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ARTICLE

A homozygous 237-kb deletion at 1p31 identified as the locus for midline cleft of the upper and lower lip in a consanguineous family

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Orofacial clefts are congenital defects that vary widely in type and severity, and can occur in isolation or in association with a variety of other defects. Herein, we describe a consanguineous family afflicted with a unique form of orofacial clefting manifesting as a facial midline defect that also involves mandibular and maxillary structures. All four affected sibs had median clefts of the upper and lower lips, tooth misalignment, and poor oral hygiene. Linkage analysis of 17 family members identified a 15.3-Mb pair recessive locus at 1p31 with a LOD score of 3.63. To the best of our knowledge, this is, to date, the first locus reported for facial midline clefting and the first recessive locus for an isolated orofacial defect. The locus harboured a novel intergenic deletion of 273 164 bp, for which all fully affected sibs were homozygous. We did not note any potentially pathogenic gene variant at the 1p31 locus via exome-sequencing analysis. The identified deletion could be harbouring a regulatory element for the gene associated with the orofacial defect. The best candidate for the putative target gene is *LHX8,* located 49 149 bp upstream of the deletion. The gene is known to be associated with facial development in several animals. Four other family members had a subclinical phenotype – a simple notch in the lower lip or an increase in the interdental distance between the lower incisors – indicative of very low-level expression of the trait.
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INTRODUCTION

Orofacial clefts are common congenital defects with a frequency of approximately 1 in 500–700 births worldwide.[1] They occur most commonly as isolated traits, but can also be part of a syndrome. The most common forms are lateral clefts, and they vary greatly in severity – from only a small notch in the vermilion to a complete cleft that in some cases extends into the palate, or the cleft can involve only the palate.[2,3] Isolated lateral clefts encompass the non-syndromic cleft lip – with or without cleft palate (CL/P) – and non-syndromic cleft palate only. Commonly, an isolated cleft lip (CL) is limited to the upper lip on either side of the philtrum (midline groove). Upper lip clefts can be unilateral or bilateral. A rare CL variant is a median cleft—a cleft in the groove. Median cleft has an incidence of <1 in a million births.[2] To the best of our knowledge, no familial isolated median clefts of both the upper lip and lower lip have been reported.

Herein, we present a family with a unique form of isolated orofacial defect – median clefts of both the upper and lower lips accompanied by dental findings. Several inheritance models were employed to search for loci contributing to the trait with variable expression. A recessive locus harbouring several genes and a large novel, intergenic deletion was identified for the full manifestation of the trait. Exome sequencing did not show any gene mutation that could potentially contribute to this very rare phenotype.

MATERIALS AND METHODS

Participants

In all, 17 members of a consanguineous family were investigated (Figure 1a). Informed consent was obtained from/for all the participants. The Boğaziçi University Institutional Review Board for Research with Human Participants approved the study protocol.

Clinical investigations

Clinical investigations included physical examination, cranial CT and abdominal ultrasonography.

Genotyping and statistical analysis

DNA was isolated from peripheral blood samples following a routine protocol. SNP genome scanning was performed in the 17 family members using an Illumina Human 370-Quad BeadChip that contained ~370 000 SNPs. A DNA sample from affected sib 405 underwent denser genome scanning using an Illumina 610-K BeadChip. Microsatellite markers D1S2137 and D1S1585 were used for further genotyping at the gene locus. UCSC Genome Browser (build GRCh37) was utilized to determine the physical positions of markers and genes.

SNP genotyping data were used for linkage analysis via easyLINKAGE v.5.08, assuming a trait allele frequency of 0.0001. GeneHunter v2.1r5 was used to calculate multipoint parametric LOD scores throughout the genome, as well as to construct haplotypes.[4]

Parental consanguinity prompted us to assume a recessive inheritance model. The capacity of the linkage programme used was not sufficient for

