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ORIGINAL ARTICLE

The *FGF* and *FGFR* Gene Family and Risk of Cleft Lip With or Without Cleft Palate

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Background: **Isolated, nonsyndromic cleft lip with or without cleft palate is a common human congenital malformation with a complex and heterogeneous etiology. Genes coding for fibroblast growth factors and their receptors (*FGF/FGFR* genes) are excellent candidate genes.**

Methods: **We tested single-nucleotide polymorphic markers in 10 *FGF/FGFR* genes (including *FGFBP1*, *FGF2*, *FGF10*, *FGF18*, *FGFR1*, *FGFR2*, *FGF19*, *FGF4*, *FGF3*, and *FGF9*) for genotypic effects, interactions with one another, and with common maternal environmental exposures in 221 Asian and 76 Maryland case-parent trios ascertained through a child with isolated, nonsyndromic cleft lip with or without cleft palate.**

Results: **Both *FGFR1* and *FGF19* yielded evidence of linkage and association in the transmission disequilibrium test, confirming previous evidence. Haplotypes of three single-nucleotide polymorphisms in *FGFR1* were nominally significant among Asian trios. Estimated odds ratios for individual single-nucleotide polymorphic markers and haplotypes of multiple markers in *FGF19* ranged from 1.31 to 1.87. We also found suggestive evidence of maternal genotypic effects for markers in *FGF2* and *FGF10* among Asian trios. Tests for gene-environment (G $\times$ E) interaction between markers in *FGFR2* and maternal smoking or multivitamin supplementation yielded significant evidence of G $\times$ E interaction separately. Tests of gene-gene (G $\times$ G) interaction using Cordell's method yielded significant evidence between single-nucleotide polymorphisms in *FGF9* and *FGF18*, which was confirmed in an independent sample of trios from an international consortium.**

Conclusion: **Our results suggest several genes in the *FGF/FGFR* family may influence risk for isolated, nonsyndromic cleft lip with or without cleft palate through distinct biological mechanisms.**

KEY WORDS: *FGF/FGFR, maternal effects, gene-environment interaction, gene-gene interaction, oral clefts*

Isolated, nonsyndromic cleft lip with or without cleft palate (iCL±P) represents one of the most common human birth defects (Mossey and Little, 2002) and has a complex and heterogeneous etiology that remains poorly understood (Jugessur and Murray, 2005). There is a strong genetic component to the etiology of this common birth defect. A multifactorial threshold model of inheritance reflecting multiple distinct causal genes is often assumed (Grosen et al., 2010). Genome-wide linkage screens in multiplex families have shown that multiple regions of the genome may harbor causal genes with a high degree of linkage heterogeneity (Marazita et al., 2004; Marazita et al., 2009). Recently, genome-wide association studies (GWAS) have identified a region on chromosome 8q24 as strongly associated with risk for iCL±P; although, it is relatively devoid

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of genes, raising the possibility that noncoding genetic regions are also critical (Birnbaum et al., 2009; Grant et al., 2009; Beaty et al., 2010). Several studies have also shown suggestive evidence for interaction between candidate genes and environmental risk factors, especially maternal smoking (Zeiger et al., 2005; Shi et al., 2007a) and nutrient intake (Shaw et al., 1998) in controlling risk for iCL±P; although, the evidence for gene-environment (G × E) interaction remains difficult to confirm. Therefore, genes and regulatory elements outside of coding regions, plus their possible interactions with each other (G × G interaction) and with environmental factors, should be considered when searching for potential causal genes for iCL±P.

Genes in the fibroblast growth factor (*FGF*) signaling pathway are excellent candidate genes for iCL±P (Nie et al., 2006; Riley et al., 2007a; Riley et al., 2007b; Riley et al., 2007c; Menezes et al., 2008). Here we tested markers in 10 *FGF* and *FGF* receptor (*FGFR*) genes for their potential role in controlling risk to iCL±P using 297 case-parent trios from four populations.

