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Further Evidence of GWAS Signals in Non-Syndromic Orofacial Clefts from Western Han Chinese

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Abstract

Background

Non-syndromic orofacial clefts (NSOCs) are the major human congenital defects with a complex etiology. Several candidate genes and environmental factors, and their interactions were assumed for the susceptibility to NSOCs. Previous GWAS have identified numerous susceptible loci from different populations. However, few loci have been replicated among Western Han Chinese yet. This study aimed to validate this findings in NSOCs from Western Han Chinese population.

Methods

We selected two SNPs (rs4147811 and rs481931) on 1p22 and recruited 440 case-parent trios with NSOCs for this study. The SNPs were genotyped by using SNPscan method. To evaluate the association, we performed transmission disequilibrium test (TDT), parent-of-origin effect and gene-gene interaction analysis.

Results

Rs481931 G allele (Z=2.05, P=0.016) and G/G homozygotes (Z=2.62, P=0.009) were over-transmitted for NSCL/P. Rs4147811 C allele (Z=2.16, P=0.030) and C/C homozygotes (Z=2.29, P=0.020) were over-transmitted for NSCL/P. Rs481931 G allele was also paternal over-transmitted for NSCL/P (P=0.030) and maternal over-transmitted for NSCPO (P=0.036).

Conclusions

Our results confirmed the previous GWAS findings of these two SNPs in the etiology of NSOCs among Western Han Chinese.

Keywords: 1p22; GWAS; Single-nucleotide polymorphisms; Transmission disequilibrium test; Non-syndromic orofacial clefts

Introduction

Non-syndromic orofacial clefts (NSOCs) are the major human congenital defects with a worldwide occurrence rate between 1/700-1/2500 [1], which varies greatly by geographic origin, ethnicity and socioeconomic status. In general, Asians and Native Americans presented the highest frequencies, sometimes at 1/500 or higher, followed by Caucasians, whereas Africans showed the lowest frequency at approximately 1/2500 [1]. Especially, Chinese newborns displayed a high frequency of NSOCs at 1.67/1000 [2].

The etiologies of NSOCs were multifactorial, with a combination of both genetic and environmental risk factors. The search for genetic factors to NSOCs had used numerous approaches including candidate genes, linkage analysis studies, genomic rearrangements and genome-wide association studies (GWAS). However, the results were still unsatisfactory [3-5]. So far, there were five GWAS of non-syndromic orofacial clefts have been reported and identified tens of susceptible loci [6-10]. The largest GWAS from Beaty et al. firstly reported the association of SNPs in 1p22 with NSCL/P, with the significance coming from Asian population [6]. And another GWAS from Ludwig et al. confirmed the association between 1p22 loci and orofacial clefts among European [7]. Significant associations were found for rs4147811 (P=3.8×10-8) and rs481931 (P=8.14×10-8) [6]. Subsequently, Yuan et al. found the association of rs481931 with NSOCs in non-Hispanic white and Hispanic NSCL/P families [11]. Fontoura et al. found evidence of the associations between rs481931 and NSOCs in Caucasian populations [12]. Yildirim et al. found a higher frequency of the most common allele of the rs481931 in Turkish born with clefts [13]. Then Liu et al. confirmed 16 susceptibility loci responsible for NSCL/P using whole-exome sequencing in a study of eight Chinese fetuses with clefts, including gene in 1p22 [14]. Meanwhile, Butali et al. observed no significant results for the association between polymorphisms (including SNPs rs481931 and rs4147811) on 1p22 and NSOCs in the Nigerian population [15]. And Mi et al. did not find association of rs481931 with risk of NSCL/P in the examined northern Chinese Han population [16]. The results of those studies above showed the inconsistence. This could be resulted from allelic heterogeneity among populations, so it is necessary to replicate GWAS findings in diverse populations.

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Hence, in this study, we selected two SNPs on 1p22 (rs4147811 and rs481931) to investigate whether these loci were associated with NSOCs in Western Han Chinese.

Materials and Methods

Ethics statement

Human subject study protocols were reviewed and approved by the Hospital Ethics Committee (HEC) of West China Hospital of Stomatology, Sichuan University. Written informed consent was obtained from each participant; for children younger than 16 years old, consents were requested from their parents or guardians.

