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Evidence of gene–environment interaction for the IRF6 gene and maternal multivitamin supplementation in controlling the risk of cleft lip with/without cleft palate

Tao Wu,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. Beijing University School of Public Health, Beijing, China

Kung Yee Liang,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Jacqueline B. Hetmanski,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Ingo Ruczinski,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Margaret Daniele Fallin,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Roxann G. Ingersoll,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. Johns Hopkins School of Medicine, Baltimore, MD, USA

Hong Wang,
Beijing University School of Public Health, Beijing, China

Shangzhi Huang,
Peking Union Medical College, Beijing, China

Xiaoqian Ye,
Mount Sinai School of Medicine, New York, NY, USA. Key Laboratory for Oral Biomedical Engineering of Ministry of Education, Hospital and School of Stomatology, Wuhan University, Wuhan, China

Yah-Huei Wu-Chou,
Chang Gung Memorial Hospital, Taoyuan, Taiwan

Philip K. Chen,
Chang Gung Memorial Hospital, Taoyuan, Taiwan

Ethylin W. Jabs,
Johns Hopkins School of Medicine, Baltimore, MD, USA. Mount Sinai School of Medicine, New York, NY, USA

Bing Shi,
West China College of Stomatology, Sichuan University, Sichuan, China

Richard Redett,
Johns Hopkins School of Medicine, Baltimore, MD, USA

Correspondence to: Terri H. Beaty, tbeaty@jhsph.edu.

Alan F. Scott, and
Johns Hopkins School of Medicine, Baltimore, MD, USA

Terri H. Beaty
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. Department of Epidemiology, School of Public Health, Johns Hopkins University, 615 N. Wolfe St., Baltimore, MD 21205, USA

Tao Wu: twu@jhsph.edu; Terri H. Beaty: tbeaty@jhsph.edu

Abstract

Although multiple genes have been identified as genetic risk factors for isolated, non-syndromic cleft lip with/without cleft palate (CL/P), a complex and heterogeneous birth defect, interferon regulatory factor 6 gene (*IRF6*) is one of the best documented genetic risk factors. In this study, we tested for association between markers in *IRF6* and CL/P in 326 Chinese case–parent trios, considering gene–environment interaction for two common maternal exposures, and parent-of-origin effects. CL/P case–parent trios from three sites in mainland China and Taiwan were genotyped for 22 single nucleotide polymorphisms (SNPs) in *IRF6*. The transmission disequilibrium test was used to test for marginal effects of individual SNPs. We used PBAT to screen the SNPs and haplotypes for gene–environment (G × E) interaction and conditional logistic regression models to quantify effect sizes for SNP–environment interaction. After Bonferroni correction, 14 SNPs showed statistically significant association with CL/P. Evidence of G × E interaction was found for both maternal exposures, multivitamin supplementation and environmental tobacco smoke (ETS). Two SNPs showed evidence of interaction with multivitamin supplementation in conditional logistic regression models (*rs2076153* nominal $P = 0.019$, *rs17015218* nominal $P = 0.012$). In addition, *rs1044516* yielded evidence for interaction with maternal ETS (nominal $P = 0.041$). Haplotype analysis using PBAT also suggested interaction between SNPs in *IRF6* and both multivitamin supplementation and ETS. However, no evidence for maternal genotypic effects or significant parent-of-origin effects was seen in these data. These results suggest *IRF6* gene may influence risk of CL/P through interaction with multivitamin supplementation and ETS in the Chinese population.

Introduction

Non-syndromic cleft lip with/without cleft palate (CL/P) is one of the most common birth defects, and Asians have higher prevalence rates compared to other racial groups (Mossey 2007). CL/P creates significant medical and psychological burdens for cases and their families (Christensen and Mortensen 2002). Recent epidemiological evidence also suggests that CL/P is associated with increased risk of overall mortality (Christensen et al. 2004). CL/P is considered to be a complex disease because both genetic and environmental risk factors contribute to its etiology (Lidral et al. 2008). Mutations of interferon regulatory factor 6 gene (*IRF6*) can lead to van der Woude syndrome (VWS), the most common Mendelian malformation syndrome which includes oral clefts as a hallmark feature (Kondo et al. 2002). Several genetic markers in *IRF6* have also yielded evidence of linkage and linkage disequilibrium (LD) in studies of non-syndromic oral clefts, and have been associated with CL/P in many populations (Zucchero et al. 2004; Blanton et al. 2005; Ghassibé et al. 2005; Scapoli et al. 2005; Srichmthong et al. 2005; Vieira et al. 2007; Park et al. 2007; Jugessur et al. 2008; Jia et al. 2009; Huang et al. 2009; Marazita et al. 2009). However, there is inconsistency across studies and one study conducted in a Chinese sample failed to replicate association between *IRF6* and CL/P (Pegelow et al. 2008; Tang et al. 2009; Paranaíba et al. 2009; Carter et al. 2009).

