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British Journal of Oral and Maxillofacial Surgery 53 (2015) 268–274

BRITISH Journal of Oral and Maxillofacial Surgery

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# Association of single-nucleotide polymorphisms in the IRF6 gene with non-syndromic cleft lip with or without cleft palate in the Xinjiang Uyghur population

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Accepted 12 December 2014

Available online 13 January 2015

**Abstract**

Our main aim was to investigate the association between the interferon regulatory factor (IRF6) gene and non-syndromic cleft lip and palate (nsCLP) in the Xinjiang Uyghur population. Twelve single nucleotide polymorphisms (SNP) were screened in a group of 100 patients with nsCLP and in a control group of 60 unaffected subjects by next generation sequencing using a MiSeq Benchtop Sequencer (Illumina). Our case–control association analysis showed that the SNP marker rs7545538 differed significantly in genotype (codominant model; CC compared with CG compared with GG; $p = 0.038$) and allele frequencies (odds ratio (OR) = 1.89, 95% CI 1.18–3.03, $p = 0.007$) between patients with nsCLP and controls. Analysis of the recessive model of inheritance showed that distribution of the recessive model of rs7545538 (GG compared with CC + GC) was significantly higher in patients with nsCLP than in controls (OR = 2.5, 95% CI 1.13–5.37, $p = 0.021$) and had a borderline association with an increased risk of nsCLP (OR = 2.5, 95% CI 1.13–5.37, $p = 0.021$). Markers rs2235377 and rs2235371 also differed significantly in dominant and over-dominant models of inheritance ($p = 0.037$) while increased G allele frequency was seen in SNP rs2235373 ($p = 0.03$). A haplotype analysis showed four common haplotypes in Block 1: CCGGT > CCGAT > CACAT > TAGAC (in frequency). The 5-marker combination haplotype CCGAT was significantly more common in patients with nsCLP than in controls ($p = 0.032$). In Block 2, the overall distribution of the haplotypes TAC and TAG predicted by the three SNP differed significantly between the patients with nsCLP and control subjects ($p = 0.009$ and 0.003, respectively). Our results showed that genetic polymorphism of the IRF6 gene is associated with increased risk of nsCLP in a Xinjiang Uyghur population.



*Keywords:* IRF6 gene; Orofacial clefts; Case–control study; Linkage disequilibrium

## Introduction

Non-syndromic cleft lip with or without cleft palate (nsCLP) is a common and debilitating congenital malformation that accounts for many birth defects.[1] Patients with these anomalies may require operation during the first year of life, as well as nutritional, dental, speech, medical, and behavioural follow-up.[2–4] The care of patients with cleft palate remains a cause for concern, which will impose a substantial economic burden on society because of its increasing occurrence and costs of medical care.[5] It is therefore important to find out the aetiology of nsCLP so that we can reduce its prevalence.

Many genes contribute to the nsCLP phenotype in a exponential fashion. However, it is still a challenge to find out

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http://dx.doi.org/10.1016/j.bjoms.2014.12.008

which are the candidate genes for nsCLP. Previous studies of linkage and candidate genes have reported the association of nsCLP with many loci throughout the genome, and many new genes have been added to the list of susceptibility genes. However, only the genetic variation of the IRF6 gene has been identified as causative, and therefore has a major aetiological part in the development of nsCLP.

The genetic variation of IRF6 in Van der Woude syndrome has led to speculation that mutation of this gene may play a part in the development of nsCLP.[6] Expression of IRF6 in the developing palatal tissue, and abnormal craniofacial development in mice with a disrupted IRF6 gene, may suggest its possible involvement in the development of the condition.[7]

The Uyghur, the second largest minority ethnic group in China with a distinct living environment, religion, racial background, customs, and socioeconomic status, live mainly in Xinjiang Uyghur Autonomous region (western China). Their population is more than 10 million with an incidence of CLP that is greater than the national level. They are of mixed genetic origin, both white and East Asian Mongolian.[8] To date, most genetic investigation of nsCLP has been done among white populations, and increasing numbers of studies are being made among the Han Chinese. However few studies have been done to look at the candidate genes associated with nsCLP among the Uyghur people, and we know of no study that has been published in English. We have therefore done a case–control association study to find out if an IRF6 polymorphism influences susceptibility to nsCLP among the Xinjiang Uyghur people.