Samples description

Our samples consisted of 440 case-parent trios, who were recruited between 2008 and 2013 from the Cleft Lip and Palate Surgery Department of West China Stomatology College, Sichuan University. With congenital anomalies or major developmental delays excluded, the cases of NSOCs were classified as isolated. All subjects were self-identified as Western Han Chinese. The types of clefts and gender were shown in Table 1.

SNPs selection and genotyping

Venous blood samples were drawn from all participants. Genomic DNA was extracted using the protein precipitation method. We chose two SNPs (rs4147811 and rs481931) based on previous GWAS findings. All the genotyping experiments were done by the Genesky Biopharm technology Company (http://www.geneskies.com/) using SNPscan technology.

Statistical analysis

For each SNP, we checked for deviations by performing Hardy-Weinberg equilibrium (HWE) test and calculated the minor allele of frequency among the normal parents. Parent-of-origin effect was assessed to distinguish the parental preference of transmission on a disease variant. Transmission disequilibrium test (TDT) was performed to evaluate the transmission of target alleles and genotypes from heterozygous parents to the affected offspring by Family-based Association Test (FBAT) program. Gene-gene interaction analysis was performed to detect the relationship between those two loci among non-syndromic orofacial clefts.

Results

Allelic and genotypic TDT analysis

TDT analysis was carried out on case-parents trios with heterozygous informative parents. Allelic TDT analyses from FBAT showed that G allele at rs481931 displayed a statistically significant evidence of over-transmission for NSCLO (P=0.028) and NSCL/P (P=0.016). At rs4147811, C allele displayed a statistically significant evidence of over-transmission for NSCL/P (P=0.031). However, the allelic TDT did not show over-transmission for NSCLP and NSCPO (Table 2).

Genotype distribution comparison in TDT analyses showed that C/C homozygotes at rs4147811 (Z=2.29 and P=0.022), G/G homozygotes at rs481931 (Z=2.62 and P=0.009) were over-transmitted for NSCL/P. A tendency towards a significant deviation was also present for the G/G homozygotes at rs481931 over-transmitted for NSCLO (Z=1.84 and P=0.066) and NSCLP (Z=1.76 and P=0.078). No evidence of association was identified in genotypic TDT analyses for NSCPO (Table 3).

	NSCLO	NSCLP	NSCL/P	NSCPO	NSOCs
Male	79	94	173	59	232
Female	57	54	111	82	193
Unknown sex	2	10	12	3	15
Total	138	158	296	144	440

Note: NSCLO, non-syndromic cleft lip only; NSCLP, non-syndromic cleft lip with cleft palate; NSCL/P, non-syndromic cleft lip with or without cleft palate (NSCL/P including NSCLO and NSCLP); NSCPO, non-syndromic cleft palate only; NSOCs, non-syndromic orofacial clefts (NSOCs including NSCLO, NSCLP and NSCPO).

Table 1: Types of non-syndromic orofacial clefts.

Phenotype	SNP	Allele	afreq	fam#	Z	P
NSCLO	rs4147811	C	0.66	101	1.93	0.054
	rs4147811	T	0.34	101	-1.93	0.054
	rs481931	**G**	**0.66**	**100**	**2.20**	**0.028**
	rs481931	**T**	**0.34**	**100**	**-2.20**	**0.028**
NSCLP	rs4147811	C	0.66	107	1.07	0.28
	rs4147811	T	0.34	107	-1.07	0.28
	rs481931	G	0.67	98	1.13	0.26
	rs481931	T	0.33	98	-1.13	0.26
NSCL/P	**rs4147811**	**C**	**0.66**	**209**	**2.16**	**0.031**
	rs4147811	**T**	**0.34**	**209**	**-2.16**	**0.031**
	rs481931	**G**	**0.67**	**199**	**2.41**	**0.016**
	rs481931	**T**	**0.33**	**199**	**-2.41**	**0.016**
NSCPO	rs4147811	C	0.65	107	-1.09	0.28
	rs4147811	T	0.35	107	1.09	0.28
	rs481931	G	0.65	99	-1.54	0.12
	rs481931	T	0.35	99	1.54	0.12
NSOCs	rs4147811	C	0.66	317	1.17	0.24
	rs4147811	T	0.34	317	-1.17	0.24
	rs481931	G	0.66	299	1.10	0.27
	rs481931	T	0.34	299	-1.10	0.27