Materials and Methods

Sample Description

As part of an international study, we collected peripheral blood, environmental exposures, and other data on iCL±P case-parent trios recruited through treatment centers at Johns Hopkins and University of Maryland in Maryland (MD), Chang Gung Memorial Hospital in Taiwan (TW), KK Women's and Children's Hospital in Singapore (SP), and Yonsei Medical Center in South Korea (KR). History of maternal exposure to cigarette smoking, alcohol consumption, and vitamin supplementation was collected from a personal interview of mothers that covered the periconceptual period (3 months before conception through the first trimester). All probands underwent clinical genetic evaluation (including checking for other congenital anomalies or major developmental delays) and were classified as having an isolated, nonsyndromic iCL±P. Research protocols were reviewed and approved by institutional review boards at each participating institution.

Single-Nucleotide Polymorphism Selection, DNA, and Genotyping

Single-nucleotide polymorphisms (SNPs) were selected in 10 *FGF*/*FGFR* genes (including *FGFBP1*, *FGF2*, *FGF10*, *FGF18*, *FGFR1*, *FGFR2*, *FGF19*, *FGF4*, *FGF3*, and *FGF9*) with a goal of identifying one SNP per 5 kilobase pairs (kb) of physical distance. Because Hapmap data were not fully available at the time our SNPs were chosen, we could not identify all possible tagging SNPs. Variants with "SNP scores" >0.6 (an assessment of design quality of the Illumina assay based on a proprietary algorithm), high validation levels in the SNP database (dbSNP) (including validation on multiple platforms), and high heterozygosity levels (particularly in multiple populations) were given priority. The SNP markers were genotyped using Illumina's GoldenGate chemistry (Oliphant et al., 2002) at the Genetic Resources Core Facility (GRCF) at Johns Hopkins. Two duplicates and four controls from the Centre d'Etude du Polymorphisme Humain collection were included on each plate to evaluate genotyping consistency within and between plates.

Statistical Analysis

Genotyping rate, minor allele frequency (MAF), pairwise linkage disequilibrium (LD), and Hardy-Weinberg equilibrium (HWE) were evaluated within each population and in three Asian populations combined. The LD was measured as r^2 for all SNPs using Haploview (Barrett et al., 2005). The SNPs were tested when the following criteria were satisfied: MAF >1%, compatibility with HWE at $p > .01$ in each group, and overall genotyping rate >80%. None of the SNPs between different *FGF*/*FGFR* genes on the same chromosome were in LD (data not shown), so the 10 *FGF*/*FGFR* genes were analyzed separately.

Statistical significance of transmission distortion from parents to the affected offspring was evaluated using the family-based association test (http://www.biostat.harvard.edu/~fbat/fbat.htm) for each individual SNP and for haplotypes of multiple SNPs (Laird and Lange, 2006). Numbers of transmitted and nontransmitted alleles for both single SNPs and haplotypes were generated using PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/, Purcell et al., 2007). The 95% confidence interval for estimated odds ratios (ORs) of overtransmission were calculated by a binomial exact test in STATA (v. 10.0, StataCorp, 2007).

Analysis of maternally mediated *in utero* effects was performed using the TRIad Multi-Marker (TRIMM) package under an assumption of mating symmetry in the population (Shi et al., 2007b). Paired difference counts (D) of the number of target alleles carried by father and mother is a key component in constructing standardized normal (Z) statistics for the mean D across all markers, and the maximum Z^2 (max_Z^2) served as a test statistic. Empiric significance of maternal genotypic effects was evaluated by permuting these max_Z^2 values over random reassignments of "father" and "mother." To optimize power, max_Z^2 and Hotelling T^2 tests were used to generate a combined p value (sum_logP) from these two tests. If the global max_Z^2 test gave a $p < 0.1$, the apparent risk allele or haplotype was used in the log-linear framework originally proposed by Weinberg et al. (1998) to estimate relative risks. Odds ratios and their significance associated with the mother's carrying one copy (S_1) of the risk allele/haplotype was assessed by a likelihood ratio test (LRT) under the log-additive model (where ORs associated with carrying two copies is simply S_1^2) as implemented in the Triad Multi-Marker relative risk