## Patients and methods

### *Study group*

The study was approved by the institutional review board of the Division of Medical Ethnics, the First Affiliated Hospital of Xinjiang Medical University, and includes 160 biologically-unrelated subjects of Xinjiang Uyghur origin, 100 who had nsCLP and 60 who had no clefts or family history of clefts and who acted as controls. They were recruited consecutively from January 2011 to December 2012 at the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China). To verify the nsCLP and to exclude known teratogenic influences, all the subjects were examined by two oral and maxillofacial surgeons for their individual phenotypic features, and their medical history was ascertained about the familial presence of other somatic and neurological disorders and the use of drugs known to cause oral clefts (phenytoin, warfarin, and ethanol). Parental informed consent was given for each study participant for both the collection of blood and subsequent genotyping.

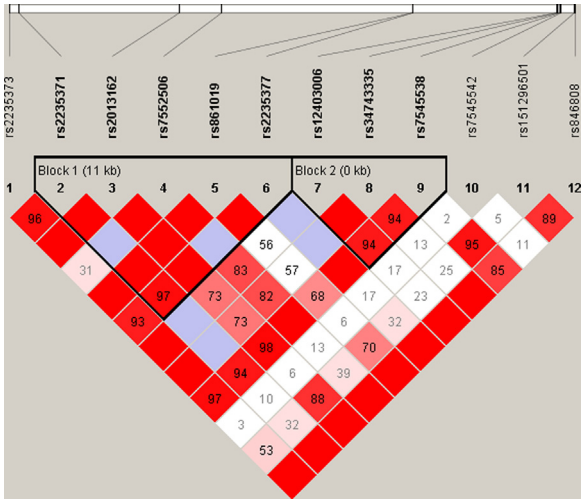


Fig. 1. The linkage disequilibrium plot consisting of 12 SNP within the gene IRF6 was generated using the genotype data from our population sample.

### *DNA extraction, library preparation, and sequencing*

Genomic DNA was isolated from 2 ml of peripheral blood taken from the subjects by following the manufacturer's standard procedure using the QIAquick® Gel Extraction kit (Qiagen, Hilden, Germany). DNA purity was then tested by calculating the ratio of absorbance at 260:280 nm using Invitrogen Qubit® Spectrophotometer. The amplification reactions were measured on a Applied Biosystems® 2720 Thermal Cycler (Life Technologies Corporation, USA) under the following conditions: the cycling programme was 95 °C for 2 min; 11 cycles of 94 °C for 20 s, 63–0.5 °C per cycle for 40 s, 72 °C for 1 min; 24 cycles of 94 °C for 20 s, 65 °C for 30 s, 72 °C for 1 min; and 72 °C for 2 min. The polymerase chain reaction (PCR) productions of the 160 samples were then ligated with DNA-adapter and specific index fragments. Consequently, a pooled library was generated by mixing all libraries equally for high-throughput sequencing. It was done on an MiSeq™ Benchtop Sequencer (Illumina Inc., San Diego, CA) in one single lane following the manufacturer's standard cluster generation and sequencing protocols. Sequence reads were subjected to demultiplexing using the CASAVA 1.8 software (Illumina, San Diego, CA) followed by quality checking using the FastQC algorithm.[9] Variants were identified using Genome Analysis Toolkit (GATK)[10] and VarScan software.[11] Coverage analysis was assessed using the Picard software Calculate HsMetrics tool. SNP and insertion/deletion (indels) analysis was done using different filtering steps. Annovar,[12] a software tool that accesses and uses information from external databases to assess implications and consequences of a given alteration in a sequence, was used to annotate the resulting list of variants.

### *Statistical analysis*

The Hardy–Weinberg equilibrium (HWE) was evaluated in both affected subjects and controls using the chi square