Gene–environment (G × E) interaction is biologically plausible when considering maternal environmental exposures and genes in the etiology of CL/P (Lidral et al. 2008). G × E

interaction has been suggested for several genes associated with non-syndromic CL/P (van den Boogaard et al. 2008; Sull et al. 2009), although few studies have investigated this question for *IRF6*. In this study, we tested for association between single nucleotide polymorphisms (SNPs) in *IRF6* and CL/P using 326 Chinese case–parent trios, while considering maternal genotypic effects and testing for G × E interactions with common maternal exposures [environmental tobacco smoke (ETS) and multivitamin supplementation].

Methods

Sample description

The "International Genetic Epidemiology of Oral Clefts" study is a multi-center, international family based study initiated in 2003 to investigate the genetic etiology of oral clefts. As part of this study, case–parent trios were recruited from three sites in mainland China (Weifang, Shandong Province; Wuhan, Hubei Province; Chengdu, Sichuan Province) and Taiwan. The majority of cases were infants seen during a surgical or postsurgical visit. All probands were examined for other congenital anomalies or major developmental delays, and were classified as having an isolated, non-syndromic CL/P. Research protocols were reviewed and approved by institutional review boards at each institution. After informed consent was obtained from parents, ethnicity and other data were obtained through structured interviews. Maternal exposure information, including cigarette smoking, ETS, multivitamin supplementation, and alcohol consumption was collected through direct interview of mothers. The proportion of infants exposed to maternal cigarette smoking and alcohol consumption was very low (around 1%), so only ETS and maternal multivitamin supplementation were analyzed. Environmental exposures were defined as being exposed from 3 months prior to pregnancy through the first trimester, except for ETS where exposure was defined as being exposed at any time from 3 months before or during entire pregnancy. Table 1 presents information on gender, family history and exposure among CL/P probands. As seen here, 93.5% of all cases had a negative family history. None of the parents of these CL/P cases were themselves affected. The proportion of exposure to ETS and multivitamin supplementation were 40.5 and 8.6%, respectively.

SNP selection and genotyping

Single nucleotide polymorphisms in and around *IRF6* on chromosome 1q32.3-q41 were identified from the literature, dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and based on previous results in this study (Park et al. 2007). A set of 22 SNPs was chosen based on the criteria of high "design scores" as provided by Illumina, Inc. (San Diego, CA), high heterozygosity, and HapMap validation (http://www.hapmap.org/index.html.en). From these 22 selected SNPs, 1 SNP (*rs12126910*) was monomorphic and was dropped. The minor allele frequencies (MAF) of the remaining 21 SNPs are shown in Table 2. The last SNP, *rs642961*, was previously suggested to cause disruption of the binding site of a transcription factor AP-2*α* and to directly influence the risk of non-syndromic CL/P (Rahimov et al. 2008). Genomic DNA samples were prepared from peripheral blood lymphocytes by the protein precipitation method (Bellus et al. 1995). Primers for each SNP were synthesized using the Oligator technology by Illumina, Inc. as part of an oligo pool for the BeadLab 1000 system. A 4 μg aliquot of each genomic DNA sample was dispensed into a bar-coded 96-well microtiter plate at a concentration of 100 ng/μl and genotyped using the Illumina Golden-GateTM chemistry at the SNP Center of the Genetic Resources Core Facility, a part of the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine. The average distance between neighboring markers was 1.795 kb (based on Build 36.1 of dbSNP). No Mendelian inconsistencies were found for these 21 SNPs. Two duplicates and four CEPH control DNA samples were included to evaluate genotyping consistency within and between plates and to insure correct plate orientation.

Statistical analysis

Minor allele frequencies were computed among parents. Pairwise linkage disequilibrium (LD) was measured as r^2 for all SNPs using the Haploview program (Barret et al. 2005), and pairwise LD was used to identify LD blocks. One independent SNP and 2 blocks of LD were identified, consisting of 3 and 17 SNPs, respectively. Clayton's extension of the transmission disequilibrium test (TDT) incorporated into STATA 9 (Spielman et al. 1993; Cordell et al. 2004) was used on individual SNPs to test for evidence of linkage or LD. The family based association test program (FBAT, http://biosun1.harvard.edu/~fbat/fbat.htm) was used for haplotype analysis (Rabinowitz and Laird 2000). FBAT uses a score test statistic to compare expected genotype among offspring under the assumption of no association between observed genotype counts and the phenotype (Laird et al. 2000; Rabinowitz and Laird 2000) and can be extended to consider haplotypes of several SNPs (Horvath et al. 2004). We used a sliding window approach for haplotype selection which analyzes systematically adjacent SNP combinations of different sizes and locations without any assumption of LD structure.