TABLE 1 **Gender and Racial Origin of 297 Cleft Lip With or Without Cleft Palate Probands From Four Populations**

	Maryland		*Taiwan*		*Singapore*		*Korea*		*Total*	
Racial Origin	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
European	41	29	0	0	1	1	0	0	42	30
African	3	3	0	0	0	0	0	0	3	3
Asian	0	0	95	51	23	10	22	18	140	79
Total	44	32	95	51	24	11	22	18	185	112

estimation program (TRIMMEST) (http://www.niehs.nih.gov/research/atniehs/labs/bb/staff/weinberg; Shi et al., 2009). A general model was used to estimate the OR for *FGF2*, where four haplotypes were present, but a more restricted model assuming HWE was used for *FGF10* where 11 haplotypes were observed (because the more general model would have required too many parameters).

Family-based association tests for individual SNPs or two-to three-SNP sliding window haplotypes incorporating a G × E interaction term with maternal smoking and vitamin supplementation were performed in a combined 2 degrees of freedom (*df*) score test for main effects of genotype (G) and G × E interaction together, followed by a 1 *df* score test for G × E interaction alone using PBAT (v3.6; http://www.biostat.harvard.edu/~clange/default.htm).

We used Cordell's (2002) LRT for possible G × G interaction assessment among markers in these 10 *FGF/FGFR* genes. Using a conditional logistic regression model, the observed two-locus genotype of the case was compared with the 15 possible "pseudo-sib" control genotypes, generating a 4 *df* test. To address the issue of multiple comparisons, we carried out permutation tests where case versus pseudo-sib control status was randomly shuffled 1000 times for each trio to generate new sets of data under the null hypothesis. An empirical p value for the most significant SNP was determined by comparing the observed test statistic with these 1000 replicates.

Although markers in these 10 *FGF/FGFR* genes were typed as part of a candidate gene study conducted before the international consortium described by Beaty et al. (2010), 157 of these 297 (52.9%) case-parent trios went into that genome-wide study. In a confirmatory analysis of G × G interaction, we removed these overlapping trios from the international consortium data set and used all remaining case-parent trios (n = 1434), which represents an independent replication sample of iCL±P case-parent trios.

Results

A total of 297 trios were collected from four populations (MD, TW, SP, and KR), and Table 1 lists gender and race of all iCL±P probands. Among the 122 SNPs genotyped in these 10 *FGF/FGFR* genes, nine SNPs were dropped due to low MAF and another two SNPs were dropped due to low genotyping call rate. Genotype distributions for the remaining 111 SNPs were all compatible with HWE (data not shown).

When analyzing transmission distortion of individual SNPs among these 10 *FGF/FGFR* genes, two independent markers in *FGF19* showed nominally significant evidence of linkage and association ($p < .05$) with iCL±P among Asian trios, and another SNP was significant only among MD trios (Table 2). Estimated ORs for carrying the apparent high-risk allele at each of these three *FGF19* SNPs ranged from 1.37 to 1.87. Analysis of sliding window haplotypes using two to five SNPs together confirmed the significance of markers in *FGF19* among Asian trios, and a three-SNP haplotype in *FGFR1* (*rs6987534*, *rs6474354*, and *rs10958700*) gave $p = .04$ (corrected $p = .30$) among Asian trios.

In testing for possible maternal genotype effects, one four-SNP haplotype in *FGF2* and another six-SNP haplotype in *FGF10* showed significant empiric evidence of an increased risk of iCL±P in offspring that depended solely upon maternal genotype among Asian trios. The corresponding ORs for the child being affected were estimated as 1.72 ($\chi^2 = 11.47$, empiric $p = .0007$) and 1.61 ($\chi^2 = 7.29$, empiric $p = .0069$) if the mother carried one copy of the risk haplotype (Table 3).

