant, in *EDNRA* (rs10519886; 0.5 (0.29, 0.86), $P = 0.034$). None of these associations met gene-wide multiple comparisons-adjusted false discovery thresholds, yet the study was relatively underpowered. The results cannot rule out a possible association of CFM risk with maternal and child variation in the endothelin pathway.

Introduction

Craniofacial microsomia (CFM) is a congenital malformation condition with an extremely variable clinical presentation. Principally, it involves asymmetrical development of facial bony structures (mandible, maxilla, zygoma, temporal bone), ear (external and middle), and muscles overlying those skeletal structures.[1,2] The cornerstone of this condition is mandibular hypoplasia and/or microtia. CFM that occurs together with extra-craniofacial anomalies, including vertebrae, upper heart, and kidneys, is called oculo-auriculo-vertebral spectrum (OAVS).[3] Some other terms have been used to describe this condition, including Goldenhar syndrome, Goldenhar-Gorlin syndrome, first arch syndrome, first and second branchial arch syndrome, otomandibular dysostosis, hemifacial microsomia.[1] The term CFM is widely used as the clinical characteristics are typically not limited to one side of the face, but tend to affect each side to a different degree. CFM is the second most common craniofacial birth defect after cleft lip and palate with birth prevalence estimated of 1 in 3,000 to 5,600 live births.[5,6]

The etiology of CFM is assumed to be heterogeneous, and hypotheses regarding possible etiologic factors must allow for the phenotypic heterogeneity. Two well-recognized theories focus on vascular disruption and cranial neural crest cell (NCC) disturbance.[7,8] The vascular disruption hypothesis posits that ischemia caused by injury to the stapedial artery—a transient artery that supplies craniofacial structures during embryogenesis—impairs development of a wide range of structures and various degrees of regional deformities.[9-11]

The cranial NCC hypothesis is plausible because normal function of cranial NCCs is required for development of the structures affected by CFM. Cranial NCCs originate from the

neural tube and migrate ventrolaterally to populate the craniofacial regions.[8,12] The intricate hierarchical pathway requires a synchrony of complex signaling network and transcription factors. Disturbances in the specification, migration, proliferation, survival and ultimate fate determination of the cranial NCCs cause craniofacial deformities in animal models.[13-15]

In addition to these general hypotheses, CFM is thought to result from a combination of genetic and environmental factors, similar to other complex diseases.[16,17] Genetic predisposition of CFM was observed in a segregation analysis.[18] Chromosomal anomalies in at least 30 CFM cases have been reported, most commonly in chromosomes 5[19], 14[20,21], 22.[22,23] Animal models also support a genetic basis for CFM. Mice lacking genes *hfm* were born with deformed craniofacial structures.[24,25] Moreover, a genome-wide association study identified 13 loci associated with the risk of CFM.[26] That some of these loci are be enriched for genes involved in NCC development and vasculogenesis, these results are consistent with the notion that genetic variations that affect NCC fate and vasculogenesis may underlie the pathogenesis of CFM. Environmental risk factors have also been described in cases born to mothers exposed to vasoactive medications, second-trimester smoking, diabetes mellitus, and multiple gestation.[8,27]

Animal models have pointed to the key regulatory molecules involved in patterning of cranial NCCs and pharyngeal arch (PA) development, and specifically in CFM pathogenesis. One such factor endothelin-1 (EDN1), is widely expressed and is required in several stages of facial development.[13,28,29] Its signaling cascades are indispensable for human ear development[30], and mice deficient for genes in the endothelin pathway exhibit a hypoplastic mandible, middle ear malformations, and facial nerve defects, all of which are characteristics

of CFM.[13] EDN1 and its receptors are necessary for NCCs to function normally, and it is also a naturally occurring vasoconstrictor responsible for controlling vascular tone and blood pressure.[31,32]

Thus, genes involved in endothelin pathway reconcile all the information from vascular disruption and aberrations in cranial NCC fate hypotheses. We, therefore, hypothesized that genetic variations particularly variants involved in endothelin pathway in mother and/or offspring, are associated with offspring CFM risk. We conducted a candidate gene association study to test this hypothesis.

We selected candidate genes on the following basis. EDN1 is expressed as an inactive precursor protein that is cleaved to its active form by endothelin-converting enzyme (ECE) and is downregulated by nitric oxide, which is produced by nitric oxide synthase (NOS3). EDN1 exerts its biological activities by binding the endothelin receptors, EDNRA and EDNRB, which are G-protein coupled receptors. EDNRA is expressed in NCCs and plays important roles in during embryogenesis.[13,33,34] Downstream signaling molecules such as distal-less homeobox 5,6(Dlx5/Dlx6) and Hand1/2 are also necessary for spatialtemporal patterning of NCCs.[35-37]

Materials and methods

Case-Parent Trios

This study is part of a larger candidate gene association study aimed to investigate association between CFM risk and genetic variations in three group of genes: retinoic-related genes, endothelin-related genes, and syndrome-related genes. CFM cases and their parents

were part of the samples from the big multi-center case-control study of CFM.[38] Study participants were recruited from 26 craniofacial centers throughout the United States and two regions in Canada. All cases were classified according to the expanded CFM spectrum, as well as the diagnosis of vascular disruption defect by craniofacial specialists. We excluded syndromic children or those with chromosomal anomalies and those older than three years of age. Mothers interviews by telephone with three years of delivery. Information obtained from telephone interviews included demographic and reproductive factors, illness, medication use, diet, and other exposures and behaviors.

Biospecimen collection

Of all 280 cases from the original interview-based study, 245 individuals were diagnosed with CFM and 35 with unilateral anotia or microtia with no facial asymmetry. Participants' parents would receive buccal cytobrushes via mail. They were asked to obtain buccal cell specimens of themselves and their child. Detailed instructions include rotating the cytobrush on the inside of the cheek for 30 seconds, before dry and place them back into the cytobrushes container. Parents were then instructed to mail the envelope back to the coordinating center in Boston. We obtained buccal cell samples from approximately 66% of cases and their parents (172 case-parent sets).

Laboratory methods

DNA extraction and whole-genome amplification

We used QIAamp DNA Mini Kit (QIAGEN Inc., Valencia, CA) to extract DNA from 172 cases-parent sets from buccal cytobrushes. After DNA extraction, we used the Quant-iT PicoGreen technique (Molecular Probes, Eugene, OR) to quantitate the extracted DNA. Following PCR, whole-genome amplification of the DNA was accomplished using the GenomiPhi DNA Amplification Kit (Amersham Biosciences; Sunnyvale, CA).

Selection of TagSNPs

We used LD-based approach to select SNPs into bins. This approach helps eliminate the need to genotype all the common variants from the three groups of genes. Instead, it estimates pairwise correlation (r^2) between loci, also a measure of linkage disequilibrium (LD). Among all SNPs, we used LDSelect to select candidate genes with minor allele frequencies > 10%. SNPs were put into bins if pairwise correlations (r^2) between SNPs are ≥ 0.8. Then, we selected at least one SNP from each bin (the tagSNPs).

Marker genotyping methods

We used BioMark™ System 96.96 Dynamic Arrays and ABI TaqMan-based genotyping assays for a high-throughput genotyping of 480 markers in 30 candidate genes. For this study, 63 SNPs in 8 candidate genes are part of endothelin pathway and 15% replicates for laboratory quality control purposes.

Data analysis

For quality control in three pathways, first, we assessed genotype and sample concordance rate in 480 SNPs from 172 case-parents set (160 triads and 12 dyads) and 78 blind replicates. Next, we removed markers (n=101) and samples (n=2) with low

concordance rates following with duplicated samples (n=77). Markers and samples that passed quality control of 98% and 95% concordance rate, respectively, were included in the additional quality control steps. Using PLINK[39], we filtered out SNPs with markers genotypes call rate of≤ 91% (n=2), a minor allele frequency< 0.02 (n=40), Mendelian error per markers of≥ 5 (n=1), Hardy-Weinberg equilibrium with a p-value < $1x10^{-6}$ (n=0). For samples, we filtered out those with individual genotype call rate of≤ 95% (n=41) and Mendelian error per individual of≥ 5 (n=102). We excluded additional samples due to missing both parents or missing offspring (n=69). Finally, a total of 336 SNPs in 88 case-parent sets (83 case-parent triads and 15 case-mother dyads) passed the quality control criteria. In this paper, we focused on the analysis of 63 SNPs in 8 candidate genes from endothelin pathway.

Statistical analyses

To investigate for association of child and maternal genetic variants with the risk of CFM, we implemented a log-linear approach in software Estimation of maternal, Imprinting and interaction effects using Multinomial modelling (EMIM).[40] By conditioning on parental mating type, this approach provides additional information compare to standard transmission disequilibrium test (TDT), as it allows independent estimation of relative risk ratio (RR) and 95% confidence interval (95% CI) for both offspring and maternal genetic associations with the disease risk. Additional assumptions include Hardy-Weinberg equilibrium, random mating, and known allele frequencies can be incorporate in the model.[40,41] We used a step-down procedure at gene-wide level as test of significant to control for false discovery rate (FDR) due to multiple comparisons.[42] Although SNPs in this

study were selected based on tagSNPs method, they still have some degree of correlation. Therefore, we use LDheatmap[43] to generate heatmaps within the gene and calculate correlation coefficients among the studied SNPs as one of our sensitivity analyses. Then we performed an adaptive permutations test using PLINK to generate empirical p-values based on gene-dropping permutation using the max (T) procedure.[39] To maximize power in this relatively small study, no correction for multiple testing across genes was done. To further investigate whether gender and ethnicity modified the disease risk, we performed the analyses separately among genders and between white and non-white probands.

Results

We examined allele distributions of 63 SNPs covering 8 candidate genes in the endothelin pathway from 98 case-parent sets (83 complete triads and 15 case-mother dyads). After quality control, we observed four SNPs that showed association with disease risk at $P<0.05$. Meeting this threshold were three maternal variants, one each in *NOS3* (rs6979482; heterozygous RR and 95% CI: 0.38 (0.19,0.76), $P = 0.018$), *ECE1* (rs4614227; 0.013 (0.15,0.97), $P = 0.022$), and *EDNRB* (rs7334914; 0.64 (0.36,1.15), $P =0.025$), and one offspring variant, in *EDNRA* (rs10519886; 0.5 (0.29, 0.86), $P = 0.034$). The second and third top hits in child genotype effects are *EDNRA* (rs2714885; 0.2 (0.04,0.94), $P =0.06$) and *HAND1* (rs283438; 0.86 (0.44,1.7), $P = 0.07$). (Table IV). None of these associations met multiple comparisons adjusted false discovery thresholds (Table V, VI).

Because the top 4 SNPs are from different genes, none of the variants appeared to be highly correlated with each other. However, there seem to be clusters of the genotyped

SNPs within genes. Gender and ethnicity did not appear to affect the association pattern or modify association results in both child and maternal genotype effect. [Unpublished data]

Discussion

We applied tagSNPs data covering important genes in endothelin pathway and downstream genes to evaluate the role of genetic variations in mother and offspring in the risk of CFM. Overall, we did not observe definitive evidence that genetic variants in maternal or offspring played a major role in susceptibility to CFM. While none of the variants analyzed in this study reached FDR threshold, we believe four genes merit further investigation. Specifically, maternal variants in *NOS3* (rs6979482, *P* = 0.018), *ECE1* (rs4614227, *P* = 0.022), *EDNRB* (rs7334914, *P* = 0.07), and child genotype variants in *EDNRA* (rs10519886, *P* = 0.034) demonstrated suggestive evidence of association with CFM risk, although these SNPs did not meet FDR thresholds for multiple comparison.

The strongest finding was from maternal main effect in *NOS3*, a gene on 7q36.1 that encodes nitric oxide synthase 3, which is an enzyme synthesize nitric oxide (NO). NO is implicated in vascular smooth muscle relaxation and anti-inflammatory.[44] NO impairment contributes to endothelial dysfunction and promotes the development of atherosclerosis, similar to the effects from cigarette smoking.[45,46] In addition, a case-control study of polymorphisms in maternal *NOS3* showed increased risk of isolated cleft lip with or without cleft palate in offspring born to the mothers with *NOS3* polymorphisms.[47]

Our next top hit in maternal main effect is *ECE1* (1p36.12). *ECE1* encodes endothelin converting enzyme 1 (ECE1), which synthesizes endothelin. Mutations in this gene are

associated with Hirschsprung disease, craniofacial anomalies, cardiac defects, and autonomic dysfunction.[48,49] During gestation, ECE1 is found abundantly in the maternal basal plate (placenta) blood vessels and is believed to be regulating uterine contraction and responsible for preterm delivery.[50] Targeted null mutation in the mouse *Ece1* exhibited craniofacial and cardiac abnormalities, similar to the defects in *Ednra*-deficient mice[33], is indicative of aberrant signals during post-migratory NCC.[13] The evidence of *ECE1* role in maternal vasculature and offspring craniofacial development has suggested a plausible association between maternal variant in this gene and the risk of CFM in offspring.