Note: NSCLO, non-syndromic cleft lip only; NSCLP, non-syndromic cleft lip with cleft palate; NSCL/P, non-syndromic cleft lip with or without cleft palate (NSCL/P including NSCLO and NSCLP); NSCPO, non-syndromic cleft palate only; NSOCs, non-syndromic orofacial clefts (NSOCs including NSCLO, NSCLP and NSCPO); SNP, Single-Nucleotide Polymorphism; afreq, allele frequency; fam#, informative family; Z, vector of the large sample Z statistic; P, P-value; Bold characters indicate the items with p-value less than 0.05.

Table 2: Allelic TDT results for SNPs in 1p22 from FBAT.

Parent-of-origin effects

Considering the parental origin of the alleles, we found no significant difference between the maternal and paternal among the SNPs for NSCLO, NSCLP, NSCL/P and NSCPO. However, we found an excess of paternal transmission of the allele G at rs481931 for NSCL/P (P=0.030) and an excess of maternal transmission of the allele G at rs481931 for NSCPO (P=0.036). Other tests showed no evidence of paternal or maternal over/under-transmission (Table 4).

Gene-gene interaction analysis

Considering the complex etiology of NSOCs, the relatedness

SNP	Genotype	NSCLO		NSCLP		NSCL/P		NSCPO		NSOCs	
		Z	P	Z	P	Z	P	Z	P	Z	P
rs4147811	**C/C**	1.71	0.086	1.43	0.15	**2.29**	**0.022**	-0.44	0.66	1.68	0.094
	C/T	-0.90	0.37	-1.26	0.21	-1.59	0.11	-0.48	0.63	-1.63	0.10
	T/T	-1.12	0.26	0	1	-0.74	0.46	1.39	0.17	0.23	0.82
rs481931	**G/G**	1.84	0.066	1.77	0.078	**2.62**	**0.009**	-0.85	0.40	1.72	0.086
	G/T	-0.80	0.42	-1.82	0.069	-1.91	0.056	-0.30	0.76	-1.79	0.073
	T/T	-1.46	0.14	0.40	0.69	-0.71	0.48	1.69	0.092	0.43	0.67

Note: NSCLO, non-syndromic cleft lip only; NSCLP, non-syndromic cleft lip with cleft palate; NSCL/P, non-syndromic cleft lip with or without cleft palate (NSCL/P including NSCLO and NSCLP); NSCPO, non-syndromic cleft palate only; NSOCs, non-syndromic orofacial clefts (NSOCs including NSCLO, NSCLP and NSCPO); SNP, Single-Nucleotide Polymorphism; Z, vector of the large sample Z statistic; P, P-value; Bold characters indicate the items with p-value less than 0.05.

Table 3: Genotypic TDT results for SNPs in 1p22 from FBAT.

Phenotype	SNP	Minor allele	Paternal		Maternal		Z	P
			T/NT	P	T/NT	P		
NSCLO	rs481931	T	26/36	0.20	26/41	0.067	0.36	0.72
	rs4147811	T	28/35	0.38	26/41	0.067	0.65	0.52
NSCLP	rs481931	T	28/43	0.075	27/25	0.78	-1.37	0.17
	rs4147811	T	33/43	0.25	29/32	0.70	-0.48	0.63
NSCL/P	**rs481931**	**T**	**54/79**	**0.030**	53/66	0.23	-0.63	0.53
	rs4147811	T	61/78	0.15	55/73	0.11	0.15	0.88
NSCPO	**rs481931**	**T**	34.5/32.5	0.81	**41.5/24.5**	**0.036**	-1.32	0.19
	rs4147811	T	35/36	0.91	42/27	0.071	-1.37	0.17
NSOCs	rs481931	T	88.5/111.5	0.10	94.5/90.5	0.77	-1.34	0.18
	rs4147811	T	96/114	0.21	97/100	0.83	-0.71	0.48