Although the rate of maternal alcohol consumption was too low to permit separate analysis, about 5% and 25% Asian mothers reported smoking and taking vitamin supplements, respectively, during the critical peri-conceptional period (Sull et al., 2009). Significant evidence of G × E interaction was seen among Asian populations for *FGFR2* (Fig. 1). For G × Smoking analysis, the most significant evidence was seen in a two-SNP haplotype (*rs2981428* and *rs3750817*) that yielded a $p = .0058$ in a 1 *df* test (Fig. 1) that was not significant after strict Bonferroni correction. For G × Vitamin interaction, the strongest evidence in *FGFR2* was seen both in 1 *df* test for a single SNP (*rs2912771*; $p = .00027$, corrected $p = .042$) and in the 2 *df* test for a three-SNP haplotype (involving *rs4752566*, *rs2912760*, and *rs3135761*; $p = .00021$, corrected $p = .033$). These p values for G × Vitamin interaction remained significant after strict Bonferroni correction for all 156 tests conducted on the 27 markers and their haplotypes in *FGFR2*. As seen in the lower panel of Figure 1, several other SNPs and haplotypes also showed nominal significance in either the 1 *df* or the 2 *df* test for G × Vitamin interaction.

A total of 16 pairs of SNPs in different *FGF* genes attained nominal significance in tests of G × G interaction (Table 4). The most significant LRT was generated by *rs2043278* in *FGF18* and *rs12870202* in *FGF9* ($p = .0001$),

TABLE 2 SNPs Yielding Significant or Marginally Significant Associations From Analysis of 111 Markers in 10 *FGF/FGFR* Genes From Family-Based Association Tests for Single SNPs and Two- to Five-SNP Haplotypes in Analysis of 76 Maryland Trios and 221 Asian Trios Separately*

Site	*Gene*	*SNP Name*	*Risk Allele/ Haplotype*	*Percentage*	*Informative Families (n)*	*T*	*NT*	*OR (95% CI)*	*p Value*
Single-SNP analysis									
Asian	*FGF19*	*rs3737463*	1	66.6	148	112	78	1.44 (1.07–1.94)	0.0136
Asian	*FGF19*	*rs948992*	2	55.4	148	114	83	1.37 (1.03–1.85)	0.0272
MD	*FGF19*	*rs1789364*	2	38.9	33	28	15	1.87 (0.96–3.76)	0.0474
MD	*FGF2*	*rs308395*	1	18.8	18	14	5	2.80 (0.95–9.93)	0.0640
Haplotype analysis									
Asian	*FGFR1*	*rs6987534* *rs6474354* *rs10958700*	2,2,1	12.4	79	28	55	0.51 (0.31–0.82)	0.0411
Asian	*FGF19*	*rs3737463* *rs948992*	1,2	45.1	145	117	82	1.43 (1.07–1.92)	0.0271
Asian	*FGF19*	*rs3737463* *rs948992* *rs1307968*	1,2,1	45.1	142	119	82	1.45 (1.09–1.95)	0.0161
Asian	*FGF19*	*rs3737463* *rs948992* *rs1307968* *rs1320706*	1,2,1,1	45.3	142	116	80	1.45 (1.08–1.95)	0.0272
Asian	*FGF19*	*rs3737463* *rs948992* *rs1307968* *rs1320706* *rs1789364*	1,2,1,1,1	36.2	126	101	77	1.31 (0.97–1.79)	0.0498

* T = number of transmitted risk alleles or risk haplotypes; NT = number of not transmitted risk alleles or risk haplotypes; OR = odds ratio; 95% CI = 95% confidence interval.

which remained significant (p = .019) after correcting for multiple comparisons via permutation tests. Only 1.9% of replicates (across the 338 separate tests for G × G interactions) generated under the null hypothesis exceeded this observed test statistic (Table 4; Fig. 2).