EDNRB is located on 13q22.3. This gene encodes endothelin receptor type B (EDNRB) which is one of the G protein-coupled receptors in endothelin signaling pathway.[51] *EDNRB* is highly expressed in head mesenchyme and is required to support NCCs migration.[52] In addition to its involvement in NCCs and mesenchymal cells development, *EDNRB* is also involved in controlling vascular tone.[53,54] Besides, *EDNRB* is among the 13 loci found to be associated with CFM risk in the first genome wide association study (GWAS) of CFM.[26] Although our results indicated the plausible maternal genotype effects instead of child main effect, these results are consistent that variants in *EDNRB* could play a role in CFM risk.

EDNRA (4q31.22-q31.23) encodes endothelin receptor type A (EDNRA), one of the important endothelin receptors responsible for controlling vascular tone. Signaling from EDNRA receptor is important for initiating multiple signaling pathways for NCCs. Craniofacial defects observed in *Ednra* knockout mice is thought to be a result of NCCs disruption.[13,33] This further supports endothelin pathogenicity in CFM individuals. Again, here we see that

EDNRA, like other genes in endothelin pathway, plays crucial roles in both vasculogenesis and NCCs development.

Among subgroup samples, both child and maternal genotype effect appear to have similar effect. No clear trend was observed among white probands. It is difficult to draw any conclusion from these findings, especially with such a limited number of probands.

Conclusion

To our knowledge, this study is the first candidate gene study designed to evaluate the role maternal genetic on offspring risk for CFM. We identified three maternal variants in *NOS, ECE1, and EDNRB* and one child variant in *EDNRA* that merit further analysis in larger studies, where greater power can be achieved. Investigating maternal and child genetic effects could provide us a better understanding in roles of genetic risk factors in susceptibility of CFM. This study is considered an initial step to fill the gap in information about genetic risk factors in CFM. Since CFM is a multifactorial disease, next steps should involve analyzing of gene and environmental interactions, as well as interactions between maternal genetics and child genetics.

Table I Candidate genes in endothelin pathway, their functions, and number of SNPs tested in this study

Candidate genes in the endothelin pathway	*Function*	*Number of SNPs tested in this study*
EDN1	A potent vasoconstrictor and involves in cranial NCC signaling	1
EDNRA	Transduce EDN1 signaling	11
EDNRB		17
ECE1	Activates the EDN1 pro-peptide	12
HAND1	Downstream signaling of endothelin in pharyngeal arches	5
HAND2		1
DLX6	Downstream signaling of endothelin in pharyngeal arches	3
NOS3	Produces nitric oxide (a potent vasodilator), EDN1 antagonist	13

Table II Variants in genes related phenotypically overlapping syndromes, total number of SNPs genotyped per gene, and number of SNPs with child genotypic associations meeting p<0.1 and p<0.05, by allele frequency.

Gene	*p>=0.1*	*p<0.1& f<0.1*	*p<0.1& f>=0.1*	*p<0.05& f<0.1*	*p<0.05& f>=0.1*	*Total*
DLX6	*3*	*0*	*0*	*0*	*0*	*3*
ECE1	*10*	*0*	*2*	*0*	*0*	*12*
EDN1	*1*	*0*	*0*	*0*	*0*	*1*
EDNRA	*7*	*1*	*3*	*0*	*1*	*11*
EDNRB	*17*	*0*	*0*	*0*	*0*	*17*
HAND1	*4*	*0*	*1*	*0*	*0*	*5*
HAND2	*1*	*0*	*0*	*0*	*0*	*1*

NOS3	*12*	*0*	*1*	*0*	*0*	*13*

Table III Variants in genes related phenotypically overlapping syndromes, total number of SNPs genotyped per gene, and number of SNPs with mother genotypic associations meeting p<0.1 and p<0.05, by allele frequency.

Gene	*p>=0.1*	*p<0.1& f<0.1*	*p<0.1& f>=0.1*	*p<0.05& f<0.1*	*p<0.05& f>=0.1*	*Total*
DLX6	*3*	*0*	*0*	*0*	*0*	*3*
ECE1	*10*	*1*	*1*	*1*	*0*	*12*
EDN1	*1*	*0*	*0*	*0*	*0*	*1*
EDNRA	*11*	*0*	*0*	*0*	*0*	*11*
EDNRB	*15*	*0*	*2*	*0*	*1*	*17*
HAND1	*5*	*0*	*0*	*0*	*0*	*5*
HAND2	*1*	*0*	*0*	*0*	*0*	*1*
NOS3	*10*	*0*	*3*	*0*	*1*	*13*

Table IV Top three SNPs from Child and Maternal genotype effects

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous RR (95% CI)*	*Homozygous RR (95% CI)*
Child genotype effect								
rs10519886	4	*EDNRA*	T	0.341	0.0335	0.219	0.5 (0.29,0.86)	0.65 (0.26,1.61)
rs2714885	4	*EDNRA*	T	0.05	0.0619	0.219	0.2 (0.04,0.94)	Undefined
rs283438	5	*HAND1*	T	0.122	0.0715	0.358	0.86 (0.44,1.7)	3.44 (0.96,12.25)
Maternal genotype effect								
rs6979482	7	*NOS3*	C	0.18	0.0132	0.172	0.38 (0.19,0.76)	0.78 (0.2,3.08)
rs4614227	1	*ECE1*	A	0.091	0.0218	0.262	0.38 (0.15,0.97)	2.69 (0.5,14.39)
rs7334914	13	*EDNRB*	T	0.208	0.0251	0.411	0.64 (0.36,1.15)	Undefined

Table V EMIM analyses for Child genetic effect in 83 triads 15 dyads

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous (95% CI)*	*Homozygous (95% CI)*
rs10252691	7	*DLX6*	C	0.129	0.1295	0.389	0.61 (0.3,1.23)	Undefined
rs10260298	7	*DLX6*	C	0.589	0.4397	0.501	1.5 (0.75,3.03)	1.33 (0.54,3.27)
rs10235199	7	*DLX6*	T	0.598	0.5007	0.501	1.43 (0.71,2.89)	1.25 (0.5,3.09)
rs2796352	1	*ECE1*	T	0.742	0.095	0.56	0.52 (0.26,1.04)	0.36 (0.15,0.9)
rs2745251	1	*ECE1*	C	0.563	0.0962	0.56	0.71 (0.38,1.34)	1.19 (0.53,2.69)
rs785195	1	*ECE1*	T	0.749	0.14	0.56	1.09 (0.36,3.3)	1.92 (0.57,6.45)
rs785198	1	*ECE1*	C	0.182	0.317	0.716	0.63 (0.34,1.16)	0.54 (0.11,2.64)
rs2745252	1	*ECE1*	C	0.094	0.4201	0.716	0.8 (0.38,1.7)	Undefined
rs2796345	1	*ECE1*	A	0.833	0.4229	0.716	1 (0.22,4.51)	1.52 (0.3,7.59)
rs10916972	1	*ECE1*	A	0.821	0.4688	0.716	0.8 (0.23,2.83)	1.15 (0.29,4.61)
rs3026792	1	*ECE1*	G	0.928	0.5357	0.716	Undefined	Undefined
rs1935574	1	*ECE1*	A	0.105	0.5375	0.716	0.66 (0.31,1.4)	0.87 (0.1,7.72)
rs4614227	1	*ECE1*	A	0.083	0.5965	0.716	0.71 (0.31,1.61)	1.42 (0.15,13.33)
rs16825276	1	*ECE1*	T	0.149	0.6746	0.736	0.84 (0.45,1.57)	0.43 (0.05,3.67)
rs1976403	1	*ECE1*	C	0.386	0.8798	0.88	0.87 (0.52,1.48)	0.88 (0.37,2.09)
rs16872377	6	*EDN1*	G	0.039	0.1054	0.105	2.41 (0.95,6.08)	Undefined
rs10519886	4	*EDNRA*	T	0.341	0.0335	0.219	0.5 (0.29,0.86)	0.65 (0.26,1.61)
rs2714885	4	*EDNRA*	T	0.05	0.0619	0.219	0.2 (0.04,0.94)	Undefined
rs1429134	4	*EDNRA*	T	0.296	0.0743	0.219	0.56 (0.32,0.97)	0.84 (0.32,2.18)
rs1346600	4	*EDNRA*	A	0.296	0.0923	0.219	0.57 (0.33,0.99)	0.85 (0.33,2.19)
rs2714886	4	*EDNRA*	A	0.161	0.1238	0.219	0.51 (0.26,1)	0.33 (0.04,2.81)
rs6537466	4	*EDNRA*	G	0.144	0.1393	0.219	0.5 (0.25,1.01)	0.42 (0.05,3.59)
rs1429131	4	*EDNRA*	T	0.144	0.1393	0.219	0.5 (0.25,1.01)	0.42 (0.05,3.59)
rs9308216	4	*EDNRA*	T	0.129	0.2189	0.301	0.53 (0.25,1.1)	0.55 (0.06,4.73)

rs10021391	4	*EDNRA*	G	0.123	0.2533	0.31	0.65 (0.31,1.34)	Undefined
rs1346601	4	*EDNRA*	T	0.128	0.3094	0.34	0.58 (0.28,1.18)	0.56 (0.06,4.85)
rs2714894	4	*EDNRA*	A	0.093	0.5333	0.533	0.65 (0.29,1.48)	1.12 (0.12,10.28)
rs2119541	13	*EDNRB*	C	0.701	0.2808	0.853	1.46 (0.54,3.96)	2.15 (0.69,6.63)
rs1441263	13	*EDNRB*	G	0.26	0.3429	0.853	0.67 (0.38,1.17)	0.81 (0.27,2.4)
rs17786237	13	*EDNRB*	G	0.206	0.3591	0.853	0.65 (0.36,1.18)	0.63 (0.16,2.47)
rs7334914	13	*EDNRB*	T	0.191	0.3594	0.853	0.65 (0.35,1.19)	0.75 (0.19,2.96)
rs17181627	13	*EDNRB*	T	0.2	0.4251	0.853	0.68 (0.37,1.22)	0.68 (0.17,2.65)
rs4884119	13	*EDNRB*	A	0.866	0.4425	0.853	0.5 (0.16,1.57)	0.41 (0.11,1.53)
rs1003093	13	*EDNRB*	A	0.863	0.4471	0.853	0.54 (0.17,1.67)	0.42 (0.11,1.57)
rs9601159	13	*EDNRB*	T	0.927	0.5721	0.853	Undefined	Undefined
rs9544956	13	*EDNRB*	G	0.878	0.6094	0.853	0.51 (0.14,1.84)	0.47 (0.11,2)
rs1441264	13	*EDNRB*	G	0.39	0.6099	0.853	1.2 (0.71,2.05)	0.95 (0.39,2.29)
rs1372177	13	*EDNRB*	G	0.539	0.6115	0.853	0.78 (0.44,1.4)	0.91 (0.41,2.02)
rs4885591	13	*EDNRB*	G	0.542	0.6356	0.853	0.89 (0.49,1.61)	1.12 (0.5,2.51)
rs9544949	13	*EDNRB*	C	0.545	0.652	0.853	0.96 (0.52,1.79)	1.24 (0.54,2.83)
rs627572	13	*EDNRB*	C	0.545	0.745	0.893	0.94 (0.51,1.72)	1.15 (0.51,2.59)
rs9601155	13	*EDNRB*	G	0.051	0.7877	0.893	0.95 (0.36,2.48)	Undefined
rs7988279	13	*EDNRB*	T	0.455	0.9313	0.973	1.09 (0.62,1.89)	1.17 (0.52,2.64)
rs646679	13	*EDNRB*	T	0.545	0.9727	0.973	0.98 (0.54,1.79)	1.04 (0.46,2.38)
rs283438	5	*HAND1*	T	0.122	0.0715	0.358	0.86 (0.44,1.7)	3.44 (0.96,12.25)
rs4958742	5	*HAND1*	A	0.711	0.3753	0.675	0.82 (0.36,1.91)	1.19 (0.44,3.2)
rs2055422	5	*HAND1*	T	0.716	0.4645	0.675	0.82 (0.35,1.89)	1.12 (0.42,3.02)
rs10477102	5	*HAND1*	G	0.532	0.5402	0.675	0.87 (0.47,1.59)	1.15 (0.5,2.68)
rs4958743	5	*HAND1*	C	0.656	0.9621	0.962	0.93 (0.46,1.91)	0.99 (0.4,2.42)
rs6825703	4	*HAND2*	T	0.551	0.8622	0.862	1.15 (0.62,2.13)	1.07 (0.46,2.47)
rs367567	7	*NOS3*	G	0.531	0.073	0.76	0.8 (0.44,1.47)	1.45 (0.66,3.2)
rs386956	7	*NOS3*	T	0.531	0.1255	0.76	0.78 (0.43,1.43)	1.31 (0.6,2.9)
rs10275580	7	*NOS3*	T	0.073	0.1753	0.76	1.98 (0.95,4.13)	2.2 (0.23,21.38)

