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Interferon regulatory factor 6 variants affect nasolabial morphology in East Asian populations

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ABSTRACT

Objective: The interferon regulatory factor 6 gene (*IRF6*) is one of the most conspicuous genes among a large number of candidate risk genes for non-syndromic cleft lip with or without cleft palate, which is considered to be a multifactorial defect. Variants of *IRF6* are also suggested to affect normal craniofacial variations, especially in the area of the nose and the upper lip. In the present study, we used lateral cephalograms to establish the relationship between *IRF6* and sagittal nasolabial morphology in healthy East Asian subjects.

Design: Genomic DNA was extracted from 215 Japanese and 226 Korean individuals, and genotyped for five *IRF6* single nucleotide polymorphisms (SNPs): rs17389541, rs642961, rs2013162, rs2235371, and rs7802. These SNPs were tested by multiple regression analyses for their association with craniofacial measurements obtained from lateral cephalometrics.

Results: We detected a significant association between the derived variants, rs2013162 and rs2235371 and the distances between a facial bone plane indicated by distance from Nasion and Point A (NA plane) to soft tissue landmarks; the Subalare (NA-Sbal) and the Subnasale (NA-Sn) in the sagittal plane.

Conclusion: Our results indicate that *IRF6* variants play an important role in the normal range of variation in nasolabial soft-tissue morphology.

1. Introduction

Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common congenital malformations that affect the craniofacial region. It is a common birth defect that is caused by genetic factors alone or by gene alterations in combination with environmental factors (Mossey, Little, Munger, Dixon, & Shaw, 2009). It affects approximately 1/700 live births, but its prevalence varies widely across different geographic locations according to ethnicity. The highest prevalence of NSCL/P has been reported in Asian and Amerindian populations, as high as 1/500, whereas European populations have intermediate prevalence rates of approximately 1/1000, while African populations have the lowest prevalence of approximately 1/2500 (Fraser, 1970). These observations indicate the importance of heritability in differential susceptibility to NSCL/P in different ethnic populations (Beaty et al., 2010). Moreover, various studies have compared the craniofacial shape and form of the cleft individuals with those of their unaffected relatives (Lu et al., 2009). A range of NSCL/P hard tissue and soft tissue microforms (a minimal manifestation or subclinical sign that is detected in non-cleft subjects, indicating a greater tendency of clefting in their offspring) have been reported in non-cleft parents (Mossey, Batra, & McIntyre, 2010). Indeed, microforms (phenotypic result from expression of the orofacial cleft genes) produce an underlying disturbance in hard and soft tissues constituting the craniofacial complex. The identification of microforms of orofacial clefting should be directed primarily at searching for characteristic phenotypic

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features that are distinctively identifiable in individuals with different types of orofacial cleft and their families. This might assist in the efforts to identify the individual cleft type etiopathogenesis, in particular, the genetic contribution to orofacial clefts.

Patients with NSCL/P have a unique soft tissue microform especially in the area of the nose and the upper lip (Yang, Chen, & Zhang, 2016; Laspos, Kyrkanides, Tallents, Moss, & Subtelny, 1997; Kyrkanides, Bellohusen, & Subtelny, 1996). Previous studies have shown that nasal asymmetry in unilateral cleft lip and palate (UCLP) individuals differ from that of non-cleft subjects (Yang et al., 2016). Previous anthropometric and anthroposcopic studies have revealed that cleft subjects have a nostril asymmetry, nasal bridge deviation, abnormally wide nose and unilaterally flat nasal tip, and wide alar base as compared to non-cleft subjects (Farkas, Posnick, Hreczko, & Pron, 1992).

It is crucial to study the link between pathological and normal variation, because many syndromes and deformities show a clear association between genetic alteration and facial variation, implying that genes involved in affected individuals might also contribute to normal facial variation (Boehringer et al., 2011). Many studies have been conducted to identify the genetic factors predisposing individuals to NSCL/P (Birnbaum et al., 2009; Mangold et al., 2009; Wu et al., 2010). Among several candidate risk genes, the interferon regulatory factor 6 (*IRF6*) is one of the most consistently identified genes in different studies (Blanton et al., 2005; Brito et al., 2012; Wu et al., 2010; Yang et al., 2016; Zucchero et al., 2004; Thomason et al., 2010).

Only few studies have highlighted the relation between the *IRF6* variants and variations in normal facial shape. For example, evaluating the soft tissues using a high-density three-dimensional (3D) image registration method demonstrated an association between an *IRF6* variant (rs642961) and the nasolabial morphology in Chinese subjects (Peng et al., 2013). An association between retrusive foreheads and *IRF6* variant (rs2235371) was also reported in healthy US citizens (Miller et al., 2014). Moreover, the geographical distribution of certain *IRF6* SNPs exhibits interregional differences in frequencies, which is consistent with the prevalence of NSCL/P in different geographical regions (Fig. 1). However, there is no study in the literature that specifically examined the relation between the normal variants of *IRF6* and the soft tissue microform in the area of the upper lip and nose, although this is one of the most affected areas in NSCL/P patients. In the present study, we used lateral cephalograms to verify the relationship between *IRF6* variants and the sagittal nasolabial morphology in healthy East Asian subjects.