To follow up on the intriguing evidence for G × G interaction between *FGF9* and *FGF18* seen here, an independent replication sample of 1434 case-parent trios was examined in a confirmatory test of G × G interaction using markers in these two genes. Only *rs2043278* in *FGF18* was included in the genome-wide marker panel, but 11 different SNPs in or near *FGF9* gave a nominally significant evidence of G × G interaction with this one SNP (Table 5). When all 38 SNPs in *FGF18* were examined with the 140 SNPs mapping to *FGF9*, several additional pairs of SNPs in these two genes showed further evidence of possible G × G interaction (see Fig. 3). The most significant pairs involved SNPs located in the intergenic region 3′ of the gene; some are as far as 500 kb away.

Discussion

In our analysis of 111 markers in 10 *FGF/FGFR* genes using 297 case-parent trios collected from an international study, SNPs in seven of these genes gave some evidence of linkage and association with unobserved causal variants for iCL±P. Genes in the *FGF/FGFR* pathway are considered good candidates for iCL±P because they play important roles in craniofacial development (Kurose et al., 2004; Rice et al., 2004; Jugessur et al., 2009) and several of them (*FGFR1*, *FGFR2*, and *FGF10*) control Mendelian malformation syndromes that can include oral clefts as a

TABLE 3 Maternal Risk Haplotypes Identified by TRIMM From Analysis of 28 Markers in the *FGF2* and *FGF10* Genes Under an Additive Model Where Maternal Genotype Alone (S_1) Controls Risk for Cleft Lip With or Without Cleft Palate in Offspring From TRIMMEST Analysis in 221 Asian Trios*

Gene (No. Chr)	*No. of SNPs*	*Sum_logP*	*SNPs*	*Risk Allele*	*Estimated Risk for Mat. Hap. S_1*	*Risk Hap. Freq*			χ^2	*p*
						0	*1*	*2*		
FGF2(4)	12	0.024	*rs3789138* *rs308388* *rs1476214* *rs1476217*	1 1 1 2	1.72	0.22	0.52	0.26	11.47	.0007
FGF10(5)	16	0.027	*rs10057630* *rs1448037* *rs593307* *rs339502* *rs1384449* *rs2973647*	1 1 1 2 2 1	1.61		0.25		7.29	.0069

* Chr = chromosome; Estimated risk for Mat. Hap. S_1 = estimated risk for maternal haplotype S_1; Risk Hap. Freq = risk haplotype frequencies.

