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Role of *ARHGAP29* nucleotide variants in the etiology of non-syndromic cleft lip with or without cleft palate

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ABSTRACT

Aim. Non-syndromic cleft lip with or without cleft palate (nsCL/P) is a common birth defect of complex and heterogeneous aetiology. Genome-wide association studies (GWAS) of nsCL/P have identified an association for the 1p22.1 chromosomal region, in which *ARHGAP29* was suggested as a candidate gene. Thus, the current study aimed to determine the contribution of the common and rare *ARHGAP29* nucleotide variants to the risk of nsCL/P in the Polish population.

Material and Methods. In total, 197 common nucleotide variants (SNVs) and 22 missense variants located within the *ARHGAP29* locus at chromosome 1p22.1 were genotyped by SNV microarray. The study was conducted in 269 individuals with nsCL/P and 569 healthy individuals.

Results. Statistical analysis revealed that 31 common nucleotide variants located at the ARHGAP29 locus were significantly associated with the increased risk of nsCL/P. The strongest individual SNV was rs2391467 with a p-value = 2.49E-06 (OR = 1.64, 95%CI: 1.34 – 2.02). Besides, one potentially deleterious missense variant (rs140877322, p. Arg348Leu) was identified in a single patient with nsCLP.

Conclusion. These findings confirm *ARHGAP29* as a strong candidate gene for nsCL/P, with both common and rare nucleotide variants of this gene involved in the aetiology of nsCL/P in the Polish population.

Introduction

Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital defects in humans, affecting approximately 1/700 live-born children worldwide [1]. The prevalence of nsCLP varies by geographic location, ethnic/racial background, and socioeconomic

status [2]. The complex aetiology of this congenital anomaly reflects the action of multiple genetic factors and environmental exposures, hence it has not been fully elucidated. NsCL/P can be divided into the cleft of the palate only (CPO), and those affecting the lip with or without the palate (CL/P) [3]. Over 500 human syndromes in which clefting is a common feature have been identified (https://www.ncbi.nlm.nih.gov/OMIM/), although most cases correspond to isolated non-syndromic clefts with the absence of other structural or cognitive abnormalities [2]. Previous studies revealed that nsCL/P might have unique aetiological features, including specific genetic associations [2,4], however, the genetic components of nsCL/P have remained elusive due to the influence of multiple environmental risk factors [5].

To date, a variety of research methods have been applied to identify genetic factors contributing to nsCL/P. Genome-wide association studies (GWAS) have been crucial in identifying 40 novel risk loci that show strong associations of single nucleotide variants (SNVs) with nsCL/P [6–10]. The most consistent results in multiple populations were observed for nucleotide variants in *IRF6* (OMIM* 607199) gene, encoding a transcription factor critically involved in craniofacial development [2] and the gene-poor region of chromosome 8q24.21 [11–14]. Also, the chromosomal region consistently associated with nsCL/P is 1p22.1, initially implicating *ABCB4* (OMIM:*601691) as a candidate gene at this locus. However, *ABCB4* was excluded due to its retinal expression and known role in a spectrum of retinal disorders [15,16]. Therefore, it has been hypothesised that a neighbouring gene, *ARHGAP29* (OMIM:*610496), maybe the aetiologic gene within the same region. During craniofacial development in murine embryos, *ARHGAP29* expression was detected in the frontonasal, lateral prominences and palatal shelves [17]. Moreover, there are reports that *IRF6* regulates keratinocyte migration through *ARHGAP29*. Cells lacking the *IRF6* gene had lower levels of *ARHGAP29* and hyperactive Rho GTPase activating protein (GAP), which is involved in crucial cellular functions essential for craniofacial development [12,18]. In addition, sequencing of *ARHGAP29* in patients with nsCL/P identified eight potentially deleterious and aetiological variants, including a frameshift and a nonsense variant [17]. Further functional studies identified a novel missense variant in *ARHGAP29* (c.1654T>C, p.Ser552Pro), showing ARHGAP29 to be a regulatory protein affecting the development of the face [19]. These findings suggest that *ARHGAP29* may play a role as the aetiologic gene at the 1p22.1 locus for nsCL/P [17]. Therefore, this study investigated whether common SNVs and rare missense variants at the *ARHGAP29* locus contribute to the risk of nsCL/P in the Polish population.

Material and Methods

The present study was designed similar to our previous cleft association studies and conducted on the same study population [9,20].

Study population

Patients with a diagnosis of nsCL/P (58.0% of males) were recruited from several Polish medical centres. Among patients, 229 (85.1%) individuals had non-syndromic cleft lip and palate (nsCLP) and 40 (14.9%) individuals had non-syndromic cleft lip only (nsCLO). The control group was composed of 569 healthy individuals (49.6% males) without any developmental anomalies. Detailed characteristics of patients and controls enrolled in the study are presented in **Table 1**. All study participants were of Polish origin. DNA was isolated from peripheral blood lymphocytes by salt extraction. All experimental protocols were approved by the Institutional Review Board of Poznan University of Medical Sciences, Poland [21]. Written and oral consent was obtained from all participants or their legal guardians.

Table 1. Characteristics of study patients and controls

	nsCL/P patients (n = 269)[a]	Controls (n = 569)[a]
Cleft type		
nsCLP	229 (85.1%)	
nsCLO	40 (14.9%)	
Gender		
male	156 (58.0%)	282 (49.6%)
female	113 (42.0%)	287 (50.4%)

[a] Final number of samples analysed in the present study after exclusion of individuals based on stringent quality control criteria
nsCL/P - non-syndromic cleft lip with or without cleft palate
nsCLP - non-syndromic cleft lip and palate
nsCLO - non-syndromic cleft lip only

Common SNV selection and genotyping

Common single nucleotide polymorphisms (SNVs) located within the *ARHGAP29* locus at chromosome 1p22.1 were genotyped with using the HumanOmniExpressExome-8 v1 array (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. After applying stringent quality control criteria (SNV call rate >0.95, minor allele frequency, MAF, >0.05, Hardy-Weinberg, HW, equilibrium p-value >0.001 in controls), 197 common SNVs were subjected to statistical analysis.

Statistical analysis

The association of *ARHGAP29* locus SNVs with nsCL/P was tested with the Cochran-Armitage trend test. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were used to assess the strength of the association. ORs were calculated for the allelic model (a vs A; a is the risk allele). The Bonferroni correction was applied to account for multiple comparisons, and p-values < 2.54E-4 (0.05 / 197 SNVs) were considered as statistically significant. The pair-wise linkage disequilibrium (LD) between the top was evaluated using the Haploview 4.2 software (www.broadinstitute.org/haploview/haploview, **Table 2**). Separate statistical analyses were conducted for individuals with nsCLP and nsCLO to assess the subgroup-specific effects of the significantly associated SNVs. In addition, separate analyses were performed in male and female groups. The effects of genotype x gender interactions were evaluated by logistic regression approach.

In silico analysis of missense variants

The SNV microarray used in the present study allowed for the genotyping of 22 *ARHGAP29* missense variants. The putative functional consequences of these missense SNVs were analysed *in silico* using SIFT (http://sift.jcvi.org/), PolyPhen (http://genetics.bwh.harvard.edu/pph2/), and Mutation Assessor (http://mutationassessor.org/r3/) tools. Additionally, for all these variants, the frequency of the minor allele was checked in the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/).

Results

Common SNVs

Statistical analysis of the 197 ARHGAP29 locus SNVs revealed that 31 were nominally associated (p_{trend} < 0.05) with the risk of nsCL/P (**Figure 1**, **Supplementary Table 1**). Three SNVs, rs11165101, rs11165110 and rs2391467, were statistically significant even after applying the strict Bonferroni correction for multiple comparisons (p_{trend} = 1.71E-04, p_{trend} = 2.19E-04 and p_{trend} = 2.56E-06, respectively). These variants located within the same recombination region were in moderate LD with each other (the mean r^2 = 0.76 and D' = 0.99; **Table 2**). Two other SNVs, rs3789688 and rs2065971, were close to the study significance level (p_{trend} = 8.34E-04 and p_{trend} = 2.91E-04, respectively). The minor allele of the strongest individual SNV (rs2391467) located 14.7 kb upstream of *ARHGAP29* was associated with a 1.64-fold increased risk of nsCL/P (95%CI: 1.34 – 2.02, p = 2.49E-06), with the allelic ORs for the other four top variants in the range of 1.43 to 1.52. For all of them, the major allele was the risk allele. Association results for tested variants are presented in **Table 3** and **Supplementary Table 1**.

Table 2. Linkage disequilibrium values D' and r^2 for the most significant SNVs located at the *ARHGAP* locus

	rs3789688	rs11165101	rs11165110	rs2065971	rs2391467
rs3789688	-	0.78	0.78	0.71	0.83
rs11165101	0.46		1.00	0.98	0.98
rs11165110	0.46	1.00	-	0.98	0.98
rs2065971	0.41		0.89		1.00
rs2391467	0.61	0.64	0.64	0.71	-

Numbers denote D' and r^2 values expressed as a percentage of maximal value (1.0). D' values are presented above diagonal.
A red-to-white gradient shows highest (1.0) to lowest (0.0) D'. r^2 vales are presented below diagonal.
A black-to-white gradient shows highest (1.0) to lowest (0.0) r^2.

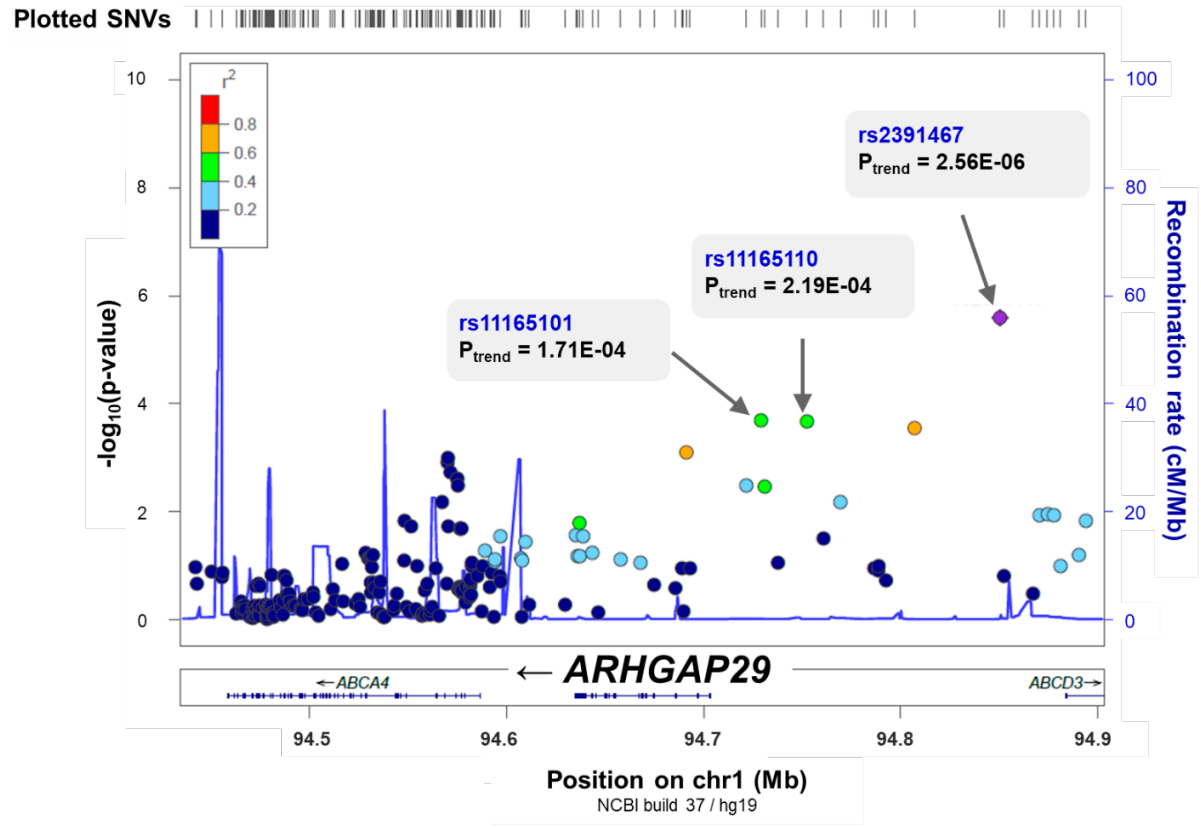

Figure 1. Regional plot of association results within the *ARHGAP29* locus

Table 3. Association results for the most significant variants at the *ARHGAP29* locus (p_{trend} values < 1.00E-03)

rs number	Location (bp)[a]	Consequence type	Gene	Alleles[b]	p_{trend}	RAF nsCL/P	RAF Controls	OR (95%CI)[d]
rs3789688	chr1: 94691240	intronic	*ARHGAP29*	T / C	8.34E-04	0.59	0.50	1.43 (1.17–1.77)
rs11165101	chr1: 94729088	intergenic	*ARHGAP29 / ABCD3*	A / C	**1.71E-04**	0.67	0.57	1.52 (1.23–1.89)
rs11165110	chr1: 94752469	intergenic	*ARHGAP29 / ABCD3*	A / G	**2.19E-04**	0.67	0.57	1.51 (1.22–1.87)
rs2065971	chr1: 94807102	intergenic	*ARHGAP29 / ABCD3*	A / C	2.91E-04	0.65	0.55	1.48 (1.20–1.83)
rs2391467	chr1: 94850443	intergenic	*ARHGAP29 / ABCD3*	A / G	**2.56E-06**	0.58	0.46	1.64 (1.34–2.02)

[a] NCBI build 37 / hg19.
[b] Underline denotes the risk allele (for all variants, exept rs2391467, the major allele is a risk allele).
[c] The ptrend values below 2.54E-04 (0.05 / 197 SNVs) were interpreted as statistically significant.
[d] Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for the allelic model (a vs A; a is the risk allele).
Significant p-values are highlighted in bold font.
RAF, risk allele frequency; nsCL/P, non-syndromic cleft lip with or without cleft palate.

The sub-phenotype analysis revealed that the most significant SNVs identified in the present study were exclusively associated with the risk of nsCLP. No significant association was identified between them and the nsCLO risk (p_{trend} values > 0.05), however, the differences in ORs between nsCLP and nsCLO subgroups were not statistically significant (heterogeneity p-values >0.05). Besides, for all these variants, the trend test p-values for nsCLP were higher than the p-values for the overall phenotype (**Table 4**). Logistic regression analysis revealed that ARHGAP29 locus variants did not show evidence of gender-dependent association with the risk of nsCL/P. No significant sex genotype interactions were detected (**Table 5**).

Table 4. Association results for oral cleft sub-phenotypes

			RAF		
rs number	Alleles[a]	p_{trend}[b]	Patients	CControls	OR (95%CI)[d]
nsCLP					
rs3789688	T / C	2.48E-03	0.59	0.50	1.41 (1.13–1.76)
rs11165101	A / C	2.62E-04	0.67	0.57	1.54 (1.23–1.93)
rs11165110	A / G	**2.23E-04**	0.67	0.57	1.55 (1.23–1.94)
rs2065971	A / C	3.25E-04	0.65	0.55	1.51 (1.21–1.89)
rs2391467	A / G	**4.28E-06**	0.58	0.46	1.67 (1.34–2.08)
nsCLO					
rs3789688	T / C	5.68E-02	0.62	0.50	1.58 (0.99–2.54)
rs11165101	A / C	1.53E-01	0.65	0.57	1.43 (0.88–2.31)
rs11165110	A / G	2.51E-01	0.64	0.57	1.33 (0.83–2.12)
rs2065971	A / C	2.22E-01	0.63	0.55	1.34 (0.84–2.14)
rs2391467	A / G	7.03E-02	0.56	0.46	1.51 (0.95–2.38)

[a] The p_{trend} values below 2.54E-04 (0.05 / 197 SNVs) were interpreted statistically significant
For all tested SNVs, there was no heterogeneity between oral cleft sub-phenotypes (heterogeneity p-values between 0.551 and 0.780).
nsCLP, non-syndromic cleft lip and palate
nsCLO, non-syndromic cleft lip only

Table 5. Gender-dependent interaction of the most significant variants at the *ARHGAP29* locus and nsCL/P

SNV	OR_{int}(95%CI)[a]	p[d]	OR_{males}(95%CI)[b]	p[d]	$OR_{females}$(95%CI)[c]	p[d]
rs3789688	0.94 (0.62–1.43)	7.77E-01	1.44 (1.09–1.91)	1.16E-02	1.36 (1.00–1.84)	4.94E-02
rs11165101	0.89 (0.58–1.37)	5.91E-01	1.56 (1.16–2.10)	3.40E-03	1.39 (1.02–1.89)	3.63E-02
rs11165110	0.93 (0.61–1.43)	7.52E-01	1.51 (1.13–2.03)	6.03E-03	1.41 (1.04–1.92)	4.80E-02
rs2065971	0.88 (0.58–1.36)	5.73E-01	1.54 (1.14–2.07)	4.32E-03	1.36 (1.00–1.86)	3.03E-02
rs2391467	1.12 (0.73–1.71)	6.05E-01	1.72 (1.28–2.31)	3.24E-04	1.54 (1.13–2.09)	7.95E-03

[a] Odds ratio for the gene x gender interaction.
[b] Odds ratio for the males.
[c] