

A type of familial cleft of the soft palate maps to 2p24.2–p24.1 or 2p21–p12

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**Running Title:** Mapping of familial cleft of the soft palate

### **Abstract**

Cleft of the soft palate (CSP) and the hard palate (CHP) are subtypes of cleft palate. Patients with either condition often have difficulty with speech and swallowing. Nonsyndromic, cleft palate isolated has been reported to be associated with several genes, but to our knowledge there have been no detailed genetic investigations of CSP. We performed a genome-wide linkage analysis using an SNP-based microarray platform and successively using microsatellite markers in a family where six members, across three successive generations, had CSP. A maximum LOD score of 2.408 was obtained at 2p24.2–24.1 and 2p21–p12, assuming autosomal dominant inheritance. Our results suggest that either of these regions is responsible for this type of CSP.

**Keywords:** cleft of the soft palate/genome-wide linkage analysis/submucous cleft palate

## Introduction

Orofacial cleft, one of the most common congenital malformations, is a heterogeneous group of complex traits. Orofacial cleft is classified into two main categories, cleft lip with or without cleft palate (CLP) and cleft palate isolated (CPI). Both clefting phenotypes can appear relating to some syndromes (syndromic orofacial cleft) or not relating to syndromes (nonsyndromic orofacial cleft). CPI is considered genetically distinct from CLP, based on epidemiological evidence and the different developmental timing of lip and palate formation. Recent molecular genetic studies<sup>1-4</sup> have identified genes or loci that are responsible for CPI. However, fewer genes and/or loci associated CPI have been reported in comparison with CLP.<sup>5</sup>

CPI is classified into mostly two subtypes morphologically: cleft of the hard palate (CHP) and cleft of the soft palate (CSP).<sup>6</sup> Submucous cleft palate (SMCP) is a small subgroup in the CPI. SMCP manifests with bifid uvula, separation of the muscle with an intact mucosa and a bony defect in the posterior edge of the hard palate.<sup>7</sup> Both CHP and CSP are caused by a failure of fusion of the palatal shelves, but little is known what causes the difference in their phenotypes. Christensen et al. suggested that CHP and CSP might be etiologically distinct.<sup>9</sup> Although the patients with CSP have serious problems in speech and deglutition as well as CHP, there have been no detailed genetic

studies performed.

We recently encountered a Japanese family that included five CSP patients and one SMCP patient. The aim of this work was to identify the CSP/SMCP predisposing locus in this family using genome-wide single nucleotide polymorphism (SNP)-based linkage analysis.

## **Materials and Methods**

### **Family and patients**

A Japanese family included five patients (I-2, II-2, II-3, III-1 and III-2) with CSP and one patient (II-5) with SMCP across three generations (Figure 1). Two patients (II-2 and II-3) were monozygotic twins. The phenotypes of two patients (III-1 and II-5) were shown in Figure 2. All the patients had no other symptoms such as mental retardation, and all family members were examined by one or two well-trained dentists.

The disease in the family was consistent with an autosomal dominant mode of inheritance. Blood samples were obtained with written informed consent from 15 co-operative family members (Figure 1). The study protocol was approved by the Committee for Ethical Issues on the Human Genome and Gene Analysis of Nagasaki

University.

### **SNP genotyping and linkage analysis**

Genomic DNA was extracted from peripheral blood lymphocytes of the 15 members, using a QIAamp<sup>TM</sup> DNA Mini Kit (QIAGEN, Hilden, Germany). Their genotypes were determined using a GeneChip<sup>TM</sup> Human Mapping 10K 2.0 Xba Array (Affymetrix, Santa Clara, CA). We used MERLIN software<sup>8</sup> to analyze compiled pedigree data sets. Mendelian errors were detected by PEDCHECK,<sup>9</sup> and SNPs with Mendelian error were not used in the data analysis. LOD scores were calculated under a parametric autosomal dominant model in which penetrance was set to 1.0 and disease allele frequency was 0.00001. Since CSP and SMCP can be categorized together because of their similar anatomical features,<sup>10</sup> the patient with SMCP (II-5) was classed as an “affected” as well as the patients with CSP for linkage score calculations.