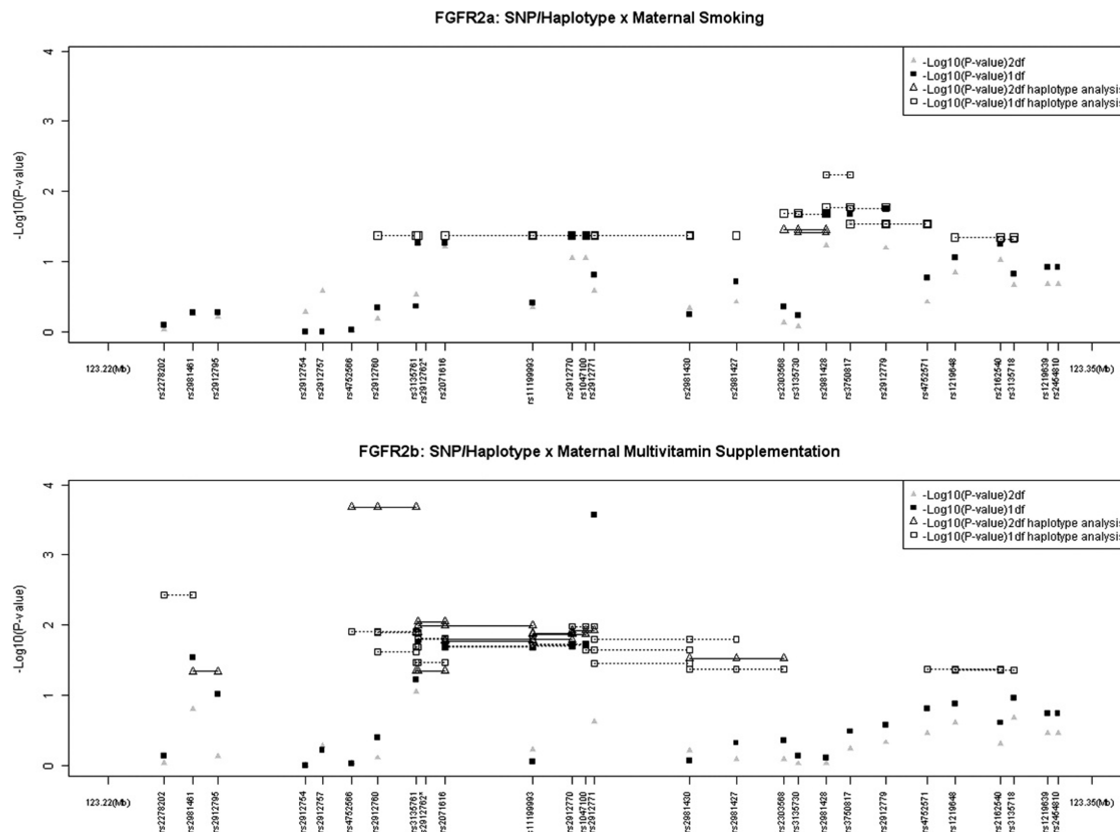

FIGURE 1 Testing for main effects (G) of individual SNPs (haplotypes) of *FGFR2* and gene-environment interaction (G × E) for two common maternal exposures in 221 CL±P case-parent trios from Asian populations. Triangles represent the 2 *df* test of G and G × E interaction; squares represent the 1 *df* test of G × E only. Haplotypes of two- and three-SNPs are connected by solid lines for the 2 *df* test and dotted lines for the 1 *df* test (only nominally significant haplotypes are shown here). *rs2912762: The distance between *rs3135761* and *rs2912762* were drawn for clarity (true distance between these two SNPs is 199 base pairs).**

TABLE 4 Significant Tests for G × G interaction Among 111 Markers in 10 Different *FGF/FGFR* Genes in 297 Cleft Lip With or Without Cleft Palate Case-Parent Trios From Four Populations Based on 4 *df* LRT Testing for Two-Way Interaction

First Gene		*Second Gene*		*Marker 1*	*Marker 2*		
Gene	*Chromosome*	*Gene*	*Chromosome*				
snp1	*snp1*	*snp2*	*snp2*	*SNP1*	*SNP2*	*Test Statistic*	*p Value*
fgf18	5	*fgf10*	5	*rs4559013*	*rs11750845*	14.255	.0065
fgf18	5	*fgf10*	5	*rs4076077*	*rs11750845*	14.300	.0064
fgf18	5	*fgf10*	5	*rs4076077*	*rs1482679*	14.156	.0068
fgf18	5	*fgf10*	5	*rs3934591*	*rs1482679*	17.743	.0014
fgf18	5	*fgfr1*	8	*rs6887323*	*rs2978073*	13.604	.0087
fgf18	5	*fgf3*	11	*rs6887323*	*rs1893047*	15.135	.0044
fgf18	5	*fgf3*	11	*rs2043278*	*rs11263592*	14.199	.0067
fgf18	5	*fgf3*	11	*rs2043278*	*rs1893047*	13.958	.0074
fgf18	5	*fgf9*	13	*rs2043278*	*rs12870202*	23.854	.0001*
fgfr2	10	*fgf10*	5	*rs2981430*	*rs593307*	13.556	.0089
fgfr2	10	*fgf10*	5	*rs2981430*	*rs339502*	13.815	.0079
fgfr2	10	*fgf10*	5	*rs2981430*	*rs1384449*	14.338	.0063
fgfr2	10	*fgfr1*	8	*rs2981427*	*rs4733930*	15.973	.0031
fgf3	11	*fgfbp1*	4	*rs11263592*	*rs732245*	13.590	.0087
fgf3	11	*fgfbp1*	4	*rs11263587*	*rs732245*	13.563	.0088
fgf9	13	*fgfr1*	8	*rs6490667*	*rs4733930*	14.010	.0073

* Only this two-SNP interaction remained significant after 1000 permutations with a *p* value of .019.