The transmission asymmetry test (TAT) suggested by Weinberg et al. (1998) was used to examine potential parent-of-origin effects. The TAT is similar to the TDT but excludes matings between two heterozygotes (where transmission can be ambiguous). The TAT was stratified into separate allelic tests for fathers and mothers. The TRIMM package (Shi et al. 2007) was used to test for maternal genotypic effects separately from effects of the child's genotype. The max_Z^2 test implemented in TRIMM compares the genotypes observed in the case to those in a hypothetical "complement" child who would have inherited the parental alleles not transmitted to the case for each SNP to identify sets of SNPs showing evidence of transmission distortion. The test statistic is obtained by leveraging the difference in the number of risk alleles inherited by cases and these "complement" controls across all trios divided by its standard error. This approach preserves LD patterns among SNPs, and generates empirical *P* values by randomly permuting "case" versus "complement" pairs for each SNP. Under the assumption of complete exchangeability between parental haplotypes in the general population, comparing maternally versus paternally transmitted combinations of SNPs provides a test for maternal genotype (or haplotype) effects on risk to the child.

We also screened for possible G × E interaction between all SNPs in *IRF6* and two common maternal exposures: ETS and multivitamin supplementation. In this analysis, we used the strategy proposed by Vansteelandt et al. (2008) where family based association tests are evaluated for individual SNPs while allowing for a potential G × E interaction in a 2 degree of freedom (*df*) test (Kraft et al. 2007), followed by a separate 1 *df* test for G × E interaction alone. This approach is implemented in the PBAT package (v3.6; http://www.biostat.harvard.edu/~clange/default.htm). The 2 *df* test examines the genetic effect of the SNP after taking into account the effects of G × E interaction, while the 1 *df* test investigates the effect of G × E interaction alone. PBAT can also perform haplotype analysis involving G × E interaction. In this analysis, we first used PBAT to screen for possible single SNP G × E interactions. We then used conditional logistic regression to estimate the odds ratios (OR) for being a case with or without environmental exposures to confirm the findings of PBAT.

Results

No SNPs showed evidence of deviating from HWE (data not shown). Figure 1 shows results of the FBAT analyses. Here $-\log_{10}$ (*P* values) are plotted over the physical distances for both single SNP (single dots) and haplotype (dots connected by lines). For clarity, only haplotypes with *P* values < 0.001 are plotted here. The lower panel of Fig. 1 shows LD across this region (as measured by r^2). Among 21 SNPs in *IRF6*, 16 SNPs showed a statistically significant association with CL/P at a nominal value of $P = 0.05$. After Bonferroni correction, 14 SNPs

remained statistically significant (Table 2). Three SNPs (*rs2013162*, *rs2236907* and *rs2294408*) had *P* values = 10^{-6} (Fig. 1). Sliding windows of haplotypes consisting of two, three, four, and five SNPs were tested. A five-SNP haplotype consisting of *rs674433*, *rs595918*, *rs17015218*, *rs2013162* and *rs7552506* showed a highly significant *P* value ($P = 1.86 \times 10^{-5}$). However, this may be driven by the single SNPs. To minimize the influence of misclassification of syndromic forms of CL/P (i.e., undiagnosed or mild forms of VWS) and non-syndromic CL/P (Birnbaum et al. 2008), we also repeated this analysis excluding cases with any positive family history (21 trios) of oral cleft, but saw no substantial difference in results (data not shown).

Maternal genotypic effects for all 21 SNPs were investigated. Parent-of-origin effects were first investigated by stratifying informative transmissions (T) and non-transmissions (NT) by parental source in the TAT (Table 3). SNP *rs1044516* showed excess maternal transmission of the minor allele (OR = 1.71, nominal $P = 0.016$), while two additional SNPs, *rs2073485* and *rs2235373*, yielded excess maternal transmission of the major allele (OR = 0.51, nominal $P = 0.004$) based on the TAT. The TRIMM max_Z^2 score was also used to test for maternal genotypic effects, however, these failed to yield significant results (nominal $P > 0.05$ for all markers).

Figure 2 shows results of screening for G × E interaction between these 21 SNPs in *IRF6* and two exposures using PBAT. Here $-\log_{10}(P$ values) are plotted over physical distance for both the 2 *df* test of G and G × E effects considered jointly (triangles) and the 1 *df* test for G × E alone (squares). SNPs showing evidence of linkage in the presence of LD without consideration of G × E are noted in bold along the X-axis. Most SNPs showed statistical significance in the 2 *df* test for G and G × E interaction, likely reflecting their marginal effects on risk.