2. Materials and methods

As described in previous reports (Kimura et al., 2015), we obtained lateral cephalometric images from 215 Japanese and 226 Korean adult subjects (≥18 years of age). Subjects with congenital disorders, such as cleft lip and palate, or with general physical diseases were excluded from the study. All of the subjects provided informed consent for their participation in this study, which was conducted with the approval of the ethics committees of Showa University, Japan, Pusan National University, South Korea, and University of the Ryukyus, Japan.

2.1. Craniofacial measurements

Lateral cephalograms of the skulls of the Japanese and the Korean subjects were obtained from Showa Dental Hospital, Japan and School of Dentistry of Pusan National University, South Korea respectively. Cephalograms were taken in natural head position. Head was fixed by fitting the ear rods of the cephalostat in the external auditory meatus. Teeth were held in centric occlusion and the lips were in the rest position. All radiographs were taken by trained technicians. The radiographic magnification ratio was 1.1. We measured the following distances: distance from the Nasion-Point A (NA) plane to the Subalare (Sbal), the Subnasale (Sn), and the Labrale superius (Ls) landmarks (Sbal-NA, Sn-NA, and Ls-NA); and distance from the Point A-Pogonion (AP) plane to the Labrale inferius (Li), and the Pogonion′ (Pgn′) landmarks (Li-AP and Pgn′-AP) (Fig. 2). Furthermore, the geometric mean of these measurements was calculated to accurately evaluate the craniofacial size of the individuals and to rule out allometric effects of the measurements (Coleman, 2008; Hennessy & Stringer, 2002). All of the values were transformed into z-scores to have a standard normal distribution, and outlier values with $|z| > 3$ were removed from the data.

2.2. Genotyping and sequencing

Saliva was taken from all subjects as a source of DNA. The Oragene DNA kit (DNA Genotek; Kanata, Ontario, Canada) was used for saliva collection, storage, and DNA purification according to the manufacturer's recommendations. Five single nucleotide polymorphisms (SNPs; rs17389541, rs642961, rs2013162, rs2235371, and rs7802) in the *IRF6* region were genotyped either using the TaqMan genotyping assay (Life Technologies, Carlsbad, CA, USA) or the DigiTag2 assay (Nishida, Tanabe, Takasu, Suyama, & Tokunaga, 2007). The frequency of the derived allele is shown in Table 1. Estimation of haplotypes was carried out using PHASE (Stephens, Smith, & Donnelly, 2001).

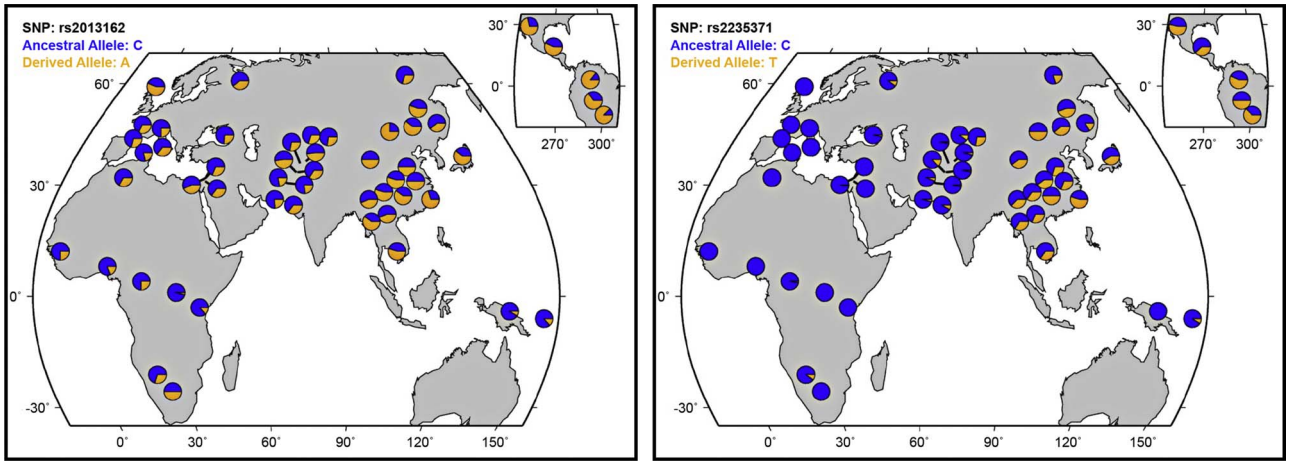

Fig. 1. Worldwide distribution of IRF6 variants in the coding region. The blue portion in the pie charts indicates ancestral alleles, C, whereas the orange portion in the pie charts indicates derived alleles, A in different geographical locations. The pie charts of the worldwide allele frequencies were downloaded from http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP/. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)