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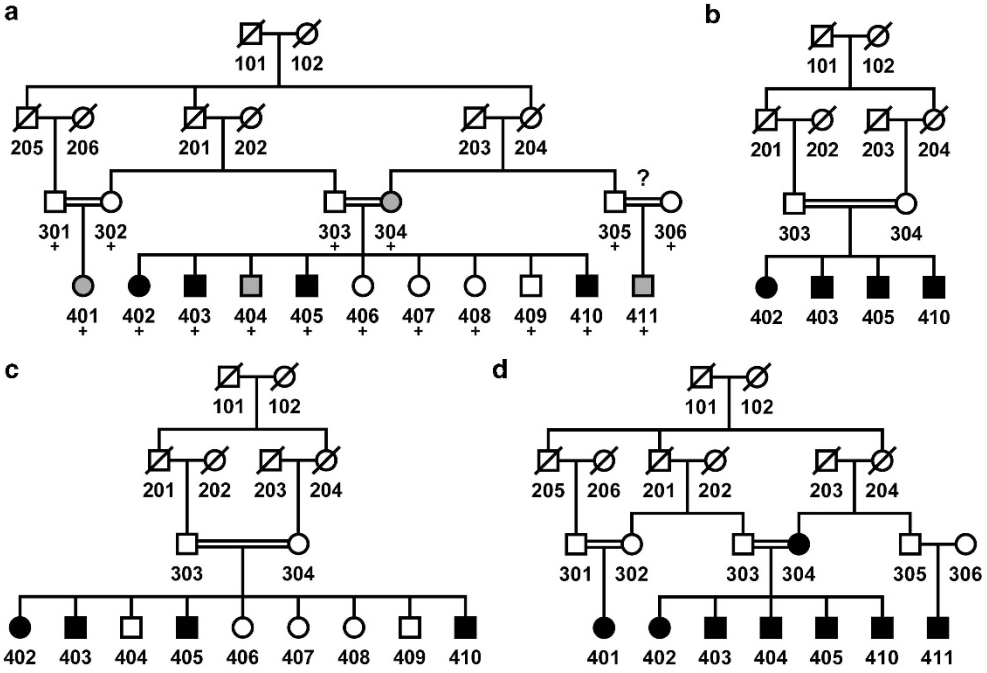

Figure 1 Pedigree drawings. (**a**) Partial pedigree (pedigree A) of the presented family. Black indicates the midline defect phenotype and grey indicates the subclinical phenotype. Individuals included in the study are indicated by +. (**b**–**d**) Pedigrees B, C and D used to calculate LOD scores.

calculating multipoint LOD scores when all the participants were included; therefore, we first calculated LOD scores for a pedigree that included only the four fully affected sibs and their parents (Figure 1b). We assumed full penetrance and selected markers at 0.1-cM intervals, using marker sets of 30. Subsequently, we performed more detailed calculations with SimWalk v.2.9 at the locus that yielded the highest LOD scores using markers selected at 0.0001 cM and in sets of 10 and pedigree C, which included all sibs in the core family (Figure 1c). The analyses were repeated assuming 70% penetrance. Additionally, a macro created in Microsoft Excel was used to detect and compare homozygous genotypes throughout the genome in all 17 family members.

Assuming a dominant model with 70% penetrance, we searched for a haplotype that might possibly harbour a second gene defect carried by the affected sibs and the subclinical members of the kindred (Pedigree D, Figure 1d). Then, more detailed calculations were performed at those loci using all of the markers.

Deletion analysis

In order to delineate the novel deletion identified at 1p31.1, we utilized seven pairs of primers designed to amplify arbitrarily selected small regions on either side of the minimal deletion region to narrow down the maximal deletion region. The sequences of the primers that were appropriate for amplification across the deletion breakpoint junction were 5′-TCTCTCCCACCCTTTT ACCA-3′ and 5′-CCTGACATATTGCTGCTGTTG-3′. The 1284-bp product was subjected to Sanger sequencing. The family members and a control panel of 121 unrelated individuals were screened for the deletion using forward primer F (5′-GCAGTGCTAATATGCAAGGAA-3′), together with reverse primers R1 (5′-TTGAACCTTCTCACTCCATCC-3′; specific to the deleted sequence) and R2 (5′-TTGTTGATCCAAACCACAGG-3′; specific to the normal sequence) in a diplex PCR reaction to amplify a 491-bp fragment specific to the deletion allele and a 292-bp fragment specific to the normal allele.