SNPs *rs2076153*, *rs17015218* and *rs861019* showed statistical significance in the explicit test of G × E interaction (with 1 *df*) for multivitamin supplementation when considered individually (*rs2076153* nominal $P = 0.035$; *rs17015218* nominal $P = 0.018$; *rs861019*: nominal $P = 0.034$) or within two- and three-SNP haplotypes. SNP *rs599021* also yielded a marginally significant *P* value in the 1 *df* test for interaction with multivitamin supplementation (nominal $P = 0.093$). After screening for G × E interaction using PBAT, we used conditional logistic regression under an additive model to estimate odds ratios (OR) for these four SNPs. Figure 3 shows ORs and 95% confidence intervals (CI) for effects of gene–multivitamin supplementation interaction for these SNPs. Solid circles represent estimated ORs of being a case with a heterozygous genotype for subjects whose mother did not take multivitamin supplementation (for *rs2076153* and *rs17015218*, the reference allele is the minor allele, but for *rs599021* and *rs861019*, the reference allele is the major allele). Open circles represent the ORs of being a similar heterozygous case whose mother did take multivitamin supplementation. *P* values from the likelihood ratio test (LRT) are listed along the *x*-axis. Being heterozygous at *rs2076153* among infants not exposed to maternal multivitamin supplementation was associated with a slightly increased risk (OR = 1.28, 95% CI = 1.01–1.63), but not among those whose mother took multivitamin supplementation (OR = 0.47, 95% CI = 0.20–1.09). The test for G × E interaction was also statistically significant ($P = 0.019$). Being exposed to maternal multivitamin supplementation in combination with the AG genotype at *rs17015218* showed a modest, but significant, reduced risk of CL/P (OR = 0.13, 95% CI = 0.02–0.99). For haplotype G × E analysis using PBAT, 7 two-SNP haplotypes and 18 three-SNP haplotypes yielded significant *P* values in 1 *df* test of G × E interaction with multivitamin supplementation.

SNP *rs1044516* showed evidence of G × E interaction with ETS using the LRT. Being a heterozygote at *rs1044516* (i.e., having the AC genotype) and being exposed to maternal ETS was associated with increased risk of CL/P (OR = 1.96, 95% CI = 1.38–2.78, LRT $P = 0.041$) compared to carrying this AA genotype and not being exposed to maternal ETS (OR = 1.22,

95% CI = 0.91–1.63). All two-SNP haplotypes involving this SNP showed evidence of both G effects and G × E interaction in the 2 *df* test using PBAT, and among these 17 two-SNP haplotypes yielded significant *P* values for G × E alone in the 1 *df* test (nominal $P < 0.05$). The two-SNP haplotype including *rs17317411* and *rs2073485* yielded the most evidence for G × E alone in the 1 *df* test (nominal $P = 3.82 \times 10^{-6}$). Twelve three-SNP haplotypes gave significant *P* values in the 1 *df* test for interaction with ETS (nominal $P < 0.05$). The most significant three-SNP haplotype included *rs3753517*, *rs1005287* and *rs17389541* (nominal $P = 2.08 \times 10^{-6}$).

Although marginal effects of *rs2076153* (ignoring maternal exposures) were not statistically significant, this SNP showed evidence of a possible interaction with maternal multivitamin supplementation in both the 2 *df* test for G and G × E combined (nominal $P = 0.026$), and in the 1 *df* test for G × E alone (nominal $P = 0.035$).

Discussion

Our results showed evidence of linkage and LD for sixteen SNPs in the *IRF6* gene and confirmed *rs642961* was significantly associated with increased risk of non-syndromic CL/P (nominal $P = 0.0004$). Single SNP analysis using conditional logistic regression and haplotype analysis using PBAT suggested the *IRF6* gene may also influence risk of CL/P through interaction with multivitamin supplementation and ETS.

The *IRF6* gene is one of nine members of a family of transcription factors (IRFs) that share a highly conserved DNA-binding domain and a less conserved protein-binding domain (Taniguchi et al. 2001). One recent study found mice deficient in *Irf6* have abnormal skin, limb and craniofacial development (Ingraham et al. 2006), and another study suggested *IRF6* acts on the cell cycle to regulate mammary epithelial cell differentiation (Bailey et al. 2008). Recently, Rahimov et al. (2008) found one SNP (*rs642961*) in the enhancer region of *IRF6* that disrupts a binding site for the transcription factor AP-2α, which may directly influence the risk of non-syndromic CL/P by altering *IRF6* transcription. Our study in Chinese case–parent trios confirmed *rs642961* was significantly associated with increased risk of non-syndromic CL/P ($P = 0.0004$).