To confirm the result of the linkage data using GeneChip<sup>TM</sup> Human Mapping 10K 2.0 Xba Array, we performed two point linkage analysis using microsatellite markers by the method described elsewhere.<sup>11</sup> Two point LOD score was calculated using MLINK program.<sup>12</sup>

## Results and Discussion

In the assay with the 10K-Array, the GeneChip<sup>TM</sup> call rates varied from 92.18 % to 99.42 % (with a mean of 97.54 %). Two regions, 2p24.2–p24.1 (CSP region 1: C SPR1) a 4.5 Mb interval between rs1545497 and rs1872325, and 2p21–p12 (CSP region 2: C SPR2) a 34.5 Mb segment between rs940053 and rs310777, were CSP candidate loci with a maximum LOD score of 2.408 (Figure 3). The LOD scores of all other regions were below 1.000. Two point LOD scores using microsatellite markers showed same scores (2.408), therefore the result of linkage analysis from SNP genotyping was reconfirmed (haplotype using microsatellite markers was shown in Figure 1). It is thus likely that a gene playing a role in palatal fusion is located within either C SPR1 or C SPR2.

Based on our knowledge of oral palate development, we chose nine genes from the candidate CSP regions and performed mutation analysis. Of the nine candidate genes, three were from C SPR1: growth/differentiation factor 7 (*GDF7*), matrilin 3 (*MATN3*) and member B of the Ras homolog gene family (*RHOB*). The other six genes were from C SPR2: calmodulin 2 (*CALM2*), bone morphologic protein 10 (*BMP10*), sprouty-related EVH1 domain-containing protein 2 (*SPRED2*), transforming growth factor, alfa (*TGFA*), ventral anterior homeobox 2 (*VAX2*; 2p13.3) and stoned B-like

factor / stonin 1 (*STONI*). Most of these genes are concerned with bone development, the TGF and mitogen-activated protein (MAP) kinase signaling pathways, or are transcription factors related to homeobox genes. However, no pathogenic mutation was found within any of its exons or intron/exon boundaries of all nine genes.

To detect structural genomic alterations that may cause CSP within the candidate regions, we performed copy number analysis with the proband's DNA using the Genome-Wide Human SNP Array 5.0 (Affymetrix). Although several copy-number alterations were detected (data was not shown), all were already registered as copy number variations on the UCSC Genome Browser (<http://genome.ucsc.edu/>) and none of them were coincided with regions with positive LOD scores.

In conclusion, this is the first report of a whole-genome linkage analysis scan for CSP. Although the LOD scores calculated are not high enough to assign the disease locus definitively, our data suggest that it lies at either 2p24.2–24.1 or 2p21–p12.

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**Titles and legends to figures**