Exome-sequencing analysis

The DNA sample obtained from affected sib 403 underwent exome-sequencing analysis using a TrueSeq DNA Sample Preparation Kit and TrueSeq Exome Enrichment Kit, covering 95% of the CCDS database. The enriched library was sequenced using an Illumina HiSeq2000 platform. Using the raw sequencing data, we aligned the reads to the reference genome (hg19) using BWA-0.5.9 software, and detected SNPs and indels using SAMTOOLS-0.1.14 and GATK. We annotated the variants for novelty after comparing them with the dbSNP (build 131) and 1000 Genomes databases using ANNOVAR software, as well as with the 22 other samples with phenotypes unrelated to cleftings that are similarly processed in our laboratory.

RESULTS

Clinical findings

The four fully affected sibs shared the same phenotypic features (Figure 2), and were aged 20 years (402 in Figure 1a), 18 years (403), 16 years (405) and 2 years (410). Four other relatives (304, 404, 401 and 411) bore some components of the orofacial defect in a less evident fashion, which could be considered subclinical. Physical examination was otherwise normal in all the study participants.

The clinical features common to all four affected sibs were as follows: incomplete median cleft of the upper lip that led to disruption of the integrity of the orbicularis oris muscle in the Cupid's bow region and absence of the philtral complex, incomplete median lower lip cleft that was limited to the vermilion (without significant muscle involvement), a double labial fraenulum between the lower lip and lower gingiva across the vestibular fold, and fusion of the upper labial mucosa and the upper gingiva, which resulted in a shallow upper vestibular fold. The dental findings were also similar: poor dental alignment, poor oral hygiene, and increased interdental distance (diastema) between the upper median incisors and, to a greater extent, between the lower median incisors. Palates were normal. Complete cranial CT did not show any other cranial or maxillofacial anomalies. No other congenital abnormalities were evident in any family member. Mental status was normal in all the study participants.

In individual 402 – the eldest of the affected sibs – cleft of the lower lip and increased interdental distance between the upper median incisors were not apparent, as lip surgery performed at another hospital and dentures partially obscured the phenotype, which was confirmed following anamnesis. In sib 410, the interdental distance between the upper median incisors was increased only slightly and that between the lower incisors was normal, most likely because permanent dentition had not yet occurred (Figure 2).

Phenotypic features in the subclinical family members were as follows: Mother 304, sib 404 and cousin 401 all had a slight sagittal depressed line in the mid-lower lip, but sib 404 had an additional 2-mm indentation of the skin into the vermilion across the vermiliocutaneous border and an approximately 1-mm-long naevoid lesion on the indented skin, and cousin 411 had only increased interdental distance between the lower median incisors (Figure 2).

Linkage analyses

We performed linkage analysis assuming various inheritance models. Recessive inheritance seemed possible, as the four fully affected subjects were siblings and the parents were first cousins. Supplementary Figure 1A shows the positive multipoint LOD scores based on a recessive model with full penetrance, using only the family members shown in pedigree B (Figure 1b). Locus 1p31 yielded a LOD score of 3.01, whereas all other loci yielded LOD scores <1.3 (Supplementary Figure 1A). Haplotype analysis at 1p31 confirmed shared homozygosity in the affected sibs, which was likely identical by descent. Including all sibs (Pedigree C) and all SNP markers at the locus, a maximum LOD score of 3.63 was obtained (Supplementary Figure 1B). The maximal homozygosity region was between rs1864569 (68 634 257 Mb and 97.34 cM) and rs11163752 (83 934 440 Mb and 109.86 cM), approximately 15.3 Mb and 12.5 cM. The results of genotyping with two microsatellite markers at the locus confirmed that the shared homozygosity was compatible with identity by descent from a recent ancestor (Table 1). Haplotype analysis showed that the two subclinical cousins did not carry the trait haplotype, indicating that the haplotype was not associated with the subclinical phenotype. To make sure that a recessive locus with incomplete penetrance was not overlooked, we performed a similar linkage analysis assuming 70% penetrance, but other than 1p31 (found by assuming full penetrance) did not find any other locus >1 cM.