Mutations in *IRF6* lead to VWS, the most common Mendelian syndrome that includes CL/P. Most patients with VWS have an oral cleft and approximately 86% of VWS cases also have pits on the lower lip. Since the pits on the lower lip are often the only way to differentiate between non-syndromic oral cleft and VWS, there could be some misclassification between VWS and non-syndromic CL/P. An estimated one in 36 non-syndromic CL/P case will actually represent VWS if one parent is also affected (Jehee et al. 2009). Whether the association between *IRF6* and non-syndromic CL/P is due to misclassification of syndromic and non-syndromic CL/P cases remains a subject of debate (Birnbaum et al. 2008). Excluding trios with any positive family could minimize the influence of this misclassification. Our analysis showed no substantial difference when we compared results of association analysis using all of 326 trios or only those trios without any positive family history of cleft. This result further validated the association between the markers in *IRF6* and non-syndromic CL/P.

Gene–environmental (G × E) interactions have been suggested for several genes associated with non-syndromic CL/P (Vieira 2006; Sull et al. 2009). However, few studies to date have focused on whether G × E interaction influences the association between *IRF6* and risk of CL/P. Our study suggests an interaction between maternal periconceptional multivitamin use and markers in *IRF6*. One SNP also showed possible G × E interactions with ETS. In this analysis, SNPs with evidence of gene effects ignoring exposures also showed evidence in the 2 *df* test of G and G × E when common maternal exposures were considered, especially in haplotype

analysis. This 2 *df* test identifies SNPs influencing risk of disease when the effect of information on exposure is incorporated into a joint test. One SNP in *IRF6* showing no significant association when exposure was ignored showed nominal significance in both the 2 *df* and 1 *df* tests when considering exposure to maternal multivitamin supplementation. This result illustrates the importance of considering possible G × E interaction in the etiology of CL/P.

Maternal vitamin use has been reported to be inversely associated with the risk of CL/P (Johnson and Little 2008) and potential interaction between candidate genes and vitamin supplementation has been suggested (Shaw et al. 1998). We found intriguing evidence of G × E interaction for maternal multivitamin supplementation at two SNPs in *IRF6*. These results suggest being exposed to maternal multivitamin supplementation and carrying certain genotypes at these two SNPs in *IRF6* may lower the risk of the baby developing CL/P. Shi et al. (2008) summarized the findings of 19 studies investigating interaction between smoking and genes and noted these findings were not consistent across studies. In this analysis, we found evidence of G × E interaction for maternal ETS. A single SNP in *IRF6* showed a significant interaction with ETS in the conditional logistic model and several two- and three-SNP haplotypes showed evidence of G × E interaction in PBAT analysis.

Maternal genotypic effects for non-syndromic CL/P have also been reported for several other candidate genes (CBS, RUNX2 and TGFA) (Rubini et al. 2005; Sull et al. 2008, 2009). In screening for parent-of-origin effects, we found suggestive evidence of excess maternal transmission for three of these SNPs (*rs1044516*, *rs2073485* and *rs2235373*) using the TAT, however, none of these could be confirmed in formal tests for maternal genotypic effects.

The case–parent trio design is robust to population stratification, and provides a unique opportunity to investigate parent-of-origin effects (Cordell et al. 2004; Starr et al. 2005). The present study found some evidence of potential maternal over-transmission for markers in *IRF6* and risk of non-syndromic CL/P. However, the evidence for association in the context of G × E interaction between markers in *IRF6* and both maternal multivitamin use and ETS tended to be stronger. Maternal multivitamin supplementation, in particular, appears to reduce risk of non-syndromic CL/P in cases carrying certain genotypes. The exposure rate of maternal vitamin supplementation was different across different sites. The mothers in mainland China had much lower use of multivitamin supplementation compared to those in Taiwan (6.8 vs. 14.1%). If this observation can be confirmed, such a G × E interaction creates opportunities for an effective intervention to reduce the risk of non-syndromic CL/P, an important public health burden.