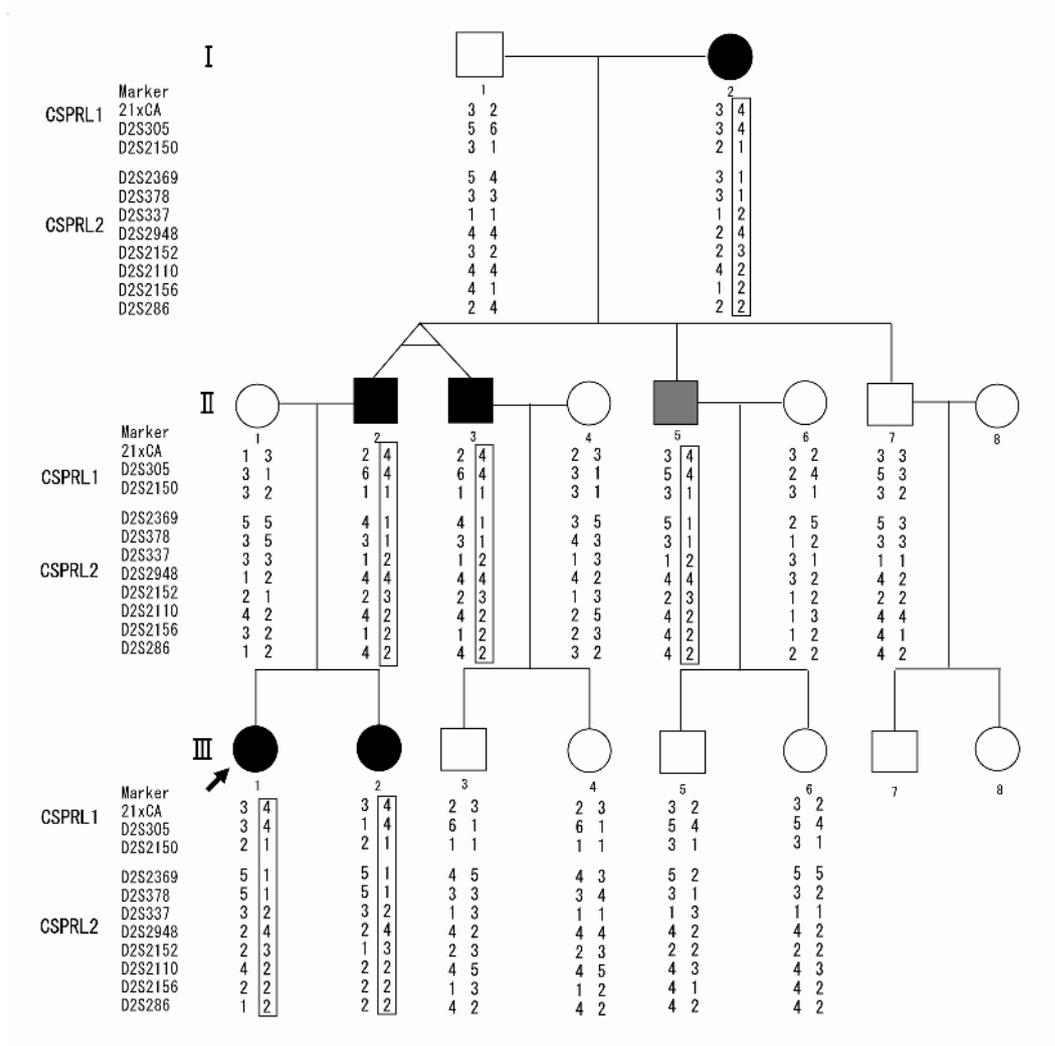


Figure 1. Family tree with haplotypes at 2p24.2–24.1 (CSPR1) and 2p21–p12 (CSPR2).

Black closed, gray closed and open symbols indicate affected with cleft of the soft palate (CSP), affected with subcutaneous cleft palate (SMCP) and unaffected, respectively. An arrow indicates the proband. Genotypes of microsatellite markers defining the candidate intervals are shown below each individual. Boxed haplotype indicates possibly disease-associated haplotype.