We also searched for a locus that might be associated with both the subclinical and the full clefting phenotypes. Assuming recessive inheritance with incomplete penetrance, we did not find any locus at which all affected sibs and the subclinical family members shared a homozygous genotype. In a dominant inheritance model with incomplete penetrance, the maximum LOD score (obtained at two loci) was 2.3, which was not significant. All parents were informative at the loci. The shared haplotypes were small, 719 kb between markers rs3795550 and rs28570494 at 1p32.2 and 509 kb between rs7767627 and rs626848 at 6q27. Both haplotypes were passed on to subclinical 411 from her mother 306, who denied a known blood relation to the kindred; this indicated that the haplotypes did not descend from a recent common ancestor. Besides the affected and subclinical subjects, several unaffected subjects carried the haplotypes (302, 305, 406 and 409 at 1p32.2, and 302, 305, 406, 408 and 409 at 6q27), further indicating that the loci are likely not related to the trait.

Deletion analysis

We analysed SNP scanning data to determine whether or not the four affected sibs shared any homozygous deletions. In all, two such deletions were identified, both at 1p31 within the gene locus and in intergenic regions, of which one was flanked by rs17095902 (75 240 211 Mb) and rs6703099 (75 534 319 Mb), and the other by rs9424981 (72 766 085 Mb) and rs2815752 (72 812 440 Mb); thus, the maximum possible sizes were 294 107 and 46 353 bp, respectively. The latter was reported as a polymorphism in the Genomic Variants database (http://projects.tcag.ca/variation/), and the SNP genotype data showed that all family members carried it in the homozygous state; therefore, it was considered a neutral variant and not

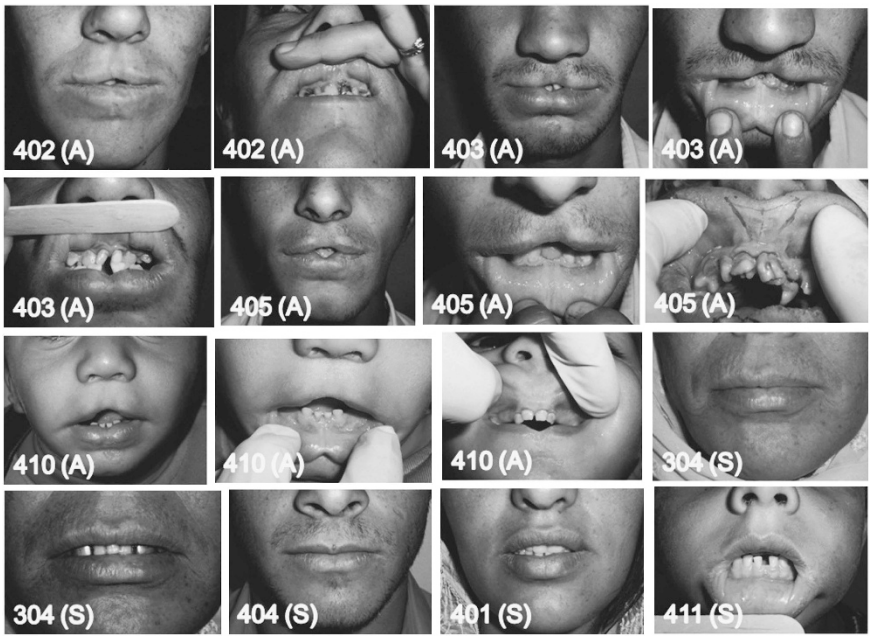

Figure 2 Photos of the affected (A) and subclinical (S) family members.

investigated further. The former deletion was novel; the breakpoint junction was identified, and it was determined that the deletion covered 273 164 nucleotides – or approximately 273 kb – spanning 75 247 714–75 520 877 bp (Supplementary Figure 2A). The deleted sequences did not encompass any known genes but harboured several small regions that were conserved across species (Supplementary Figure 2B).

Inheritance of the 273-kb deletion throughout the family was investigated and the results confirmed the haplotype data: all four affected sibs were homozygous for the deletion, whereas all other sibs and the father of subclinical cousin 411 carried one copy. The subclinical cousins did not carry the deletion haplotype. The haplotypes around the deletion are presented in Table 1. None of the 121 individuals from the general population we screened for the breakpoint junction carried the deletion.