rs7795959	7	*NOS3*	C	0.562	0.5802	0.981	0.84 (0.46,1.54)	1.06 (0.47,2.39)
rs6979482	7	*NOS3*	C	0.157	0.5956	0.981	0.72 (0.38,1.36)	0.77 (0.15,3.87)
rs6963048	7	*NOS3*	G	0.159	0.6129	0.981	0.87 (0.47,1.6)	0.38 (0.05,3.23)
rs10952308	7	*NOS3*	T	0.494	0.6138	0.981	0.96 (0.54,1.71)	1.27 (0.57,2.83)
rs2286418	7	*NOS3*	C	0.055	0.7515	0.981	0.97 (0.39,2.42)	Undefined
rs2240628	7	*NOS3*	C	0.703	0.8081	0.981	1.02 (0.46,2.24)	0.85 (0.32,2.26)
rs367191	7	*NOS3*	A	0.133	0.8093	0.981	0.85 (0.44,1.63)	0.56 (0.06,4.78)
rs2108726	7	*NOS3*	C	0.682	0.9302	0.981	0.93 (0.45,1.94)	0.85 (0.34,2.13)
rs2159158	7	*NOS3*	A	0.545	0.9349	0.981	0.99 (0.55,1.79)	0.9 (0.39,2.04)
rs13234689	7	*NOS3*	T	0.525	0.9811	0.981	0.96 (0.54,1.71)	0.92 (0.41,2.08)

Table VI EMIM analyses for Maternal genetic effect in 83 triads 15 dyads

Marker name	***Chr***	***Gene***	***Minor allele***	***MAF***	***P-value***	***BH***	***Heterozygous (95% CI)***	***Homozygous (95% CI)***
rs10235199	7	*DLX6*	T	0.639	0.2711	0.505	0.61 (0.33,1.12)	0.54 (0.24,1.24)
rs10260298	7	*DLX6*	C	0.631	0.3364	0.505	0.64 (0.35,1.17)	0.57 (0.25,1.3)
rs10252691	7	*DLX6*	C	0.093	0.6318	0.632	1.09 (0.53,2.25)	2.43 (0.43,13.59)
rs4614227	1	*ECE1*	A	0.091	0.0218	0.262	0.38 (0.15,0.97)	2.69 (0.5,14.39)
rs1976403	1	*ECE1*	C	0.381	0.0695	0.417	0.64 (0.37,1.1)	1.14 (0.51,2.55)
rs3026792	1	*ECE1*	G	0.952	0.1239	0.496	0.13 (0.02,0.69)	0.11 (0.02,0.77)
rs1935574	1	*ECE1*	A	0.097	0.2436	0.731	0.67 (0.3,1.47)	2.37 (0.44,12.78)
rs2745252	1	*ECE1*	C	0.081	0.4686	0.861	1.14 (0.54,2.43)	Undefined
rs785198	1	*ECE1*	C	0.172	0.5098	0.861	0.81 (0.45,1.48)	0.35 (0.04,2.85)
rs2796352	1	*ECE1*	T	0.712	0.5432	0.861	1 (0.46,2.18)	0.74 (0.28,1.93)
rs16825276	1	*ECE1*	T	0.134	0.6292	0.861	0.89 (0.47,1.71)	1.67 (0.4,6.98)
rs2796345	1	*ECE1*	A	0.87	0.7121	0.861	1 (0.22,4.48)	0.77 (0.15,3.99)
rs2745251	1	*ECE1*	C	0.601	0.7174	0.861	0.78 (0.42,1.45)	0.73 (0.32,1.67)
rs10916972	1	*ECE1*	A	0.832	0.9045	0.979	1.2 (0.27,5.35)	1.35 (0.27,6.66)
rs785195	1	*ECE1*	T	0.785	0.9786	0.979	1.04 (0.35,3.13)	1.1 (0.32,3.75)

rs16872377	6	*EDN1*	G	0.059	0.6585	0.659	0.94 (0.38,2.3)	2.89 (0.28,29.29)
rs2714886	4	*EDNRA*	A	0.13	0.2194	0.621	1.06 (0.56,2)	Undefined
rs6537466	4	*EDNRA*	G	0.114	0.2605	0.621	1.11 (0.57,2.15)	Undefined
rs1429131	4	*EDNRA*	T	0.114	0.2605	0.621	1.11 (0.57,2.15)	Undefined
rs1346601	4	*EDNRA*	T	0.108	0.3263	0.621	1.03 (0.52,2.04)	Undefined
rs9308216	4	*EDNRA*	T	0.104	0.3325	0.621	1.09 (0.55,2.18)	Undefined
rs10021391	4	*EDNRA*	G	0.099	0.3387	0.621	1.14 (0.56,2.29)	Undefined
rs10519886	4	*EDNRA*	T	0.286	0.4464	0.625	0.9 (0.53,1.55)	1.46 (0.59,3.63)
rs1429134	4	*EDNRA*	T	0.25	0.5002	0.625	1 (0.58,1.73)	1.65 (0.63,4.33)
rs2714894	4	*EDNRA*	A	0.082	0.5116	0.625	1.06 (0.49,2.28)	Undefined
rs1346600	4	*EDNRA*	A	0.255	0.604	0.664	0.98 (0.57,1.69)	1.5 (0.57,3.94)
rs2714885	4	*EDNRA*	T	0.027	0.8744	0.874	1.21 (0.36,4.09)	Undefined
rs7334914	13	*EDNRB*	T	0.208	0.0251	0.411	0.64 (0.36,1.15)	Undefined
rs7988279	13	*EDNRB*	T	0.404	0.0687	0.411	1.59 (0.88,2.88)	2.62 (1.15,5.93)
rs627572	13	*EDNRB*	C	0.508	0.1124	0.411	1.93 (0.97,3.86)	2.39 (0.99,5.72)
rs646679	13	*EDNRB*	T	0.497	0.1162	0.411	1.83 (0.93,3.59)	2.41 (1.02,5.69)
rs17786237	13	*EDNRB*	G	0.216	0.134	0.411	0.64 (0.36,1.14)	0.21 (0.03,1.72)
rs2119541	13	*EDNRB*	C	0.699	0.1587	0.411	2.32 (0.68,7.87)	3.2 (0.85,11.96)
rs17181627	13	*EDNRB*	T	0.211	0.1693	0.411	0.66 (0.37,1.18)	0.23 (0.03,1.84)
rs9544956	13	*EDNRB*	G	0.887	0.2344	0.498	0.34 (0.11,1.1)	0.29 (0.08,1.14)
rs1003093	13	*EDNRB*	A	0.867	0.2823	0.533	0.44 (0.14,1.36)	0.33 (0.09,1.22)
rs4884119	13	*EDNRB*	A	0.87	0.3183	0.541	0.46 (0.15,1.44)	0.35 (0.09,1.3)
rs9601159	13	*EDNRB*	T	0.929	0.6186	0.956	Undefined	Undefined
rs1441263	13	*EDNRB*	G	0.253	0.7476	0.964	0.82 (0.47,1.41)	0.73 (0.24,2.26)
rs9544949	13	*EDNRB*	C	0.544	0.7517	0.964	1.25 (0.66,2.36)	1.36 (0.59,3.14)
rs9601155	13	*EDNRB*	G	0.049	0.7935	0.964	Undefined	Undefined
rs1441264	13	*EDNRB*	G	0.388	0.8786	0.964	0.96 (0.56,1.64)	1.14 (0.49,2.63)
rs4885591	13	*EDNRB*	G	0.541	0.9072	0.964	1.1 (0.6,2.04)	1.2 (0.53,2.73)
rs1372177	13	*EDNRB*	G	0.533	0.9954	0.995	1.01 (0.55,1.83)	1.03 (0.46,2.33)

rs4958743	5	*HAND1*	C	0.639	0.1353	0.677	0.68 (0.34,1.35)	1.08 (0.46,2.52)
rs283438	5	*HAND1*	T	0.108	0.3348	0.792	1.62 (0.85,3.1)	2.02 (0.37,10.87)
rs2055422	5	*HAND1*	T	0.743	0.6263	0.792	0.67 (0.3,1.49)	0.69 (0.26,1.83)
rs4958742	5	*HAND1*	A	0.742	0.6339	0.792	0.67 (0.3,1.5)	0.7 (0.27,1.86)
rs10477102	5	*HAND1*	G	0.554	0.8685	0.869	0.85 (0.47,1.55)	0.85 (0.37,1.92)
rs6825703	4	*HAND2*	T	0.519	0.3362	0.336	1.56 (0.81,3.02)	1.84 (0.78,4.31)
rs6979482	7	*NOS3*	C	0.18	0.0132	0.172	0.38 (0.19,0.76)	0.78 (0.2,3.08)
rs7795959	7	*NOS3*	C	0.538	0.0528	0.246	0.76 (0.41,1.42)	1.44 (0.65,3.17)
rs367191	7	*NOS3*	A	0.156	0.0567	0.246	0.44 (0.22,0.89)	0.71 (0.14,3.55)
rs2240628	7	*NOS3*	C	0.716	0.2826	0.888	0.55 (0.27,1.13)	0.53 (0.22,1.29)
rs2108726	7	*NOS3*	C	0.641	0.421	0.888	1.13 (0.52,2.49)	1.59 (0.63,4.02)
rs367567	7	*NOS3*	G	0.543	0.4886	0.888	0.93 (0.5,1.72)	1.29 (0.58,2.88)
rs10952308	7	*NOS3*	T	0.5	0.5381	0.888	0.87 (0.49,1.56)	1.17 (0.53,2.59)
rs6963048	7	*NOS3*	G	0.126	0.5736	0.888	1.3 (0.69,2.45)	2.1 (0.49,8.94)
rs386956	7	*NOS3*	T	0.538	0.6921	0.888	1 (0.54,1.84)	1.26 (0.56,2.84)
rs13234689	7	*NOS3*	T	0.541	0.7268	0.888	0.83 (0.47,1.48)	0.72 (0.32,1.62)
rs2286418	7	*NOS3*	C	0.054	0.7513	0.888	1.03 (0.41,2.57)	Undefined
rs10275580	7	*NOS3*	T	0.092	0.8333	0.903	1.24 (0.61,2.52)	1.4 (0.16,12.7)
rs2159158	7	*NOS3*	A	0.538	0.9706	0.971	0.94 (0.52,1.72)	0.98 (0.44,2.21)

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CHAPTER III

Craniofacial microsomia and variants in genes related phenotypically overlapping syndromes

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Abstract

Craniofacial microsomia (CFM) is a complex developmental disorder involving asymmetric formation of the ears and facial structures, particularly derivatives of the first and second pharyngeal arches. Familial cases have been reported. Overlapping phenotypes can be found in several birth defect syndromes. One method to identify the genetic causes of complex disease such as CFM is to investigate candidate genes responsible for craniofacial syndromes characterized by anomalies of the ear and jaw. Here we evaluated 147 tagSNPs (tagged single nucleotide polymorphisms) from 8 candidate genes in 6 developmental syndromes: Brachiootorenal syndrome (*EYA1, SIX1,5, TBX1*), Treacher Collins syndrome (*TCOF1)*, Townes-Brocks syndrome (*SALL1),* Auriculocondylar syndrome (*PLCB4, GNAI3)* and Miller syndrome (*DHODH*). We applied a log-linear approach via the software Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling (EMIM) to estimate the relative risk of CFM associated with offspring and maternal genotypes in 98 case-parent sets. After correction for multiple comparisons, offspring variants in *TBX1* (rs429738) were associated with CFM [heterozygous and homozygous relative risks [95% CI]: 0.4 (0.2, 0.6) and 0.7 (0.3, 1.6) respectively]. None of the maternal variants were associated

with CFM, but the top three SNPs were all in *PLCB4* (rs6118558, rs5011374, rs6118616: *P*= 0.0076, 0.0146, 0.0155 respectively). These results provide evidence supporting a possible role of *TBX1* in CFM development. Identification of genes from syndromes with overlapping phenotypes is the first step toward a better understanding of genetic basis in complex traits. Our results supported the genetic predisposition in susceptibility to CFM and has provided additional information in underlying mechanisms of this disease. As always, positive results must be followed up with confirmatory studies. Exome sequencing or bigger genetic epidemiology are necessary to validate the results.