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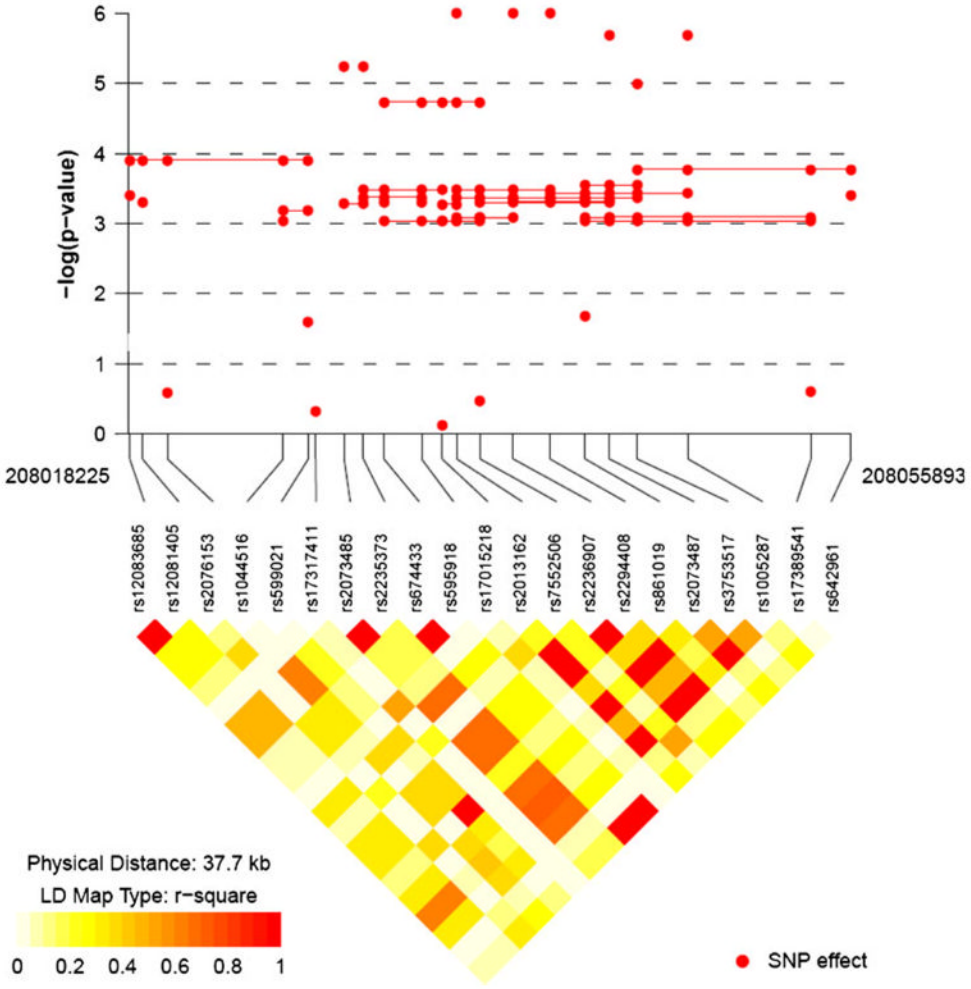

Fig. 1.

Significance of individual single nucleotide polymorphisms (SNPs) and sliding window haplotypes for the interferon regulatory factor 6 (IRF6) gene in 326 nonsyndromic cleft lip with or without cleft palate trios. The −log10 (empirical *P* value) for the overall test for an individual SNP (*dots*) and for sliding windows of haplotypes of two to five SNPs (*dots connected by lines*) is presented. Only haplotypes with *P* values < 0.001 are shown here. The plot was produced using snp.plotter (Luna and Nicodemus 2007)