Exome-sequencing findings

Based on exome-sequencing analysis in affected sib 403 at the recessive locus 1p31, only the variants with frequencies ≤ 0.01 were considered. The reason why we assumed such a cutoff value was that the predicted homozygous frequency for 0.01 allele frequency is 0.0001, which is the trait allele frequency we assumed. The variants are presented in Supplementary Table 1, together with predictions on protein structure and function. Both of the exonic variants were assessed as weak candidates for the pathogenic mutation underlying the trait in the family. All four novel 3′UTR variants were predicted not to affect poly-adenylation.

DISCUSSION

The patients presented herein have facial midline defects, a unique form of orofacial clefting. The four fully affected sibs had incomplete median cleft of both the upper and lower lips, accompanied by dental findings. To the best of our knowledge, to date, no familial isolated median clefts of both the upper and lower lips together have been reported. In contrast to the common lateral CL/P phenotype, which is characterized by discontinuity of the entire lip elements in the cleft site of the upper lip, the phenotype in the presented patients was characterized by a discontinuous upper vermilion and loss of orbicularis integrity in a midline segment of the upper lip, accompanied by a shallow upper vestibular fold, absent philtral complex, and coexisting lower lip median cleft. Another important feature of the presented phenotype is the absence of any other clinical entity involving the cranium, extremities or mental status, in contrast to other orofaciodigital syndromes.

Because the four affected subjects were siblings and the parents were first cousins, we first searched for loci with recessive effect and

Table 1 Haplotypes for selected SNP markers and two microsatellites around the 273-kb deletion at 1p31