Introduction

Craniofacial microsomia (CFM; MIM number 164210), a congenital disorder involving asymmetric development of facial structures, is the second most common craniofacial birth defect with birth prevalence estimated of 1 in 3,000 to 5,600 live births.[1,2] CFM is known for its heterogeneous manifestations and wide range of severity, with isolated microtia representing the mildest form.[2-4] The hallmark characteristics of CFM are asymmetric ear anomalies, lower jaw asymmetry and ocular defects, all of which involve derivatives of first and second pharyngeal arches.[5]

Heterogeneity has complicated clarification of the etiology of CFM. Environmental risk factors are related to maternal exposure to vasoactive or teratogenic substances.[6,7] In addition, the CFM phenotype has been noted in infant born to diabetic mothers[8-10], women living in high altitude regions[11], or to pregnant women exposed to thalidomide, and retinoic acid[9,12,13]. Familial occurrence of CFM, implicating genetic transmission, was also observed. A

study of pedigree data found that 45% of the 97 CFM probands have relatives with similar clinical manifestations.[13] Additional families with CFM involving more than one generation have been identified.[14-16] Segregation analysis of 74 families has suggested autosomal dominant inheritance pattern associated with CFM.[17] Additional evidence of genetic predisposition for CFM comes from the identification of chromosomal anomalies in several regions including translocation breakpoint on chromosome 4[18], 5p15.33[19], 12p13.33[20], 14q32.1[21], 14q23.1[22], and 22q[23,24]. This complex trait is most probably caused by the combination of genetic and environmental influences that result in the failure of proper function of cranial neural crest cells (NCCs) during embryogenesis.

Microtia and deformity of the lower jaw, the two common phenotypes in CFM, can occur in an isolated, non-syndromic manner or in association with other first and second pharyngeal arch syndromes[18,25-31]. Although the role of the genes responsible for morphogenesis of ear and jaw is not explicitly understood, it shows that single gene disorders can cause these common phenotypes by interfering in the signaling or genetic pathway necessary for normal development of first and second pharyngeal arches. While Mendelian disease is a result of single gene defect, genetic risk factors of CFM are most likely due to multiple mutations with small effects or interacting single nucleotide polymorphisms (SNPs). We hypothesized that variation in genes responsible for first and second pharyngeal arch syndromes could be potential candidates for CFM risk.

For this study, therefore, we evaluated nine candidate genes from six pharyngeal arch syndromes that have phenotypic characteristics of this spectrum (Table 1)

First and second pharyngeal arch syndromes for candidate gene selection

Branchiootorenal syndrome (MIM number 113650; *EYA1*, *SIX1*, *SIX5* mutations**)** is characterized by structural defects of the inner ear; sensorineural, middle ear; conductive, or mixed hearing loss; branchial fistulas or cysts; ear pits and preauricular ear tags; and renal anomalies. Mutations in either *EYA1, SIX1*, and *SIX5* were reported in cases diagnosed with Branchiootorenal syndrome.[32-39]

DiGeorge syndrome (MIM number 188400; *TBX1* mutation), now known as chromosome 22q11.2 deletion (*del22q11*) syndrome, is characterized by craniofacial, external ear, cardiovascular, thymus, and parathyroid defects.[40-42] In 22q11.2 deletion syndrome, as in CFM, both anomalous migration of NCCs[43] and disruption of the embryonic vasculature[44] have been implicated as possible mechanisms. In humans, the gene located within the microdeletion on chromosome 22 is *TBX1,* a member of T-box transcription factors.[45,46] The *Tbx1* gene is required for proper migration of NCCs into the pharyngeal arches.[47] The *Tbx1-Six1/Eya1-Fgf8* genetic pathway has also been identified in cardiovascular development and craniofacial morphogenesis in mammals, implicating overlapping pathogenesis of BOR and 22q11.2 deletion syndrome.[48]

Treacher Collins syndrome (MIM number 154500; *TCOF1* mutations) is an autosomal dominant craniofacial condition caused by mutation of *TCOF1* on chromosome 5q32-33.1[49-52] *TCOF1* mutations cause haploinsufficiency of the protein Treacle, which in turn leads to a reduction in cranial NCC proliferation and deficient NCC migration to the first and second pharyngeal arches.[53,54] Clinical features include coloboma of the lower eyelid, downslanting of palpebral fissures, malar and maxillary hypoplasia, and ear malformations with conductive

hearing loss due to atresia of the external ear canal.[55] Clinical characteristics are often bilateral and can exhibit a remarkably broad spectrum. No molecular mechanism has yet been identified to account for the extent of clinical variability.[56]

Auriculocondylar syndrome (MIM number 602483, *PLCB4*, *GNAI3* mutations) is a rare, autosomal dominant malformation syndrome.[57] Asymmetry of the affected structures can be observed, with wide range of severity and clinical manifestations that can overlap with CFM.[58,59] Missense mutations in *PLCB4* on chromosome 20 and a gain-of-function of *GNAI3* mutations on chromosome 1 have been identified in individuals with ACS.[29,60] *PLCB4* and *GNAI3* are also directly involved as core signaling molecules of the endothelin pathway, *EDN1-DLX5/DLX6* pathway, which is critical for cranial NCC signaling during craniofacial morphogenesis.[60-62]

Townes-Brocks syndrome (MIM number 107480; *SALL1* mutation) is an autosomal dominant disorder with multiple malformations characterized by external ear anomalies (microtia, preauricular ear tags or pits) with sensorineural hearing loss, hand malformations, anorectal malformations, and familial aggregation.[63,64] Variable expressivity has been observed within affected families.[65] Patients with Townes-Brocke syndrome and *SALL1* mutation tend to exhibit epibulbar dermoid, a hallmark of CFM, implicating a possible common genetic basis for the two conditions.[28,66]

Postaxial acrofacial dysostosis syndrome (MIM number 263750; *DHODH* mutation), also known as Miller syndrome, is a rare mendelian disorder characterized by severe micrognathia, malar hypoplasia, orofacial clefts, cup-shaped ears, and postaxial limb deformities.[67,68] Several characteristics are similar to Treacher Collins syndrome, but

postaxial acrofacial dysostosis is caused by a mutation of *DHODH*.[69,70] The underlying cellular basis is due to deficient formation migration of NCCs.[71,72]

Materials and methods

Case-Parent Trios

This study is part of a larger investigation that also included genetic variants in two groups of genes not described here, retinoic acid-related and endothelin-related genes. Buccal swab samples were collected from CFM probands and their parents whom participated in a previous multi-center case-control study of CFM.[6,73] Study participants were recruited through craniofacial specialists from 26 centers throughout the United States and two regions in Canada. In this current study, cases were classified according to the severity of each CFM-related characteristics, by applying the OMENS classification. [5] Children with diagnoses of syndromes or chromosomal anomalies were excluded. (Table I-IV in the previous chapter) Available a data from the original study e.g. structured interviews and food frequency questionnaires of the mothers were incorporated. Additional data include maternal smoking and alcohol exercise during pregnancy, the mothers' weight, the infant's birthweight, pregnancy complications such as bleeding and preeclampsia, maternal and paternal demographic information, and maternal reproductive history.

Biospecimen collection

Of the original 280 cases, 245 had been diagnosed with CFM and 35 with unilateral anotia or microtia without evidence of facial asymmetry. The participants' parents received a

mailed buccal cytobrush to obtain buccal cell specimens for themselves and their child. The parents were instructed to rotate the cytobrush on the inside of the cheek for 30 seconds, dry the brushes, place them back into the cytobrush containers, and send them back to the coordinating center in Boston through the mail. We obtained buccal cell samples from the total of 172 case-parent set which were approximately 66% of cases and their parents recruited.

Laboratory methods

DNA extraction and whole-genome amplification

We extracted DNA from buccal cytobrushes of 172 cases-parent sets using QIAamp DNA Mini Kit (QIAGEN Inc., Valencia, CA) according to manufacturer's protocol. Following extraction, DNA was quantitated by the Quant-iT PicoGreen technique (Molecular Probes, Eugene, OR) and whole-genome amplification was accomplished using the GenomiPhi DNA Amplification Kit (Amersham Biosciences; Sunnyvale, CA).

Selection of TagSNPs

We implemented a linkage disequilibrium (LD)-based approach (r^2) to identify a subset of SNPs (tagSNPs) for genotyping, excluding SNPs with minor allele frequency <10%. There is a great deal of redundancy across SNPs, often because they are located near each other within a gene.[74] To increase cost efficiency and avoid genotyping SNPs providing redundant information we selected tagSNPs selection by using LDSelect.[75] First we estimated the pairwise correlations among SNPs. We selected at least one SNP from each bin (the tagSNPs) based on $r^2 \geq 0.8$ as a threshold.

Marker genotyping methods

We conducted medium-throughput genotyping of 480 markers in 37 candidate genes, of which 147 SNPs in 8 candidate genes are related to first and second pharyngeal arch syndromes, by using BioMark^{TM} System 96.96 Dynamic Arrays and ABI TaqMan-based genotyping assays. We also included 15% blind replicates for laboratory quality control purposes.

Data analysis

Data quality control

We assessed genotyping and sample concordance rate in 480 SNPs in samples from 172 case-parent sets and 78 blind replicates. First, we removed low-concordance markers (n=101) and samples (n=2) and then excluded the remaining duplicated samples (n=77). Only markers and samples that passed concordance rates of 98% and 95%, respectively, were used in the present analyses. The following quality control criteria implemented in PLINK[76] were applied to filter out more SNPs: SNPs call rate of ≤91% (n=2), sample genotype call rate of ≤95% (n=41), minor allele frequency <0.02 (n=40), Mendelian error per markers of ≥5 (n=1), and Mendelian error per individual of ≥5 (n=102). No samples were out of Hardy-Weinberg equilibrium with a p-value < $1x10^{-6}$. We excluded additional samples due to missing both parents or missing offspring (n=69). After quality control, we have 336 SNPs in 279 samples (83 case-parent triads and 15 case-mother dyads). In this paper, we focus on the analysis of 147 SNPs in 8 candidate genes that are related to first and second pharyngeal arch syndromes.

Statistical analyses

We estimated relative risk (RR) of CFM associated with offspring and maternal genotype effects case-parent sets. We performed a log-linear approach through the software PREMIM and EMIM.[77] The child genotype RR parameters were estimated on the basis of observed counts of genotype combinations in case-parent trios. The default EMIM setting assumes an additive model when estimate R1 and R2 parameters (heterozygous RR, homozygous RR, respectively). The model was also used to model maternal genotype effect and estimate S1 and S2 parameters (heterozygous RR, homozygous RR, respectively). We performed all analyses under Hardy-Weinberg equilibrium assumption.[78]. Maximum likelihood estimated were obtained from each model and a likelihood ratio test for each SNP was performed to assess the significance among nested models.

For gene-wide test of association, we used a stepdown approach to account for multiple comparison within each gene[79]. No correction for multiple testing across genes was performed so as to minimize the chance of missing any true associations. For sensitivity analyses, we performed label-switching permutation procedure in PLINK[76] to generate empirical significance levels. Additionally, we repeated analyses under the conditional on exchangeable parental genotype" (CEPG) while relaxing Hardy-Weinberg assumption. Then, we assessed degree of correlation between genotyped SNP, r^2 value with heat map for each gene were generated using LDheatmap.[80] To study whether the association between SNPs and CFM risk differ depends on cases' characteristics, we performed subgroup analyses stratified by gender and ethnicity (white vs non-white probands).