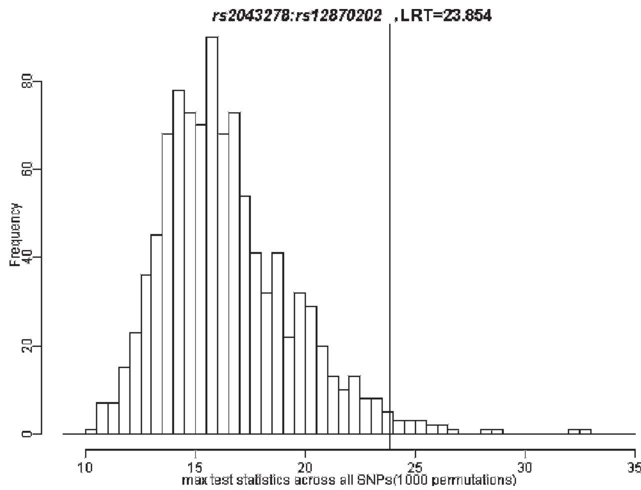

FIGURE 2 Distribution of maximum LRT values over 1000 replicates. Histograms represent the frequency of maximum test statistics generated under the null hypothesis of no G × G interaction. The vertical line shows the position of observed test statistic of G × G interaction between *rs12870202* and *rs2043278*. About 1.9% of permutated test statistics exceeded this observed value, giving an empiric *p* value corrected for multiple testing (*p* = .019).

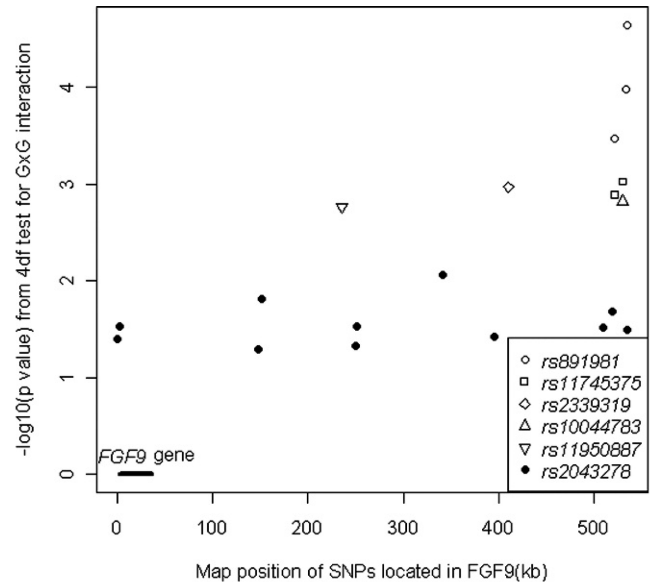

FIGURE 3 Significance [as $-\log_{10}(p)$] from the 4 *df* LRT for Cordell's test of G × G interaction between markers in *FGF18* and *FGF9* plotted against the physical position in *FGF9*. SNP (*rs2043278*) in *FGF18* is an intronic marker located at 170815212 (Build 36) and showed moderately significant *p* values for 11 different SNPs in *FGF9* (solid circles), most of which were located in the 3′UTR (some as far as 500 kb away from the coding region of *FGF9*). Five additional SNPs in *FGF18* also yielded strong evidence of G × G interaction with SNPs in the intergenic region 3′ of the *FGF9* coding region (open symbols).

hallmark feature (Slaney et al., 1996; Dodé et al., 2003; Entesarian et al., 2005). Genes can contribute to the etiology of nonsyndromic forms of oral clefts as well as Mendelian malformation syndromes, such as interferon regulatory factor 6 (Kondo et al., 2002; Zucchero et al., 2004).