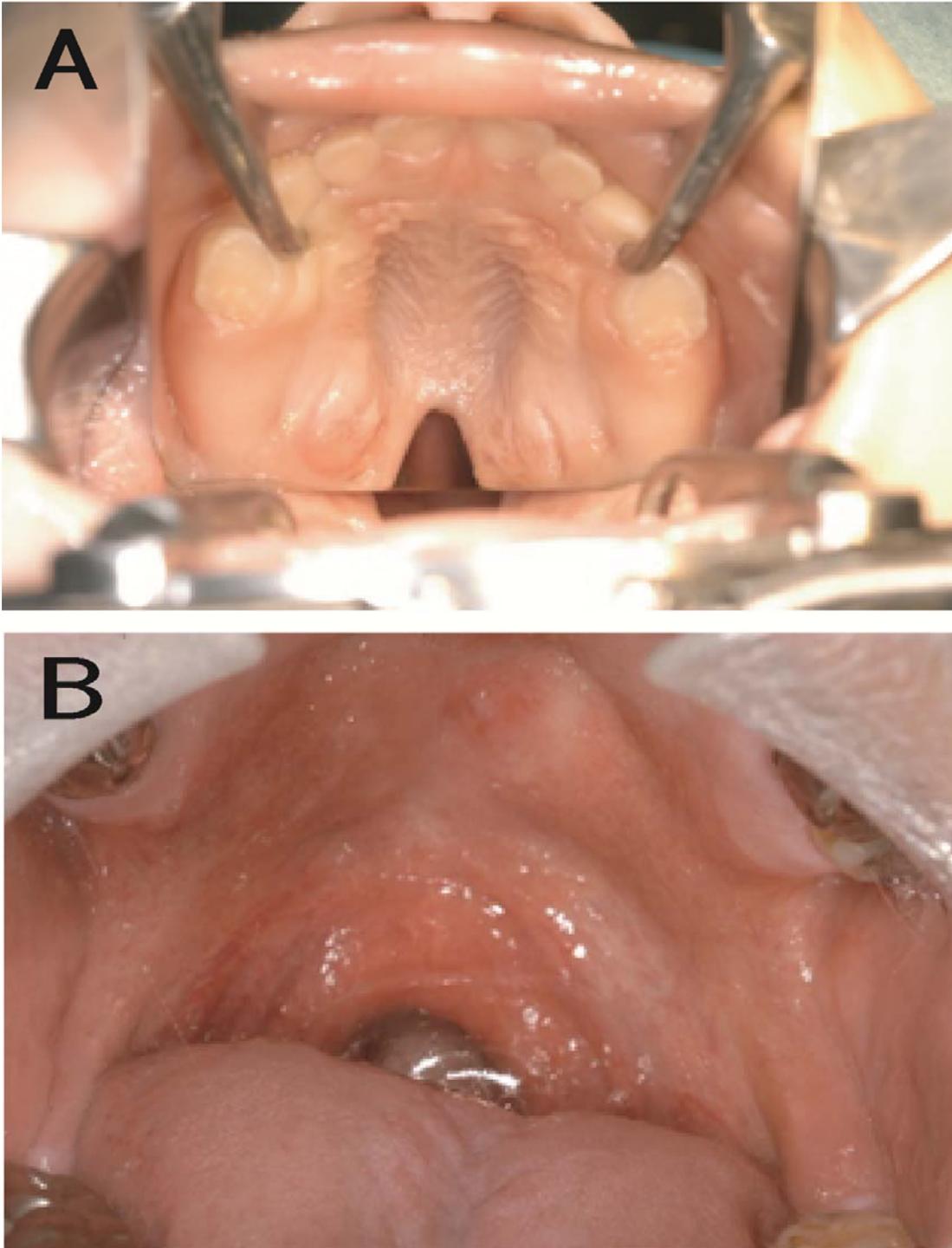


Figure 2. Views of palates. The palate of individual III-1 with CSP (A) showing a cleft limited to the soft palate, and that of individual II-5 with SMCP (B) showing a translucent zone in the soft palate resulting from a separation of the muscle.

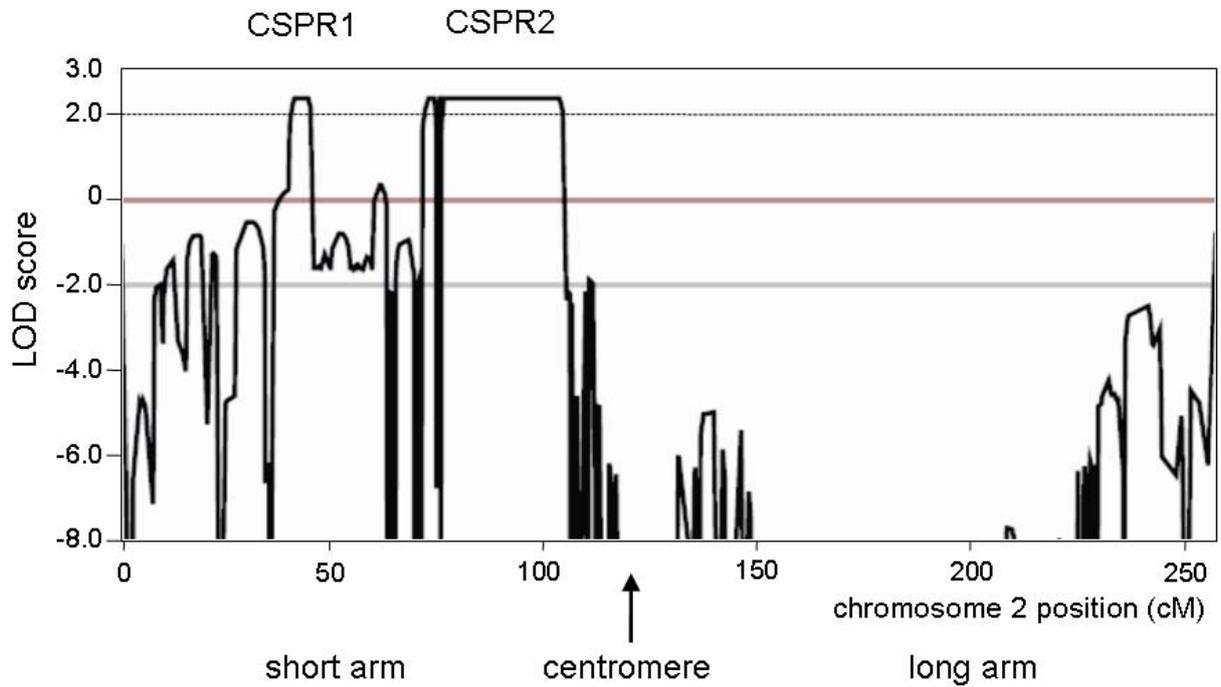


Figure 3. Multipoint LOD scores on chromosome 2. A 4.5 Mb (physical position, 18281893–22775527) interval from rs1545497 to rs1872325 corresponds to CSR1, and a 34.5 Mb interval (45834656–80355227) from rs940053 to rs310777 corresponds to CSR2.