Results

Of 98 cases, 58% were male probands and 53% were of Caucasian ancestry (Table II, chapter 2). From 279 markers, the top hits for proband main effects were on *TBX1* (rs429738, *P*= 0.0004), *EYA1* (rs1905039, *P*= 0.0098), and *PLCB4* (rs6118616, *P*= 0.0155). After correction for multiple testing, one SNP from child genotype analysis reached the gene-wide FDR threshold. *TBX1* (rs429738) were associated with CFM [heterozygous and homozygous relative risks [95% CI]: 0.4 (0.2, 0.6) and 0.7 (0.3, 1.6) respectively] (Table IV, V).

We separated maternal genetic effects from child genetic effects by conducting a likelihood ratio test comparing between multinomial maximum likelihood models that includes child and maternal genetic effects and child genetic effects only. The top SNPs for maternal main effects were all from *PLCB4* (rs6118558, rs5011374, rs6118616: *P*= 0.0076, 0.0146, 0.0155 respectively). None of the variants from maternal genotype effects met the FDR threshold. (Table VI)

Repeating analyses via permutation testing altered the p-values only negligibly. We also obtained similar RR estimates and p-values after relaxing the Hardy-Weinberg assumption. We also assessed LD coefficients analysis between genotyped SNPs, no strong correlations between the top SNPs from both child and maternal genetic effects. We observed only negligible differences in results between genders and white and non-white probands for both child and maternal effect. [Unpublished data]

Discussion

We investigated eight candidate genes in six syndromes of which malformations of ear and/or jaw are primary manifestations and that have a known genetic cause. Using this method, we identified associations between CFM risk and child genetic variants in *TBX1* previously implicated in del22q11.

CFM and del22q11 were thought to have common pathogenic risk factors. Moreover, it was suggested that that clinical expression of CFM should be included within the wide phenotypic expression of del22q11.[81] This notion supports our results that genetic variation in *TBX1* is implicated in the pathogenesis of CFM, as it is in del22q11.

The fact that the top three variants in maternal genetic effects are in *PLCB4* is promising, despite the negative findings. It is undeniable that none of our results met FDR threshold, however, we cannot rule out the possibility that maternal genetic variants may contribute to CFM in some degree. Alternatively, the inability to detect any association may be a result of low study power.

Missense mutations in *PLCB4* were known to cause Auriculocondylar syndrome.[60] *PLCB4* was found to function downstream of endothelin pathway which are core signaling molecules for pharyngeal arch patterning.[60,61] Although Auriculocondylar syndrome cases present with distinctive ear malformations from CFM, some cases identified with *PLCB4* mutations were found to have mildly dysplastic lower jaws.[62] In contrast, the same study could not identify mutations in *PLCB4* in those presented with question mark ears without small mandibles.[62] Whether CFM is genetically distinct form Auriculocondylar syndrome, or whether it represents variable expression of the same disorder remains to be determined. To date, no studies have identified association between maternal variants in *PLCB4* and

Auriculocondylar syndrome in offspring, but familial transmission with incomplete penetrance in some families suggested a direction to follow up.[62]

In this study, we used different approach to identify candidate genes. Instead of selecting from biological mechanism of the genes, we selected candidate genes based on syndromes with overlapping phenotypes. Similar method of identifying genes responsible for non-syndromic disorder from syndromic individuals have been successfully demonstrated in non-syndromic cleft lip/palate. *PVRL1*[82] and *IRF6*[83] are known to involve in cleft lip/palate-ectodermal dysplasia (MIM number 225060) and van der Woude syndrome (MIM number 119300), respectively. Genetic variations in both genes are also risk factors for non-syndromic cleft lip/palate.[84-86]

Conclusion

In summary, we investigated the association between CFM risk and the role of variants in genes related to phenotypically overlapping syndromes. We found association in variant in *TBX1* in offspring and CFM risk. Although we found no association in maternal main effect, the top three variants in *PLCB4* should not be overlooked. Overall, these results extend our knowledge of the genetic basis of phenotypically overlapping craniofacial syndromes in relation to CFM. Further analyses of the genes associated with phenotypic subgroups may provide additional information regarding underlying genetic pathways involved in CFM susceptibility.

Table I List of syndromes with overlapping phenotypes to CFM and corresponding genetic defect (AD – Autosomal Dominant, AR – Autosomal recessive)

Location	*Phenotype*	*Phenotype MIM number*	*Inheritance*	*Gene/ Locus*	*Gene/ Locus MIM number*	*Number of SNPs in this study*
8q13.3	Branchiootorenal syndrome 1	113650	AD	*EYA1*	601653	34
14q23.1	Branchiootorenal syndrome 3	608389	AD	*SIX1*	601205	5
22q11.21	22q11.2 deletion syndrome	188400	AD	*TBX1*	602054	12
5q32-q33	Treacher Collins syndrome 1	154500	AD	*TCOF1*	606847	10
1p13.3	Auriculocondylar syndrome 1	602483	AD	*GNAI3*	139370	13
20p12.3-p12.2	Auriculocondylar syndrome 2	614669	AD, AR	*PLCB4*	600810	63
16q12.1	Townes-Brocks syndrome 1	107480	AD	*SALL1*	602218	3
16q22.2	Postaxial acrofacial dysostosis (Miller syndrome)	263750	AR	*DHODH*	126064	7

Table II Variants in genes related phenotypically overlapping syndromes, total number of SNPs genotyped per gene, and number of SNPs with child genotypic associations meeting p<0.1 and p<0.05, by allele frequency.

Gene	*p≥0.1*	*p<0.1& f<0.1*	*p<0.1& f≥0.1*	*p<0.05& f<0.1*	*p<0.05& f≥0.1*	*Total*
DHODH	6	0	1	0	1	7
EYA1	30	2	2	0	1	34
GNAI3	12	1	0	1	0	13
PLCB4	60	1	2	0	2	63
SALL1	3	0	0	0	0	3
SIX1	5	0	0	0	0	5
TBX1	9	0	3	0	2	12
TCOF1	7	0	3	0	2	10

Table III Variants in genes related phenotypically overlapping syndromes, total number of SNPs genotyped per gene, and number of SNPs with mother genotypic associations meeting p<0.1 and p<0.05, by allele frequency.

Gene	*p≥0.1*	*p<0.1& f<0.1*	*p<0.1& f≥0.1*	*p<0.05& f<0.1*	*p<0.05& f≥0.1*	*Total*
DHODH	7	0	0	0	0	7
EYA1	32	1	1	1	0	34
GNAI3	13	0	0	0	0	13
PLCB4	52	1	10	0	9	63
SALL1	3	0	0	0	0	3
SIX1	5	0	0	0	0	5
TBX1	12	0	0	0	0	12
TCOF1	9	1	0	1	0	10

Table IV Top three SNPs from Child and Maternal genotype effects

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous RR (95% CI)*	*Homozygous RR (95% CI)*
Child genotype effect								
rs429738	22	*TBX1*	A	0.393	0.0004	0.005	0.36 (0.21, 0.63)	0.69 (0.3, 1.55)
rs1905039	8	*EYA1*	C	0.437	0.0098	0.333	0.49 (0.29, 0.85)	0.85 (0.39, 1.86)
rs6118616	20	*PLCB4*	G	0.294	0.0155	0.944	1.45 (0.87, 2.44)	0.4 (0.11, 1.54)
Maternal genotype effect								
rs6118558	20	*PLCB4*	A	0.659	0.0076	0.295	4.87 (1.14, 20.89)	7.5 (1.61, 34.93)
rs5011374	20	*PLCB4*	C	0.387	0.0146	0.295	1.9 (1.04, 3.47)	3.4 (1.48, 7.83)
rs6118616	20	*PLCB4*	G	0.294	0.0155	0.944	1.45 (0.87, 2.44)	0.4 (0.11, 1.54)

Table V Analyses for Child genetic effect in 83 triads 15 dyads

Marker name	*Chr*	*Gene*	*Minor allele*	*MAF*	*P-value*	*BH*	*Heterozygous RR (95% CI)*	*Homozygous RR (95% CI)*
rs9888988	16	*DHODH*	T	0.307	0.0275	0.193	0.9 (0.52, 1.55)	2.2 (0.95, 5.05)
rs10871310	16	*DHODH*	C	0.413	0.1765	0.595	0.91 (0.52, 1.58)	1.57 (0.71, 3.47)
rs1345872	16	*DHODH*	G	0.139	0.2551	0.595	1.34 (0.72, 2.46)	2.96 (0.85, 10.23)
rs12925165	16	*DHODH*	C	0.369	0.3537	0.619	1.11 (0.65, 1.9)	1.72 (0.76, 3.92)
rs8047267	16	*DHODH*	C	0.322	0.4937	0.691	1.13 (0.66, 1.92)	1.66 (0.7, 3.93)
rs12446956	16	*DHODH*	C	0.139	0.8054	0.868	0.97 (0.52, 1.82)	0.52 (0.06, 4.45)
rs6564255	16	*DHODH*	A	0.189	0.8676	0.868	0.91 (0.51, 1.65)	0.67 (0.14, 3.21)
rs1905039	8	*EYA1*	C	0.437	0.0098	0.333	0.49 (0.29, 0.85)	0.85 (0.39, 1.86)
rs10504502	8	*EYA1*	T	0.064	0.0534	0.661	2.19 (1.01, 4.76)	Undefined
rs980299	8	*EYA1*	C	0.227	0.0907	0.661	0.57 (0.31, 1.02)	1.01 (0.34, 3)
rs10504491	8	*EYA1*	C	0.069	0.0951	0.661	1.44 (0.65, 3.19)	7.06 (1.37, 36.44)
rs12675959	8	*EYA1*	C	0.301	0.1123	0.661	0.96 (0.57, 1.61)	0.31 (0.08, 1.18)
rs17778089	8	*EYA1*	C	0.096	0.1167	0.661	1.87 (0.96, 3.65)	3.91 (0.85, 18.02)
rs1077434	8	*EYA1*	C	0.209	0.2681	0.93	0.66 (0.37, 1.19)	1.04 (0.32, 3.31)
rs1481784	8	*EYA1*	C	0.906	0.2703	0.93	0.3 (0.08, 1.11)	0.28 (0.06, 1.3)
rs2219989	8	*EYA1*	C	0.072	0.3369	0.93	1.39 (0.64, 3)	4.23 (0.7, 25.6)
rs1000377	8	*EYA1*	G	0.072	0.3608	0.93	1.1 (0.49, 2.44)	4.02 (0.66, 24.35)
rs6981565	8	*EYA1*	A	0.52	0.3617	0.93	0.72 (0.41, 1.27)	0.91 (0.41, 2.02)
rs7824431	8	*EYA1*	A	0.081	0.4202	0.93	0.67 (0.29, 1.58)	Undefined
rs16937466	8	*EYA1*	C	0.078	0.4287	0.93	0.67 (0.29, 1.57)	Undefined
rs6999672	8	*EYA1*	A	0.096	0.4332	0.93	0.86 (0.41, 1.8)	Undefined
rs16937407	8	*EYA1*	C	0.084	0.4654	0.93	1.35 (0.65, 2.8)	3.11 (0.54, 18)
rs16937372	8	*EYA1*	A	0.081	0.4817	0.93	0.74 (0.32, 1.72)	Undefined
rs7828540	8	*EYA1*	G	0.799	0.5812	0.93	1.18 (0.4, 3.53)	0.9 (0.26, 3.13)
rs11990153	8	*EYA1*	A	0.488	0.6143	0.93	0.77 (0.44, 1.33)	0.84 (0.38, 1.88)
rs7018254	8	*EYA1*	A	0.448	0.6309	0.93	0.89 (0.52, 1.51)	0.67 (0.29, 1.57)