Fine-mapping linkage scans in the 8p11-23 chromosomal region (Riley et al., 2007c), association, and sequencing studies (Riley et al., 2007a; Riley et al., 2007b) offered further support for *FGFR1* as a good candidate for iCL±P. Our analysis of markers in *FGFR1* gene confirmed these previous reports. However, neither results from our or Riley's studies would retain statistical significance if strict Bonferroni correction for multiple testing were used. Although there is no previous evidence that *FGF19* influences risk for iCL±P, our results combined with evidence from animal models (Kurose et al., 2004) suggest further investigation of this gene may be warranted.

TABLE 5 Tests for G × G interaction between *rs2043278* in *FGF18* and 11 SNPs in *FGF9* that achieved nominal significance in 1434 case-parent trios from the International Cleft Consortium

SNP Pair	*Position in FGF9*	*Statistic*	*Nominal p*
rs2043278 : *rs829209*	21133584	10.05	.0396
rs2043278 : *rs9634328*	21136546	10.71	.0300
rs2043278 : *rs672905*	21281253	9.42	.0514
rs2043278 : *rs7999069*	21284490	12.27	.0154
rs2043278 : *rs17073403*	21383899	9.61	.0475
rs2043278 : *rs7338014*	21384015	10.77	.0293
rs2043278 : *rs9580272*	21474452	13.59	.0087
rs2043278 : *rs12853883*	21529175	10.16	.0378
rs2043278 : *rs725600*	21643202	10.70	.0301
rs2043278 : *rs9552612*	21652578	11.60	.0205
rs2043278 : *rs17326684*	21667539	10.52	.0325

Mutations in noncoding regions of *FGF2* and *FGF10* can result in impaired transcription (Riley et al., 2007b). Our analysis showed intriguing evidence for maternal genotypic effects controlling the offspring's risk of iCL±P for markers in these two genes among Asians, though fetal effects identified in two previous studies for SNPs in *FGF10* (Riley et al., 2007b; Menezes et al., 2008) were not confirmed here. Maternal genes control the *in utero* environment, so potential maternal genotype effects of markers in *FGF2* and *FGF10* genes seen among our Asian trios may be important (Boyles et al., 2009).

Sequence analysis identified several rare mutations in coding regions of *FGFR2*, which may be causal (Riley et al., 2007a; Riley et al., 2007b). In our study, SNPs in *FGFR2* showed suggestive evidence of G × E interaction with maternal smoking and significant evidence of interaction with vitamin supplementation (even after Bonferroni correction). Evidence from association studies with markers in *FGFR2* across studies has been inconsistent (Riley et al., 2007a; Menezes et al., 2008), but our suggestion of G × E interaction deserves further investigation given the potential for public health intervention with modifiable environmental risk factors.

Potentially important mutations in noncoding regions of *FGF9* were identified in a separate sequencing study (Riley et al., 2007a; Riley et al., 2007b). Our analysis of 297 trios

showed intriguing evidence for possible G × G interaction between *FGF18* and *FGF9* that may be important for iCL±P. Using independent trios from the International Cleft Consortium described by Beaty et al. (2010), we confirmed the significance of G × G interaction between markers in *FGF9* and *FGF18* identified in these 297 trios, although the strongest evidence was seen for markers distal to the 3′ end of *FGF9* (well away from coding regions). The combination of statistical evidence from these 297 trios and from the 1434 independent trios from the GWAS makes it more likely these findings are biologically meaningful. Very few GWAS signals are actually found in coding sequences (Hindorff et al., 2009), and the strongest association signal for iCL±P in Europeans lies in an apparent "gene desert" on chromosome 8q24 (Birnbaum et al., 2009; Grant et al., 2009; Beaty et al., 2010). Similar regulators may underlie these associations, and one can imagine a shared enhancer sequence could explain the suggested interaction between these two *FGF* genes.

Our association results confirmed some previous findings from published linkage, association, and sequencing analysis for various *FGF/FGFR* genes and provided new clues about how these different genes may act through potential maternal genotypic effects and G × E and G × G interactions to control risk of iCL±P. Although some of the statistical evidence presented here did not retain significance after strict Bonferroni correction, mechanisms of G × G interaction in particular require further investigation.

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