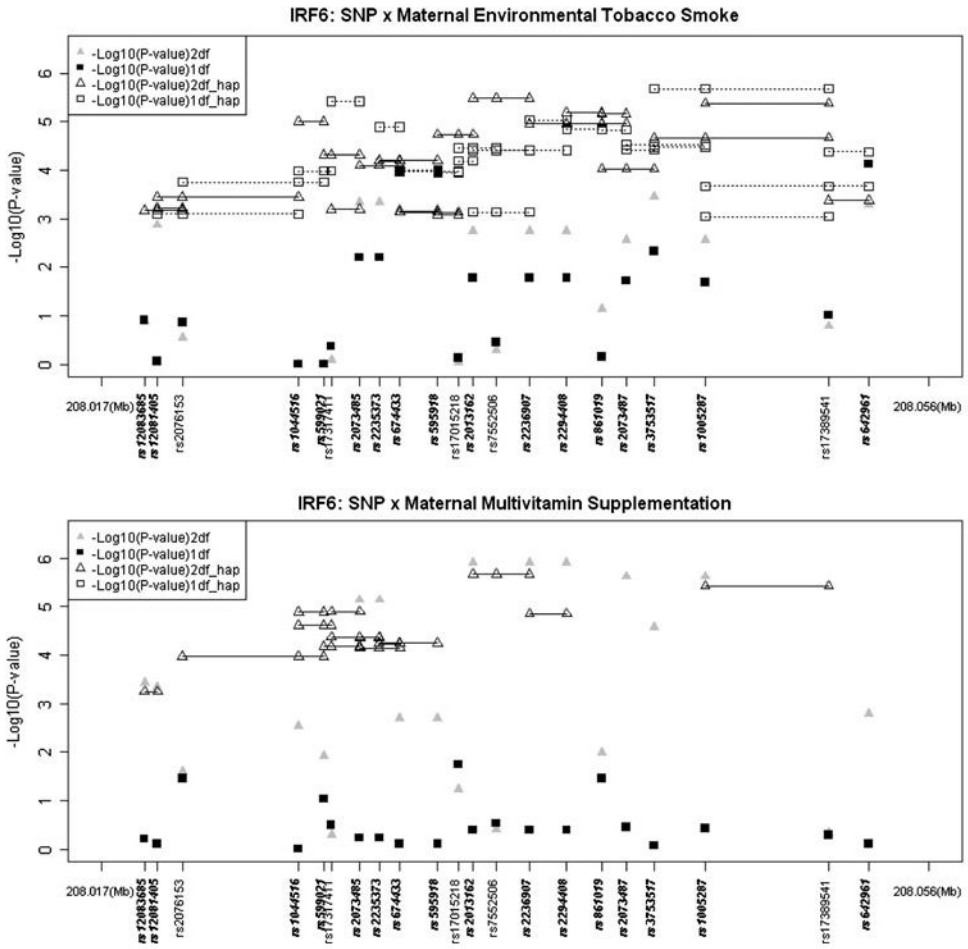

Fig. 2.

Testing for gene–environment interaction (G × E) for common maternal exposures in 326 CL/P case–parent trios from Chinese populations. *Triangles* represent the gene–environmental interaction 2 *df* test of G and G × E interaction, *squares* represent the 1 *df* test of G × E only. Haplotypes of 2- and 3-loci are connected by *dashed lines* (only nominally significant haplotypes $P < 10^{-3}$). SNPs in *bold* showed significant *P* values (nominal $P < 0.05$) for genetic effects ignoring environmental exposures

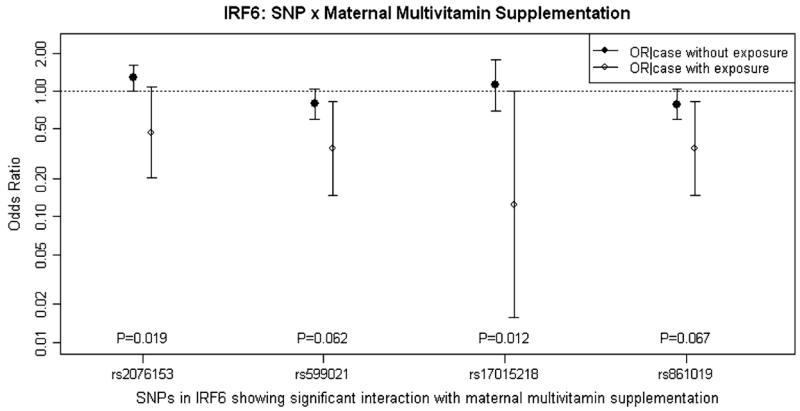

Fig. 3.

Odds ratios (OR) and 95% confidence intervals (CI) for estimated effects of gene–multivitamin supplementation interaction at four SNPs in *IRF6*. Odds ratios and CIs are drawn on a logarithmic scale. The ORs and 95% CI were obtained from conditional logistic regression using additive model. *Filled circles* represent the ORs of being a case with one copy of the risk allele and without exposure to maternal vitamin supplementation (for *rs2076153* and *rs7552506* the reference allele is the minor allele and for *rs599021* and *rs861019*, the reference allele is the major allele). *Open circles* represent the odds ratios of being a case with one copy of the risk allele and being exposed to maternal vitamin supplementation. *P* values from the likelihood ratio test (LRT) with 1 degree of freedom testing the significance of gene–multivitamin supplementation interaction are showed for each SNP

Table 1

Characteristics of 326 Chinese non-syndromic cleft lip with or without cleft palate (CL/P) cases

Number of probands	Cleft lip only	Cleft lip with cleft palate	Total
Gender			
Male	60	165	225
Female	36	65	101
Family history			
Sporadic	87	218	305
Familial	9	12	21
Exposure			
Multivitamin supplementation	9	19	28
Environmental tobacco smoke	45	87	132

Table 2

TDT analysis of 21 SNPs in IRF6 gene among 326 Chinese non-syndromic CL/P case–parent trios

No.	SNP name	Position[a]	MAF	TDT					
				T	NT	OR[b]	χ^2	*P* value	Corrected *P* value[c]
1	rs12083685	208018225	0.262	95	150	0.633	12.35	0.000442	0.009282
2	rs12081405	208018889	0.263	95	149	0.638	11.95	0.000546	0.011466
3	rs2076153	208020194	0.466	166	146	1.137	1.28	0.257518	–
4	*rs1044516*	*208026237*	*0.493*	*193*	*133*	*1.451*	*11.04*	*0.000890*	*0.018690*
5	rs599021	208027545	0.278	140	105	1.333	5.00	0.025347	0.532287
6	rs17317411	208027937	0.039	22	27	0.815	0.51	0.475051	–
7	rs2073485	208029417	0.400	112	191	0.586	20.60	0.000006	0.000126
8	rs2235373	208030426	0.400	112	191	0.586	20.60	0.000006	0.000126
9	rs674433	208031498	0.226	137	85	1.612	12.18	0.000483	0.010143
10	rs595918	208033466	0.226	137	85	1.612	12.18	0.000483	0.010143
11	rs17015218	208034538	0.068	40	43	0.930	0.11	0.741934	–
12	rs2013162	208035307	0.496	118	206	0.573	23.90	0.000001	0.000021
13	rs7552506	208036525	0.159	86	99	0.869	0.91	0.339184	–
14	rs2236907	208038251	0.496	118	206	0.573	23.90	0.000001	0.000021
15	rs2294408	208040172	0.496	118	206	0.573	23.90	0.000001	0.000021
16	rs861019	208042009	0.278	139	103	1.350	5.36	0.020659	0.433839
17	rs2073487	208043269	0.499	121	207	0.585	22.55	0.000002	0.000042
18	rs3753517	208044721	0.341	110	186	0.591	19.51	0.000010	0.000210
19	rs1005287	208047380	0.499	121	207	0.585	22.55	0.000002	0.000042
20	rs17389541	208053795	0.090	49	61	0.803	1.31	0.252559	–
21	rs642961	208055893	0.226	138	85	1.624	12.60	0.000364	0.007644

Non-italicized values indicates inferred LD blocks

T transmitted, *NT* not transmitted, *OR* odds ratio, *CL/P* cleft lip with or without cleft palate, *TDT* transmission disequilibrium test, *LD* linkage disequilibrium, *SNP* single nucleotide polymorphism

[a] Based on NCBI Human Genome build 36.1

[b] Odds Ratio of transmission of minor allele

[c] *P* values after Bonferroni correction

Table 3

Number of transmitted (T) or non-transmitted (NT) minor alleles in 326 CL/P trios

No.	SNP name	Paternal				Maternal					
		TAT				TAT				Maternal genotypic effect	
		T	NT	*P* value	OR[a]	*T*	NT	*P* value	OR[a]	*Z*	*P* value
1	rs12083685	29	45	0.629	0.64	31	48	0.056	0.65	0.446	0.70
2	rs12081405	29	44	0.079	0.66	31	48	0.056	0.65	−0.507	0.65
3	rs2076153	41	39	0.823	1.05	39	33	0.480	1.18	0.056	1.00
4	rs1044516	44	32	0.169	1.38	53	31	0.016	1.71	−0.162	0.91
5	rs599021	46	36	0.269	1.28	47	36	0.227	1.31	0.475	0.69
6	rs17317411	8	16	0.102	0.50	13	10	0.532	1.30	−0.144	1.00
7	rs2073485	35	50	0.104	0.70	27	53	0.004	0.51	−0.054	1.00
8	rs2235373	35	50	0.104	0.70	27	53	0.004	0.51	−0.054	1.00
9	rs674433	44	24	0.153	1.83	32	30	0.800	1.07	−0.658	0.56
10	rs595918	44	24	0.153	1.83	32	30	0.800	1.07	0.658	0.56
11	rs17015218	15	21	0.317	0.71	21	16	0.411	1.31	0.553	0.66
12	rs2013162	35	46	0.222	0.76	33	40	0.413	0.83	−0.107	0.96
13	rs7552506	30	37	0.392	0.81	33	27	0.439	1.22	0.400	0.75
14	rs2236907	35	46	0.222	0.76	33	40	0.413	0.83	−0.107	0.96
15	rs2294408	35	46	0.222	0.76	33	40	0.413	0.83	−0.107	0.96
16	rs861019	45	36	0.317	1.25	47	36	0.227	1.31	0.536	0.64
17	rs2073487	35	47	0.185	0.74	32	40	0.346	0.80	0.000	1.00
18	rs3753517	26	48	0.011	0.54	30	54	0.009	0.56	0.230	0.87
19	rs1005287	48	34	0.122	1.41	32	40	0.346	0.80	0.000	1.00
20	rs17389541	20	27	0.307	0.74	23	24	0.884	0.96	0.193	0.93
21	rs642961	44	23	0.010	1.91	32	30	0.799	1.07	0.593	0.60

OR (transmission) odds ratio of transmission of minor allele