rs7001898	8	*EYA1*	A	0.175	0.6611	0.93	1.07 (0.59, 1.92)	1.73 (0.52, 5.73)
rs10504497	8	*EYA1*	G	0.607	0.6653	0.93	1.34 (0.65, 2.78)	1.5 (0.61, 3.73)
rs1481806	8	*EYA1*	G	0.594	0.7233	0.93	0.79 (0.43, 1.46)	0.86 (0.38, 1.95)
rs988143	8	*EYA1*	C	0.587	0.7422	0.93	0.82 (0.44, 1.51)	0.91 (0.4, 2.07)
rs10103397	8	*EYA1*	A	0.6	0.7671	0.93	1.05 (0.53, 2.05)	1.27 (0.54, 2.99)
rs4738110	8	*EYA1*	A	0.36	0.7794	0.93	0.83 (0.49, 1.4)	0.79 (0.32, 1.95)
rs737534	8	*EYA1*	G	0.174	0.7833	0.93	0.83 (0.46, 1.52)	0.63 (0.13, 3.15)
rs7830811	8	*EYA1*	C	0.409	0.7925	0.93	0.84 (0.49, 1.42)	0.88 (0.38, 2.03)
rs12675825	8	*EYA1*	A	0.212	0.8027	0.93	0.83 (0.46, 1.48)	0.9 (0.26, 3.18)
rs11785920	8	*EYA1*	T	0.136	0.8164	0.93	0.88 (0.46, 1.68)	0.54 (0.06, 4.6)
rs10095316	8	*EYA1*	A	0.208	0.8202	0.93	0.92 (0.52, 1.64)	1.24 (0.38, 4.05)
rs17708659	8	*EYA1*	G	0.03	0.8821	0.945	1.15 (0.34, 3.88)	Undefined
rs11779364	8	*EYA1*	C	0.508	0.9003	0.945	1.05 (0.59, 1.89)	1.19 (0.53, 2.68)
rs4072400	8	*EYA1*	A	0.831	0.9176	0.945	1.02 (0.29, 3.56)	0.9 (0.22, 3.65)
rs4579563	8	*EYA1*	G	0.57	0.9707	0.971	0.93 (0.51, 1.72)	0.96 (0.42, 2.18)
rs485074	1	*GNAI3*	G	0.077	0.0253	0.329	0.4 (0.15, 1.07)	3.16 (0.54, 18.67)
rs6674178	1	*GNAI3*	A	0.185	0.1719	0.839	0.61 (0.33, 1.12)	0.25 (0.03, 2.1)
rs7524737	1	*GNAI3*	T	0.254	0.1936	0.839	0.8 (0.47, 1.38)	0.28 (0.06, 1.32)
rs2274536	1	*GNAI3*	G	0.266	0.4514	0.944	1.02 (0.6, 1.76)	1.68 (0.66, 4.28)
rs11102002	1	*GNAI3*	T	0.074	0.5288	0.944	1.15 (0.52, 2.52)	Undefined
rs12126552	1	*GNAI3*	G	0.928	0.5937	0.944	Undefined	Undefined
rs1887547	1	*GNAI3*	C	0.635	0.7821	0.944	1.01 (0.49, 2.05)	1.21 (0.5, 2.93)
rs12061779	1	*GNAI3*	A	0.203	0.8446	0.944	1.04 (0.6, 1.82)	0.74 (0.19, 2.92)
rs6684608	1	*GNAI3*	C	0.635	0.8682	0.944	1.03 (0.51, 2.09)	1.19 (0.49, 2.87)
rs6537876	1	*GNAI3*	T	0.184	0.8788	0.944	1.12 (0.64, 1.98)	0.93 (0.23, 3.72)
rs6681343	1	*GNAI3*	C	0.157	0.9203	0.944	0.96 (0.52, 1.76)	1.23 (0.3, 5.03)
rs1109112	1	*GNAI3*	G	0.192	0.944	0.944	1.02 (0.58, 1.8)	0.83 (0.21, 3.29)
rs3814310	1	*GNAI3*	T	0.192	0.944	0.944	1.02 (0.58, 1.8)	0.83 (0.21, 3.29)
rs6118616	20	*PLCB4*	G	0.294	0.0155	0.944	1.45 (0.87, 2.44)	0.4 (0.11, 1.54)

rs6118498	20	*PLCB4*	A	0.551	0.0355	0.944	0.48 (0.28, 0.84)	0.56 (0.26, 1.23)
rs6086834	20	*PLCB4*	C	0.055	0.0927	0.944	0.27 (0.07, 1.01)	Undefined
rs4816145	20	*PLCB4*	T	0.124	0.1313	0.944	1.28 (0.68, 2.41)	Undefined
rs2299680	20	*PLCB4*	C	0.067	0.1477	0.944	1.8 (0.84, 3.87)	Undefined
rs16995654	20	*PLCB4*	T	0.066	0.1675	0.944	0.46 (0.17, 1.28)	2.18 (0.22, 21.58)
rs6056654	20	*PLCB4*	G	0.142	0.1866	0.944	1.55 (0.85, 2.84)	0.6 (0.07, 5.11)
rs16996017	20	*PLCB4*	T	0.136	0.1891	0.944	1.57 (0.86, 2.87)	0.63 (0.07, 5.39)
rs16995763	20	*PLCB4*	A	0.182	0.19	0.944	0.66 (0.35, 1.25)	1.4 (0.43, 4.61)
rs2232257	20	*PLCB4*	T	0.122	0.2289	0.944	0.91 (0.46, 1.78)	2.75 (0.72, 10.55)
rs13039478	20	*PLCB4*	G	0.112	0.3226	0.944	0.96 (0.48, 1.89)	Undefined
rs6056647	20	*PLCB4*	A	0.077	0.3537	0.944	1.34 (0.64, 2.83)	Undefined
rs6077545	20	*PLCB4*	C	0.084	0.3831	0.944	1.23 (0.59, 2.57)	Undefined
rs6118618	20	*PLCB4*	C	0.071	0.3861	0.944	1.36 (0.63, 2.94)	Undefined
rs6077510	20	*PLCB4*	G	0.337	0.3943	0.944	1.43 (0.84, 2.44)	1.33 (0.54, 3.27)
rs6086904	20	*PLCB4*	A	0.044	0.4045	0.944	1.65 (0.66, 4.14)	Undefined
rs7268671	20	*PLCB4*	G	0.081	0.4108	0.944	0.61 (0.25, 1.46)	1.46 (0.15, 13.82)
rs17482999	20	*PLCB4*	A	0.067	0.4146	0.944	1.37 (0.62, 3.04)	Undefined
rs2327164	20	*PLCB4*	C	0.175	0.4152	0.944	0.85 (0.46, 1.57)	1.69 (0.51, 5.64)
rs6077536	20	*PLCB4*	T	0.067	0.421	0.944	1.37 (0.62, 3.02)	Undefined
rs2206138	20	*PLCB4*	A	0.546	0.4489	0.944	0.87 (0.47, 1.61)	1.21 (0.54, 2.73)
rs6056448	20	*PLCB4*	T	0.804	0.4531	0.944	0.75 (0.29, 1.92)	0.54 (0.17, 1.67)
rs7265290	20	*PLCB4*	G	0.05	0.4693	0.944	0.85 (0.32, 2.29)	4.06 (0.37, 44)
rs2072954	20	*PLCB4*	G	0.297	0.4728	0.944	0.79 (0.46, 1.35)	1.12 (0.44, 2.84)
rs7261773	20	*PLCB4*	A	0.067	0.4849	0.944	1.58 (0.72, 3.43)	2.42 (0.24, 23.99)
rs6086797	20	*PLCB4*	A	0.849	0.4919	0.944	0.95 (0.27, 3.31)	0.66 (0.16, 2.74)
rs13041524	20	*PLCB4*	A	0.096	0.4976	0.944	1.11 (0.54, 2.28)	Undefined
rs6140909	20	*PLCB4*	A	0.066	0.5126	0.944	0.72 (0.29, 1.77)	2.25 (0.23, 22.31)
rs6077537	20	*PLCB4*	A	0.213	0.5232	0.944	0.9 (0.51, 1.57)	0.43 (0.09, 2.09)
rs16995573	20	*PLCB4*	G	0.061	0.535	0.944	1.29 (0.56, 2.96)	Undefined

rs2064183	20	*PLCB4*	G	0.033	0.5359	0.944	1.66 (0.58, 4.71)	Undefined
rs6039432	20	*PLCB4*	C	0.555	0.5418	0.944	1.04 (0.55, 1.96)	1.4 (0.61, 3.21)
rs6039393	20	*PLCB4*	C	0.388	0.5467	0.944	0.75 (0.45, 1.27)	0.68 (0.28, 1.64)
rs6086799	20	*PLCB4*	A	0.815	0.5719	0.944	0.8 (0.29, 2.19)	0.6 (0.18, 1.97)
rs17389980	20	*PLCB4*	A	0.05	0.5793	0.944	0.62 (0.22, 1.81)	Undefined
rs2876163	20	*PLCB4*	A	0.052	0.5794	0.944	1.34 (0.54, 3.31)	Undefined
rs6108263	20	*PLCB4*	G	0.24	0.5997	0.944	0.75 (0.43, 1.31)	0.79 (0.25, 2.48)
rs6039443	20	*PLCB4*	T	0.184	0.6024	0.944	0.76 (0.41, 1.38)	0.55 (0.11, 2.72)
rs13044386	20	*PLCB4*	G	0.847	0.6034	0.944	2.45 (0.32, 18.78)	2.3 (0.27, 19.5)
rs7263079	20	*PLCB4*	A	0.045	0.6345	0.944	1.35 (0.52, 3.48)	Undefined
rs12479503	20	*PLCB4*	T	0.236	0.6481	0.944	0.77 (0.44, 1.34)	0.83 (0.27, 2.61)
rs6056500	20	*PLCB4*	C	0.149	0.6629	0.944	0.84 (0.44, 1.59)	1.37 (0.33, 5.68)
rs2224357	20	*PLCB4*	C	0.15	0.6636	0.944	0.83 (0.44, 1.56)	0.43 (0.05, 3.62)
rs6086835	20	*PLCB4*	C	0.178	0.6769	0.944	0.84 (0.46, 1.52)	1.23 (0.35, 4.4)
rs6086808	20	*PLCB4*	G	0.506	0.6829	0.944	0.79 (0.46, 1.37)	0.72 (0.32, 1.63)
rs6056551	20	*PLCB4*	G	0.778	0.6893	0.944	1.66 (0.48, 5.69)	1.62 (0.42, 6.32)
rs6056656	20	*PLCB4*	A	0.352	0.723	0.967	1.22 (0.72, 2.06)	1.1 (0.45, 2.69)
rs6039487	20	*PLCB4*	G	0.343	0.7572	0.967	1.05 (0.62, 1.78)	0.8 (0.31, 2.09)
rs6086908	20	*PLCB4*	A	0.039	0.7576	0.967	1.26 (0.45, 3.49)	Undefined
rs3746548	20	*PLCB4*	G	0.115	0.7698	0.967	0.77 (0.38, 1.57)	0.74 (0.08, 6.56)
rs4141984	20	*PLCB4*	C	0.034	0.7827	0.967	1.29 (0.43, 3.83)	Undefined
rs10485731	20	*PLCB4*	C	0.048	0.8095	0.972	0.95 (0.34, 2.63)	Undefined
rs6118558	20	*PLCB4*	A	0.707	0.8237	0.972	1.12 (0.47, 2.66)	1.3 (0.47, 3.63)
rs7269241	20	*PLCB4*	C	0.792	0.8625	0.972	1.36 (0.39, 4.68)	1.45 (0.37, 5.67)
rs5011374	20	*PLCB4*	C	0.474	0.8821	0.972	0.87 (0.5, 1.5)	0.86 (0.38, 1.95)
rs1883488	20	*PLCB4*	T	0.097	0.9092	0.972	1.17 (0.58, 2.38)	1.14 (0.12, 10.39)
rs6133686	20	*PLCB4*	C	0.145	0.9136	0.972	0.87 (0.46, 1.65)	0.94 (0.18, 4.79)
rs6133690	20	*PLCB4*	T	0.145	0.9136	0.972	0.87 (0.46, 1.65)	0.94 (0.18, 4.79)
rs6140908	20	*PLCB4*	G	0.145	0.9136	0.972	0.87 (0.46, 1.65)	0.94 (0.18, 4.79)

rs6056552	20	*PLCB4*	T	0.835	0.9363	0.972	0.95 (0.27, 3.34)	0.86 (0.21, 3.49)
rs6039442	20	*PLCB4*	T	0.794	0.9407	0.972	0.97 (0.32, 2.93)	1.07 (0.31, 3.74)
rs1028337	20	*PLCB4*	G	0.089	0.9759	0.985	1.01 (0.48, 2.11)	1.28 (0.14, 11.84)
rs4816144	20	*PLCB4*	T	0.133	0.9853	0.985	1.02 (0.54, 1.93)	1.16 (0.22, 6.01)
rs3112625	16	*SALL1*	A	0.517	0.3349	0.54	0.85 (0.47, 1.54)	1.25 (0.56, 2.77)
rs3112633	16	*SALL1*	T	0.225	0.483	0.54	0.77 (0.43, 1.35)	1.13 (0.38, 3.38)
rs4784230	16	*SALL1*	C	0.191	0.5401	0.54	0.82 (0.46, 1.48)	1.34 (0.41, 4.34)
rs813337	14	*SIX1*	G	0.022	0.1784	0.595	1.46 (0.4, 5.3)	Undefined
rs17098866	14	*SIX1*	A	0.083	0.311	0.595	0.79 (0.35, 1.75)	2.88 (0.5, 16.68)
rs710065	14	*SIX1*	T	0.072	0.3568	0.595	1.1 (0.5, 2.45)	4.07 (0.67, 24.65)
rs710066	14	*SIX1*	G	0.056	0.6579	0.666	1.08 (0.44, 2.64)	3.31 (0.32, 34.64)
rs2255146	14	*SIX1*	A	0.159	0.666	0.666	1.21 (0.67, 2.21)	1.79 (0.49, 6.55)
rs429738	22	*TBX1*	A	0.393	0.0004	0.005	0.36 (0.21, 0.63)	0.69 (0.3, 1.55)
rs383331	22	*TBX1*	G	0.621	0.0207	0.124	0.45 (0.24, 0.85)	0.67 (0.3, 1.5)
rs408718	22	*TBX1*	T	0.605	0.0866	0.346	0.72 (0.37, 1.39)	1.23 (0.54, 2.82)
rs421390	22	*TBX1*	A	0.542	0.1329	0.379	0.59 (0.34, 1.05)	0.78 (0.35, 1.71)
rs428702	22	*TBX1*	G	0.601	0.1662	0.379	0.59 (0.32, 1.07)	0.74 (0.33, 1.65)
rs9620784	22	*TBX1*	A	0.154	0.1895	0.379	1.09 (0.57, 2.06)	2.89 (0.87, 9.55)
rs6519749	22	*TBX1*	A	0.049	0.2575	0.441	0.66 (0.21, 2.02)	5.49 (0.52, 57.55)
rs2075271	22	*TBX1*	T	0.077	0.3355	0.503	0.56 (0.22, 1.41)	Undefined
rs439792	22	*TBX1*	C	0.279	0.3947	0.515	0.71 (0.41, 1.22)	0.92 (0.34, 2.46)
rs400946	22	*TBX1*	T	0.274	0.4293	0.515	0.73 (0.43, 1.26)	0.97 (0.36, 2.61)
rs9613607	22	*TBX1*	A	0.778	0.7167	0.782	0.73 (0.3, 1.78)	0.64 (0.22, 1.84)
rs2106262	22	*TBX1*	T	0.872	0.8503	0.85	1.68 (0.22, 13.08)	1.59 (0.18, 13.75)
rs2748220	5	*TCOF1*	T	0.692	0.0385	0.193	0.75 (0.39, 1.44)	0.38 (0.16, 0.93)
rs1864959	5	*TCOF1*	G	0.695	0.0386	0.193	0.76 (0.39, 1.47)	0.38 (0.16, 0.94)
rs1560661	5	*TCOF1*	C	0.497	0.0774	0.258	1.66 (0.9, 3.04)	1.08 (0.45, 2.61)
rs1864958	5	*TCOF1*	T	0.545	0.1459	0.365	0.68 (0.4, 1.17)	0.44 (0.19, 1)
rs2295223	5	*TCOF1*	T	0.07	0.346	0.692	0.71 (0.29, 1.74)	3.21 (0.36, 28.36)

rs2071368	5	*TCOF1*	G	0.077	0.5298	0.734	0.69 (0.29, 1.61)	1.64 (0.17, 15.56)
rs6891122	5	*TCOF1*	A	0.044	0.5704	0.734	0.58 (0.18, 1.83)	Undefined
rs7702237	5	*TCOF1*	T	0.067	0.6605	0.734	0.98 (0.42, 2.27)	Undefined
rs7722480	5	*TCOF1*	A	0.067	0.6605	0.734	0.98 (0.42, 2.27)	Undefined
rs1056400	5	*TCOF1*	C	0.105	0.9459	0.946	1.11 (0.56, 2.19)	0.94 (0.11, 8.43)

Table VI Analyses for Maternal genetic effect in 83 triads 15 dyads

Marker name	***Chr***	***Gene***	***Minor allele***	***MAF***	***P-value***	***BH***	***Heterozygous RR (95% CI)***	***Homozygous RR (95% CI)***
rs1345872	16	*DHODH*	G	0.151	0.514	0.924	1.38 (0.77, 2.5)	1.04 (0.21, 5.22)
rs6564255	16	*DHODH*	A	0.189	0.5417	0.924	0.76 (0.42, 1.39)	1.14 (0.33, 3.96)
rs10871310	16	*DHODH*	C	0.444	0.6125	0.924	0.81 (0.47, 1.4)	1 (0.44, 2.23)
rs12446956	16	*DHODH*	C	0.125	0.676	0.924	1.25 (0.66, 2.36)	0.74 (0.09, 6.37)
rs9888988	16	*DHODH*	T	0.335	0.8015	0.924	0.96 (0.56, 1.62)	1.21 (0.5, 2.92)
rs8047267	16	*DHODH*	C	0.351	0.8961	0.924	0.91 (0.53, 1.54)	1.01 (0.42, 2.43)
rs12925165	16	*DHODH*	C	0.397	0.9235	0.924	0.96 (0.56, 1.64)	1.09 (0.47, 2.5)
rs17778089	8	*EYA1*	C	0.087	0.0268	0.844	2.1 (1.06, 4.17)	6.57 (1.56, 27.7)
rs980299	8	*EYA1*	C	0.235	0.091	0.844	0.53 (0.3, 0.96)	0.76 (0.25, 2.38)
rs7828540	8	*EYA1*	G	0.753	0.1083	0.844	0.68 (0.26, 1.76)	1.21 (0.42, 3.52)
rs6999672	8	*EYA1*	A	0.109	0.1803	0.844	0.59 (0.27, 1.26)	Undefined
rs7018254	8	*EYA1*	A	0.398	0.2152	0.844	1.62 (0.92, 2.86)	1.44 (0.61, 3.43)
rs1905039	8	*EYA1*	C	0.459	0.2309	0.844	0.68 (0.4, 1.15)	0.5 (0.21, 1.16)
rs737534	8	*EYA1*	G	0.191	0.2706	0.844	0.62 (0.33, 1.13)	0.48 (0.1, 2.35)
rs7001898	8	*EYA1*	A	0.203	0.2841	0.844	0.67 (0.37, 1.22)	1.11 (0.35, 3.54)
rs11785920	8	*EYA1*	T	0.154	0.2847	0.844	0.61 (0.31, 1.17)	0.38 (0.05, 3.2)
rs12675959	8	*EYA1*	C	0.253	0.3147	0.844	1.47 (0.86, 2.51)	1.15 (0.39, 3.39)
rs1481806	8	*EYA1*	G	0.577	0.3366	0.844	0.76 (0.4, 1.43)	1.07 (0.47, 2.4)
rs10504502	8	*EYA1*	T	0.105	0.35	0.844	0.8 (0.38, 1.65)	Undefined
rs2219989	8	*EYA1*	C	0.086	0.3663	0.844	1.24 (0.6, 2.56)	Undefined

rs10504491	8	*EYA1*	C	0.091	0.3791	0.844	1.16 (0.57, 2.37)	Undefined
rs988143	8	*EYA1*	C	0.589	0.3844	0.844	0.69 (0.37, 1.27)	0.85 (0.38, 1.9)
rs7830811	8	*EYA1*	C	0.365	0.397	0.844	1.43 (0.83, 2.49)	1.66 (0.7, 3.92)
rs4579563	8	*EYA1*	G	0.573	0.46	0.885	0.72 (0.4, 1.32)	0.87 (0.39, 1.94)
rs11990153	8	*EYA1*	A	0.448	0.4687	0.885	1.42 (0.79, 2.56)	1.56 (0.68, 3.6)
rs1481784	8	*EYA1*	C	0.876	0.5197	0.892	1.1 (0.14, 8.69)	1.64 (0.19, 14.21)
rs16937372	8	*EYA1*	A	0.076	0.585	0.892	0.72 (0.31, 1.69)	1.7 (0.18, 16.17)
rs11779364	8	*EYA1*	C	0.503	0.591	0.892	1.37 (0.74, 2.52)	1.35 (0.59, 3.11)
rs10095316	8	*EYA1*	A	0.204	0.603	0.892	0.91 (0.51, 1.63)	1.48 (0.49, 4.49)
rs7824431	8	*EYA1*	A	0.07	0.6374	0.892	0.78 (0.33, 1.86)	2.02 (0.21, 19.56)
rs16937466	8	*EYA1*	C	0.07	0.6664	0.892	0.87 (0.37, 2.02)	Undefined
rs10504497	8	*EYA1*	G	0.65	0.7055	0.892	0.83 (0.42, 1.62)	0.7 (0.29, 1.65)
rs17708659	8	*EYA1*	G	0.038	0.763	0.892	0.71 (0.22, 2.29)	Undefined
rs1000377	8	*EYA1*	G	0.086	0.7891	0.892	0.84 (0.38, 1.83)	1.57 (0.17, 14.21)
rs4072400	8	*EYA1*	A	0.816	0.8032	0.892	1.6 (0.36, 7.03)	1.64 (0.33, 8.05)
rs6981565	8	*EYA1*	A	0.506	0.833	0.892	0.97 (0.54, 1.74)	1.15 (0.51, 2.57)
rs16937407	8	*EYA1*	C	0.092	0.8333	0.892	1.24 (0.61, 2.52)	1.4 (0.16, 12.7)
rs12675825	8	*EYA1*	A	0.196	0.8389	0.892	0.99 (0.56, 1.76)	1.36 (0.42, 4.37)
rs4738110	8	*EYA1*	A	0.33	0.8399	0.892	1.08 (0.63, 1.85)	1.31 (0.54, 3.18)
rs10103397	8	*EYA1*	A	0.609	0.884	0.911	0.97 (0.5, 1.91)	1.11 (0.47, 2.61)
rs1077434	8	*EYA1*	C	0.196	0.9482	0.948	0.96 (0.54, 1.7)	0.8 (0.2, 3.14)
rs6674178	1	*GNAI3*	A	0.12	0.2067	0.82	1.45 (0.77, 2.75)	3.46 (0.91, 13.23)
rs11102002	1	*GNAI3*	T	0.063	0.2327	0.82	1.15 (0.49, 2.74)	5.78 (0.9, 37.23)
rs1887547	1	*GNAI3*	C	0.668	0.4699	0.82	0.65 (0.33, 1.27)	0.65 (0.28, 1.53)
rs6684608	1	*GNAI3*	C	0.668	0.5023	0.82	0.67 (0.34, 1.3)	0.64 (0.27, 1.5)
rs6537876	1	*GNAI3*	T	0.212	0.5222	0.82	0.72 (0.4, 1.28)	0.65 (0.17, 2.49)
rs6681343	1	*GNAI3*	C	0.174	0.5279	0.82	0.85 (0.47, 1.55)	0.34 (0.04, 2.85)
rs12061779	1	*GNAI3*	A	0.224	0.5533	0.82	0.75 (0.43, 1.32)	0.58 (0.15, 2.24)
rs485074	1	*GNAI3*	G	0.059	0.576	0.82	1.26 (0.54, 2.92)	Undefined

rs12126552	1	*GNAI3*	G	0.93	0.6281	0.82	Undefined	Undefined
rs3814310	1	*GNAI3*	T	0.208	0.6942	0.82	0.78 (0.44, 1.39)	0.7 (0.18, 2.71)
rs1109112	1	*GNAI3*	G	0.208	0.6942	0.82	0.78 (0.44, 1.39)	0.7 (0.18, 2.71)
rs2274536	1	*GNAI3*	G	0.297	0.8626	0.934	0.87 (0.51, 1.47)	0.81 (0.3, 2.22)
rs7524737	1	*GNAI3*	T	0.23	0.9343	0.934	0.95 (0.55, 1.64)	0.8 (0.24, 2.71)
rs6118558	20	*PLCB4*	A	0.659	0.0076	0.295	4.87 (1.14, 20.89)	7.5 (1.61, 34.93)
rs5011374	20	*PLCB4*	C	0.387	0.0146	0.295	1.9 (1.04, 3.47)	3.4 (1.48, 7.83)
rs6086808	20	*PLCB4*	G	0.413	0.0232	0.295	1.59 (0.87, 2.92)	3.04 (1.35, 6.88)
rs2206138	20	*PLCB4*	A	0.495	0.027	0.295	1.45 (0.74, 2.84)	2.8 (1.21, 6.49)
rs6133686	20	*PLCB4*	C	0.141	0.0347	0.295	0.64 (0.32, 1.28)	2.67 (0.79, 9.02)
rs6133690	20	*PLCB4*	T	0.141	0.0347	0.295	0.64 (0.32, 1.28)	2.67 (0.79, 9.02)
rs6140908	20	*PLCB4*	G	0.141	0.0347	0.295	0.64 (0.32, 1.28)	2.67 (0.79, 9.02)
rs6039432	20	*PLCB4*	C	0.514	0.0374	0.295	1.68 (0.83, 3.4)	2.94 (1.23, 7)
rs6086797	20	*PLCB4*	A	0.815	0.0466	0.326	Undefined	Undefined
rs6056500	20	*PLCB4*	C	0.138	0.0771	0.486	0.78 (0.4, 1.53)	2.92 (0.85, 10)
rs3746548	20	*PLCB4*	G	0.083	0.086	0.493	1.81 (0.89, 3.68)	0 (0, 0)
rs2224357	20	*PLCB4*	C	0.141	0.1286	0.675	0.68 (0.35, 1.34)	2.12 (0.58, 7.72)
rs6086835	20	*PLCB4*	C	0.209	0.1721	0.834	0.65 (0.36, 1.16)	0.24 (0.03, 1.93)
rs2299680	20	*PLCB4*	C	0.109	0.1884	0.835	0.59 (0.28, 1.27)	Undefined
rs2072954	20	*PLCB4*	G	0.276	0.2025	0.835	0.87 (0.5, 1.52)	1.72 (0.69, 4.27)
rs6056448	20	*PLCB4*	T	0.744	0.2183	0.835	2.73 (0.63, 11.81)	3.42 (0.72, 16.23)
rs7269241	20	*PLCB4*	C	0.766	0.2402	0.835	1.3 (0.38, 4.53)	2.09 (0.55, 8)
rs13044386	20	*PLCB4*	G	0.819	0.2536	0.835	2.16 (0.28, 16.6)	3.35 (0.41, 27.48)
rs6108263	20	*PLCB4*	G	0.201	0.2943	0.835	1.05 (0.59, 1.85)	2.14 (0.76, 6.01)
rs10485731	20	*PLCB4*	C	0.043	0.3144	0.835	0.85 (0.29, 2.47)	7 (0.65, 75.37)
rs6039442	20	*PLCB4*	T	0.813	0.3177	0.835	1.99 (0.46, 8.66)	1.44 (0.29, 7.09)
rs1028337	20	*PLCB4*	G	0.092	0.3369	0.835	0.79 (0.36, 1.71)	2.73 (0.5, 15)
rs6086908	20	*PLCB4*	A	0.038	0.3666	0.835	1.02 (0.35, 2.99)	7.2 (0.6, 86.26)
rs7268671	20	*PLCB4*	G	0.059	0.3967	0.835	1.49 (0.66, 3.4)	Undefined

rs7261773	20	*PLCB4*	A	0.1	0.4051	0.835	0.6 (0.27, 1.35)	1.18 (0.13, 10.36)
rs6077537	20	*PLCB4*	A	0.177	0.411	0.835	1.1 (0.61, 1.99)	2.15 (0.69, 6.7)
rs16996017	20	*PLCB4*	T	0.137	0.4187	0.835	1.18 (0.63, 2.21)	2.52 (0.68, 9.32)
rs13039478	20	*PLCB4*	G	0.124	0.4314	0.835	0.63 (0.31, 1.28)	0.62 (0.07, 5.32)
rs12479503	20	*PLCB4*	T	0.201	0.4381	0.835	1.07 (0.61, 1.89)	1.96 (0.68, 5.66)
rs2876163	20	*PLCB4*	A	0.048	0.4396	0.835	1.57 (0.64, 3.82)	Undefined
rs17482999	20	*PLCB4*	A	0.087	0.4542	0.835	0.77 (0.35, 1.69)	Undefined
rs6086799	20	*PLCB4*	A	0.777	0.4549	0.835	2.31 (0.53, 10.02)	2.41 (0.5, 11.56)
rs13041524	20	*PLCB4*	A	0.113	0.459	0.835	0.63 (0.3, 1.32)	0.74 (0.08, 6.47)
rs6086904	20	*PLCB4*	A	0.059	0.4739	0.835	0.74 (0.29, 1.9)	2.85 (0.28, 28.92)
rs6077536	20	*PLCB4*	T	0.086	0.4935	0.835	0.77 (0.35, 1.69)	Undefined
rs6039487	20	*PLCB4*	G	0.319	0.5047	0.835	1.01 (0.59, 1.73)	1.55 (0.65, 3.72)
rs16995654	20	*PLCB4*	T	0.065	0.5154	0.835	0.66 (0.26, 1.68)	Undefined
rs6118498	20	*PLCB4*	A	0.533	0.5373	0.835	0.98 (0.55, 1.75)	0.7 (0.3, 1.61)
rs4816145	20	*PLCB4*	T	0.119	0.545	0.835	1.04 (0.53, 2.02)	2.27 (0.53, 9.74)
rs1883488	20	*PLCB4*	T	0.107	0.5734	0.835	0.82 (0.39, 1.7)	1.78 (0.33, 9.71)
rs7265290	20	*PLCB4*	G	0.043	0.5736	0.835	1.44 (0.56, 3.72)	Undefined
rs2327164	20	*PLCB4*	C	0.196	0.574	0.835	0.73 (0.4, 1.32)	0.79 (0.2, 3.1)
rs6056551	20	*PLCB4*	G	0.792	0.6012	0.835	1.51 (0.44, 5.18)	1.23 (0.32, 4.81)
rs17389980	20	*PLCB4*	A	0.032	0.6046	0.835	1.56 (0.54, 4.53)	Undefined
rs6056656	20	*PLCB4*	A	0.348	0.6086	0.835	0.99 (0.58, 1.69)	1.4 (0.6, 3.25)
rs6140909	20	*PLCB4*	A	0.07	0.6183	0.835	0.77 (0.33, 1.84)	Undefined
rs6077510	20	*PLCB4*	G	0.337	0.6297	0.835	1.26 (0.73, 2.16)	1.49 (0.62, 3.55)
rs16995573	20	*PLCB4*	G	0.07	0.6365	0.835	0.96 (0.42, 2.19)	Undefined
rs6056552	20	*PLCB4*	T	0.831	0.6769	0.87	0.6 (0.2, 1.85)	0.65 (0.18, 2.31)
rs6077545	20	*PLCB4*	C	0.098	0.7081	0.892	0.74 (0.34, 1.58)	1.01 (0.11, 9.06)
rs6118618	20	*PLCB4*	C	0.086	0.7341	0.907	0.77 (0.35, 1.71)	1.33 (0.14, 12.3)
rs2064183	20	*PLCB4*	G	0.048	0.782	0.937	0.78 (0.28, 2.17)	Undefined
rs6039393	20	*PLCB4*	C	0.344	0.7895	0.937	1.11 (0.65, 1.9)	1.36 (0.57, 3.21)

rs4141984	20	*PLCB4*	C	0.033	0.8035	0.937	1.37 (0.46, 4.07)	Undefined
rs7263079	20	*PLCB4*	A	0.048	0.8314	0.938	1.16 (0.45, 2.95)	Undefined
rs2232257	20	*PLCB4*	T	0.135	0.8366	0.938	0.98 (0.52, 1.85)	0.55 (0.06, 4.71)
rs6086834	20	*PLCB4*	C	0.032	0.8491	0.938	1.2 (0.39, 3.69)	Undefined
rs6039443	20	*PLCB4*	T	0.158	0.8637	0.938	1.15 (0.63, 2.09)	0.93 (0.19, 4.64)
rs16995763	20	*PLCB4*	A	0.177	0.8915	0.952	0.87 (0.48, 1.58)	0.94 (0.24, 3.75)
rs6056654	20	*PLCB4*	G	0.164	0.9088	0.954	0.91 (0.5, 1.68)	1.13 (0.28, 4.55)
rs6056647	20	*PLCB4*	A	0.086	0.9237	0.954	0.93 (0.43, 1.99)	1.37 (0.15, 12.61)
rs6118616	20	*PLCB4*	G	0.288	0.9515	0.959	1.05 (0.61, 1.78)	1.17 (0.45, 3.04)
rs4816144	20	*PLCB4*	T	0.134	0.9594	0.959	0.99 (0.52, 1.87)	1.24 (0.24, 6.31)
rs3112633	16	*SALL1*	T	0.228	0.4683	0.706	0.74 (0.42, 1.3)	1.06 (0.34, 3.27)
rs4784230	16	*SALL1*	C	0.207	0.6146	0.706	0.75 (0.42, 1.34)	0.69 (0.18, 2.68)
rs3112625	16	*SALL1*	A	0.546	0.7063	0.706	0.78 (0.44, 1.4)	0.82 (0.37, 1.84)
rs2255146	14	*SIX1*	A	0.2	0.3106	0.823	0.81 (0.45, 1.43)	0.25 (0.03, 2.07)
rs17098866	14	*SIX1*	A	0.065	0.4717	0.823	1.59 (0.72, 3.48)	2.61 (0.26, 25.9)
rs710066	14	*SIX1*	G	0.054	0.5593	0.823	1.38 (0.58, 3.28)	Undefined
rs813337	14	*SIX1*	G	0.027	0.7829	0.823	1.43 (0.44, 4.63)	Undefined
rs710065	14	*SIX1*	T	0.075	0.8233	0.823	1.14 (0.53, 2.49)	2.09 (0.22, 19.6)
rs383331	22	*TBX1*	G	0.589	0.1072	0.781	2.09 (0.97, 4.49)	1.74 (0.67, 4.51)
rs428702	22	*TBX1*	G	0.576	0.1317	0.781	1.87 (0.92, 3.81)	1.48 (0.6, 3.67)
rs9620784	22	*TBX1*	A	0.148	0.2261	0.781	1.67 (0.92, 3.03)	1.33 (0.26, 6.73)
rs6519749	22	*TBX1*	A	0.038	0.3982	0.781	1.16 (0.41, 3.31)	7.1 (0.59, 85.11)
rs2075271	22	*TBX1*	T	0.049	0.4718	0.781	1.54 (0.63, 3.76)	Undefined
rs9613607	22	*TBX1*	A	0.773	0.5389	0.781	1.37 (0.46, 4.04)	1.05 (0.31, 3.57)
rs439792	22	*TBX1*	C	0.277	0.5442	0.781	0.75 (0.44, 1.29)	0.91 (0.33, 2.48)
rs408718	22	*TBX1*	T	0.621	0.5765	0.781	0.82 (0.42, 1.63)	1.06 (0.45, 2.46)
rs400946	22	*TBX1*	T	0.272	0.5859	0.781	0.77 (0.45, 1.33)	0.96 (0.35, 2.63)
rs2106262	22	*TBX1*	T	0.876	0.905	0.952	0.7 (0.15, 3.18)	0.69 (0.13, 3.59)
rs421390	22	*TBX1*	A	0.516	0.9116	0.952	1.13 (0.62, 2.07)	1.18 (0.52, 2.69)

rs429738	22	*TBX1*	A	0.344	0.9522	0.952	1.04 (0.62, 1.76)	0.94 (0.38, 2.34)
rs2071368	5	*TCOF1*	G	0.054	0.0265	0.265	1.07 (0.43, 2.67)	10.98 (1.97, 61.14)
rs2295223	5	*TCOF1*	T	0.048	0.1287	0.644	1.45 (0.59, 3.58)	9.25 (1.33, 64.32)
rs6891122	5	*TCOF1*	A	0.048	0.3214	0.943	0.43 (0.13, 1.43)	Undefined
rs2748220	5	*TCOF1*	T	0.607	0.6454	0.943	1.28 (0.62, 2.64)	1.52 (0.62, 3.7)
rs7702237	5	*TCOF1*	T	0.07	0.7071	0.943	0.85 (0.37, 1.98)	Undefined
rs7722480	5	*TCOF1*	A	0.07	0.7071	0.943	0.85 (0.37, 1.98)	Undefined
rs1864959	5	*TCOF1*	G	0.612	0.7089	0.943	1.25 (0.61, 2.58)	1.45 (0.6, 3.54)
rs1864958	5	*TCOF1*	T	0.475	0.7542	0.943	1.04 (0.58, 1.86)	1.29 (0.58, 2.89)
rs1560661	5	*TCOF1*	C	0.508	0.8863	0.985	1.03 (0.58, 1.85)	0.9 (0.39, 2.05)
rs1056400	5	*TCOF1*	C	0.108	0.9938	0.994	1.04 (0.52, 2.06)	0.98 (0.11, 8.59)

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