Marker	Genetic position (Mb)	301		302		401		303		304		402		403		404		405		406		407		408		409		410		305		306		411	
		F	UA	M	UA	S	Sc	F	UA	M	Sc	S	A	S	A	S	Sc	S	A	S	UA	S	UA	S	UA	S	UA	S	A	F	UA	M	UA	S	Sc
rs1926289	68.47	2	2	1	2	2	2	1	2	1	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	1	2	1	1	1	2	1	1	1
rs7529360	68.61	2	2	1	2	2	2	1	2	2	1	2	2	2	2	2	1	2	2	2	1	2	1	2	1	1	1	2	2	2	2	1	1	2	1
rs3748705	68.62	2	1	2	1	1	1	2	1	2	1	1	2	1	2	1	1	1	2	1	1	1	1	1	1	2	2	1	2	2	1	2	2	1	2
rs4233320	68.64	1	2	2	2	2	2	2	**1**	**1**	2	**1**	**1**	**1**	**1**	**1**	2	**1**	**1**	**1**	2	**1**	2	**1**	2	2	**1**	**1**	**1**	**1**	2	1	1	2	1
rs2225019	68.84	1	2	1	1	1	1	1	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	1	**2**	**2**	**2**	**2**	1	1	1	1	1
D1S2137	68.88	2	3	2	2	2	2	2	**2**	**2**	2	**2**	**2**	**2**	**2**	**2**	2	**2**	**2**	**2**	2	**2**	2	**2**	2	2	**2**	**2**	**2**	**2**	3	3	1	2	1
rs868402	69.89	2	2	2	2	2	2	2	**1**	**1**	2	**1**	**1**	**1**	**1**	**1**	2	**1**	**1**	**1**	2	**1**	2	**1**	2	2	**1**	**1**	**1**	**1**	*2*	2	2	2	2
rs2820549	71.16	2	1	2	1	2	2	2	**1**	**1**	2	**1**	**1**	**1**	**1**	**1**	2	**1**	**1**	**1**	2	**1**	2	**1**	2	2	**1**	**1**	**1**	**1**	*1*	1	1	1	1
rs12405821	71.88	1	2	1	2	1	1	2	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	2	**2**	**2**	**2**	**2**	*2*	2	2	2	2
rs12123766	72.86	2	1	2	2	2	2	2	**1**	**1**	2	**1**	**1**	**1**	**1**	**1**	2	**1**	**1**	**1**	2	**1**	2	**1**	2	2	**1**	**1**	**1**	**1**	*2*	1	2	2	2
rs11210072	73.25	1	2	1	1	1	1	1	**2**	**2**	2	**2**	**2**	**2**	**2**	**2**	*2*	**2**	**2**	**2**	*2*	**2**	*2*	**2**	*2*	1	**2**	**2**	**2**	**2**	*1*	1	2	2	2
rs1340434	74.37	2	2	2	1	2	2	1	**1**	**1**	2	**1**	**1**	**1**	**1**	**1**	2	**1**	**2**	**1**	2	**1**	2	**1**	2	*1*	**1**	**1**	**1**	**1**	*2*	2	1	2	1
rs11485264	75.20	1	1	1	1	1	1	1	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	1	**2**	**2**	**2**	**2**	*1*	1	1	1	1
rs1409785	75.25	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	2	2	2	2
rs1590639	75.26	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	2	2	2	2
rs277384	75.27	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	2	1	2	1
rs277366	75.30	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	1	1	2	1
rs1413994	75.37	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	1	1	2	1
rs12121720	75.39	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	1	1	2	1
rs3845358	75.45	1	1	2	1	1	1	1	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**	1	**0**	1	1	**0**	**0**	**0**	**0**	2	1	1	2	1
rs6703099	75.53	1	1	1	2	1	1	1	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	1	**2**	**2**	**2**	**2**	2	1	1	2	1
rs11163185	75.81	2	2	2	2	2	2	2	**1**	**1**	1	**1**	**1**	**1**	**1**	**1**	1	**1**	**1**	**1**	1	**1**	1	**1**	1	2	**1**	**1**	**1**	**1**	2	2	1	2	1
rs6696780	76.73	2	2	2	1	2	2	2	**1**	**1**	2	**1**	**1**	**1**	**1**	**1**	2	**1**	**1**	**1**	2	**1**	2	**1**	2	2	**1**	**1**	**1**	**1**	*1*	1	1	1	1
rs1933393	77.46	1	2	2	2	2	2	1	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	1	**2**	**2**	**2**	**2**	*2*	2	1	2	2
rs2352522	79.26	2	1	1	2	1	1	1	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	1	**2**	**2**	**2**	**2**	1	2	1	1	1
D1S1585	80.13	4	4	4	4	4	4	4	**5**	**5**	1	**5**	**5**	**5**	**5**	**5**	1	**5**	**5**	**5**	1	**5**	1	**5**	1	4	**5**	**5**	**5**	**5**	3	2	4	3	2
rs10493631	80.48	1	2	2	1	2	2	1	**2**	**2**	2	**2**	**2**	**2**	**2**	**2**	2	**2**	**2**	**2**	2	**2**	2	**2**	2	1	**2**	**2**	**2**	**2**	2	1	2	2	1
rs12409530	81.40	2	1	1	1	1	1	1	**2**	**2**	2	**2**	**2**	**2**	**2**	**2**	2	**2**	**2**	**2**	2	**2**	2	**2**	2	1	**2**	**2**	**2**	**2**	2	1	2	2	1
rs2148916	81.72	2	1	1	2	1	1	1	**2**	**2**	2	**2**	**2**	**2**	**2**	**2**	2	**2**	**2**	**2**	2	**2**	2	**2**	2	1	**2**	**2**	**2**	**2**	1	1	1	1	1
rs709749	82.80	1	2	1	2	1	1	2	**2**	**2**	1	**2**	**2**	**2**	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	1	2	**2**	**2**	**2**	**2**	1	2	1	1	2
rs11163752	83.93	1	2	1	1	1	1	1	**2**	**2**	1	**2**	**2**	**2**	1	**2**	1	**2**	**2**	**2**	1	**2**	2	**2**	1	1	**2**	**2**	**2**	**2**	2	2	1	2	2
rs11163981	84.95	2	1	2	1	2	2	2	**1**	**1**	2	**1**	**1**	**1**	2	**1**	2	**1**	**1**	**1**	2	**1**	1	**1**	2	2	**1**	**1**	**1**	**1**	2	2	2	2	2

Abbreviations: A, affected; F, father; M, mother; S, sib; Sc, subclinical; UA, unaffected.
Allele 0 denotes deletion. The trait haplotype is shown in grey.

identified a large locus. The identified locus harboured a 273-kb deletion, which was not found in the 121 control subjects, showing with >80% power that the deletion was not a normal sequence variant in our population.[5] Exome sequencing did not identify at the locus any mutations that could be associated with the phenotype. Therefore, it was hypothesized that the novel intergenic deletion contained the genetic defect underlying the trait. Alternatively, the mutation could be in a region such as an intron or an intergenic region, or of a kind that could not be detected by the exome-sequencing method employed, such as an exonic duplication, or even missed due to incomplete coverage of the analysis.

As the 273-kb deletion sequences do not contain any known coding sequences, they might contain gene-regulatory elements, such as enhancers and silencers. Such elements are difficult to recognize, as they do not have consensus sequences. A signature of gene-regulatory elements is conservation across species. Several short sequences within the deletion region are highly conserved across primates, and some are also conserved across other vertebrates (Supplementary Figure 2B). The large size of the deletion discouraged us from initiating a molecular study. Whether the deletion might contain an element that affects the expression of a gene involved in facial development was investigated via ENCODE.[6] The most noteworthy region is 75.2–75.4 Mb, possibly containing a variety of regulatory elements (Supplementary Figure 2B). An example of such a gene that could be regulated by a putative *cis* element within the deletion sequences is *LHX8*, also called *LHX7* (*LIM homeobox 8*, MIM: 604425), which is located 73 242 bp upstream of the deletion and belongs to the LIM homeobox gene family, members of which encode transcription regulators that are required for patterning during embryonic development.[7] In mice *Lhx8* is expressed in the mesenchyme of the palatal structures during development, and knockout mice exhibited cleft palate with other normal craniofacial structures.[8] The gene also has a role in tooth formation and development.[8,9] The orthologue of the gene was suggested to have a role in upper lip development in chicken embryoes.[10] Loss of function of lhx8 (together with msx2) in frogs resulted in a median cleft in the upper lip and primary palate; both genes were suggested to be regulated by retinoic acid signalling.[11] Thus, animal studies indicate that the gene has a role in orofacial development and is a very good candidate for the trait observed in the presented family. Additional research could help determine whether or not the 273-kb deletion sequences contain any elements that regulate *LHX8*. *LHX8* is expressed in only a few organs – the highest level of expression is in the skin and it is lower in the connective tissue, pancreas, brain and testis, as well as in the embryo (UniGene). Unfortunately, no tissue sample from the affected subjects was available to investigate whether *LHX8* expression was altered.

We employed several inheritance models in search for a locus that could possibly unravel the genetic basis of the subclinical phenotype. No recessive locus having low penetrance was found. Recessive mutations that are incompletely penetrant are known, albeit rarely; one example is in SHFM6, where a homozygous missense mutation in *WNT10B* was not penetrant in one of the 13 homozygous individuals.[12] Considering dominant inheritance, the obvious question is whether the haplotype at 1p31 could act in a semidominant fashion with low penetrance, but two of the subclinical subjects did not carry the haplotype. We searched for a putative second locus that could be associated with the subclinical phenotype by itself and with the clefting in concert with the recessive genetic defect at 1p31. Assuming a dominant model with 70% penetrance, we searched for a haplotype that might be harbouring a second gene defect carried by the affected sibs and the subclinical members of the kindred. The LOD scores did not reach significance, because the pedigree was not sufficiently large to be informative. The haplotypes could just be common haplotypes in the inbred community and not related to the trait considering the small sizes of the shared haplotypes at the two loci with the highest LOD score; the high frequency of the haplotypes in the kindred and inheritance in one subclinical cousin from a parent who at the best is distantly related to the kindred ruled out inheritance from a recent common ancestor. Small common haplotypes are frequent in inbred communities.[13]

In conclusion, the facial midline defect in the four sibs presented herein is an exciting, very rare phenotype, and 1p31 is an autosomal recessive locus, not yet described for facial development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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