Note: NSCLO, non-syndromic cleft lip only; NSCLP, non-syndromic cleft lip with cleft palate; NSCL/P, non-syndromic cleft lip with or without cleft palate (NSCL/P including NSCLO and NSCLP); NSCPO, non-syndromic cleft palate only; NSOCs, non-syndromic orofacial clefts (NSOCs including NSCLO, NSCLP and NSCPO); SNP, Single-Nucleotide Polymorphism; T/NT, transmitted/non-transmitted; Z, vector of the large sample Z statistic; P, P-value; Bold characters indicate the items with p-value less than 0.05.

Table 4: Parent-of-origin effects for SNPs in 1p22 by FBAT.

between disease and candidate genes and/or their interactions was ignored frequently. For this reason, the analysis of gene-gene interaction was performed. However, we did not find any interaction between those two loci (data not shown).

Discussion

NSOCs are a complex hereditary disease of heterogeneous etiology, which cannot be explained with a single gene or environment model. It is well known that different pathogenic factors may act in different populations. Searching for the broader implications of risk for orofacial clefts in diverse ethnic populations is important to provide more insight into the underlying etiology. To date, much progress had been successfully made in the identification of several putative candidate genes for NSOCs. However, replication researches on those candidate genes to other populations are still limited [1].

The transmission/disequilibrium test, whereby transmissions and non-transmissions of an allele of interest to an affected offspring are stratified according to parental origin, statistically significantly restricted to fathers but not mothers may be interpreted as evidence for non-expression of the maternally derived allele, which may reflect underlying imprinting [17]. Our replication study showed that rs481931 and rs4147811 presented significant association with different subtypes in the conventional allelic TDT analysis and the genotypic TDT analysis. Parent-of-origin effects may occur when the phenotypic effect of an allele depends on whether it is inherited from an individual's mother or father [18]. Therefore, we designed our family-based samples in this study. The results showed no significant difference between the maternal and paternal effects. However, when stratified by phenotype, allele G at rs481931 was observed over-transmission in different subtypes, including NSCL/P and NSCPO (Table 4). These results were not consistent with the findings of Butali et al. [15] and Mi et al. [16], but consistent with the findings of Beaty et al. [6] and Yuan et al. [11].

The two SNPs, in this study, reside in the intron of ABCA4 gene, with which there is no evidence showed association with the orofacial morphogenesis [6,19]. ARHGAP29, encoding Rho GTPase activating protein (GAP) 29, is located adjacent to ABCA4, which was identified strongly expression in the medial and lateral nasal processes at E10.5 in murine embryos [20]. Although the intron did not influence the protein structure, many studies had reported that the presence of an intron and the act of its removal can influence almost every step in gene expression from transcription to mRNA export, localization, translation, and decay [21,22]. And there also might exist regulatory elements in the intron, which play roles on the ABCA4 gene or adjacent ARHGAP29 gene [23].

In the current study, Rs4147811 and rs481931 in our samples deviated from HWE (P<0.05). When stratified by phenotype, the SNPs were closely compatible with HWE. HWE usually had been used for understanding genetic characteristics of populations. However, some association researches did not consider departures from HWE in patients to indicate genotyping error but prefer to assume a biological explanation in a patient sample [24]. Especially, there was a higher genetic load in families related to probands leading to the departures from HWE in the case-parents design of this study. Indeed, we found evidence of genotypic and allelic association for the two SNPs with NSCL/P.

Our study still had several limitations. First, sample size is a potential confounding factor. Our study subjects are homogeneous Western Han Chinese populations resulting to insufficient of samples. Especially, samples showed deviation from HWE in our study. It is necessary to amplify samples in the subsequent researches. Second, the selected SNPs of our study may influence results. However, we believe that this is unlikely to be a significant issue.

In summary, our results provide additional evidence for the association between 1p22 and NSOCs in a population from Western Han Chinese, which is consistent with previous GWAS findings, especially relating to Chinese population.

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