

SHORT REPORT

Linkage disequilibrium analysis of two genes mapping on OFC3: *PVR* and *PVRL2*

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Clefts of the lip with or without cleft palate (CL/P) are one of the most common birth defects, occurring in 1/700–1/1000 infants born alive. The nature of the genetic contribution is still to be clarified; however, some chromosome regions and candidate genes have been proposed for this malformation. Recently, a couple of genes, *PVR* and *PVRL2*, mapping in the candidate region OFC3 on chromosome 19q13.31, have been investigated because of their homology to *PVRL1*, a gene previously shown to cause the Margarita Island CL/P-ectodermal dysplasia syndrome. In the present work, we investigated *PVR* and *PVRL2* genes by family-based linkage disequilibrium analysis using a sample collected from the Italian population. In contrast to previous analyses on other populations, we could not find any statistically significant association between the markers alleles and non-syndromic clefting.

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Introduction

Clefts of the lip and/or palate (CL/P) are a common craniofacial anomaly having a multifactorial aetiology with both genetic and environmental implications. The large number of candidate genes and loci that seem to be involved attest to the complexity of this malformation.¹ One example of a clefting gene is the poliovirus receptor related-1 (*PVRL1*), which encodes nectin-1, a cell-to-cell adhesion molecule. Its loss of function was shown to cause the CL/P-ectodermal dysplasia (CLPED1; MIM 225060), a rare autosomal recessive syndrome.²

Recently, our group investigated 146 CL/P probands and 100 unaffected controls,³ all from an Italian population,

but failed to detect the common *PVRL1* nonsense mutation W185X, which is seen as a genetic risk factor for non-syndromic CL/P in Northern Venezuela.⁴ In a further screening of the same sample, we found three rare sequence variants in *PVRL1* exon 3.⁵

Interestingly, two *PVRL1*-related genes, *PVR* and *PVRL2*, which alike encode nectin-related Ig-class cell–cell adhesion molecules that serve as viral receptors, both map in chromosome segment 19q13.31–q13.32, close to *BCL3*. Here is located OFC3, one of several non-syndromic CL/P susceptibility loci mapped in humans.^{6,7}

We previously analysed the OFC3 candidate region using a sample of 40 multiplex pedigrees and microsatellite markers, and obtained evidence of linkage and linkage disequilibrium with APM and TDT analyses.⁸

Recently, Warrington *et al*⁹ searched for an association between orofacial clefts and mutations in the *PVR* and *PVRL2* genes, using samples from different populations. They found a statistically significant association in the South American and Iowa populations for an allelic variant

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Table 1 SNPs information and TDT results

<i>dbSNP ID</i>	<i>Genome position^a</i>	<i>Gene position</i>	<i>Alleles^b</i>	<i>MAF^c</i>	<i>T/NT^d</i>	<i>P-value^e</i>
rs35385129	chr19:49854029	PVR-intron 6	C/A	0.13	59/42	0.09
rs1871047	chr19:50043586	PVRL2-intron1	A/G	0.45	92/82	0.45

^aUCSC Genome Browser on Human May 2004 Assembly.

^bSNP alleles in coding frame, major allele first.

^cMinor allele frequency (MAF) calculated from all parental chromosomes.

^dTransmission/Non-Transmission (T/NT) counts are given for the major allele.

^eP-value for TDT.

in the *PVR* gene, while the *PVR* marker did not show any significant association in the Danish sample.

Materials and methods

For the present work, we tested a sample of 230 affected patients and their parents, selected after informed consent from an Italian population, excluding syndromic cases. In order to classify the CL/P as non-syndromic and to exclude potential teratogenic influences, a careful anamnesis was carried out to evaluate the presence of any other somatic or neurological disorder in the family or the use of clefting substances, such as phenytoin, warfarin, and ethanol, during pregnancy. Eighty-five nuclear triads (father–mother–affected child) were enrolled, one for each multiplex pedigree, while 145 trios (father–mother–affected child) pertained to sporadic cases. DNA was extracted from peripheral blood samples as previously described.¹⁰

To investigate the linkage disequilibrium with *PVR* gene, we used the pre-designed TaqMan assay C_1828143_10 for SNP having public ID rs35385129 at dbSNP at NCBI, while to test *PVRL2* we chose the SNP assay C_1846421_10 (rs1871047). SNP analyses were performed using TaqMan SNPs genotyping assay (Applied Biosystems, Monza, Milan, Italy) run on an Applied Biosystems 7500 Sequence Detection System (Applied Biosystems, Monza, Milan, Italy). Linkage disequilibrium between SNP alleles and disease was tested with the transmission disequilibrium test (TDT), which examines the transmission of alleles from heterozygous parents to affected offspring.¹¹

Results

Both markers employed were in Hardy–Weinberg equilibrium and none showed evidence of linkage disequilibrium. Table 1 summarises the information about the two markers used and the results of linkage disequilibrium analyses.

Discussion

Non-syndromic cleft lip with or without cleft palate is a complex genetic trait, and little is known about it.

Different chromosome regions and candidate genes have been proposed as causes in the aetiology of such congenital malformation. The OFC3 region has recently been further investigated for the presence of two genes, *PVR* and *PVRL2*, because of their homology to *PVRL1*, a gene seen to be involved in a form of cleft. We investigated the two candidate genes for the presence of linkage disequilibrium, analysing the same two SNPs previously used by Warrington *et al.*⁹

In our investigation, no significant departure from random sharing was observed for both polymorphisms. A borderline over-transmission of the most frequent allele C ($P=0.09$) at SNP rs35385129 was noticed, while the previous study showed significant overtransmission of the minor allele A.⁹ The investigation by Warrington *et al* was conducted with three different samples having comparable size with that used in the present study. The first from South America consisted of 434 patients and their mothers, the second from Iowa consisted of 205 affected/parents triads, and the third was composed by 147 triads from Denmark. Two populations, from South America and Iowa, showed significant linkage disequilibrium between oral clefts and *PVR* marker (P -value 0.0007 and 0.0009, respectively), while Danish population did not (P -value 0.44). In summary, our results partially confirm those reported by Warrington *et al*, since the analysis of Italian population did not show association between the *PVR* polymorphism and the cleft, as observed for Danish. Altogether, data from both studies do not support *PVR* involvement in CL/P onset among Europeans; however, it could not be excluded that one or more susceptibility alleles, not associated with rs35385129 alleles, might play a role in clefting. This evidence still reflects the complex aetiology of the CL/P malformation, which involves different genetic and environmental factors in distinct populations.

The results reported give additional strength to the fact that this disease presents genetic heterogeneity and in occasions allelic heterogeneity, which reflect the complex aetiology of CL/P. Therefore, ethnic and geographic variables are of paramount importance and must be considered if we are to have a precise definition of the onset of this congenital malformation.

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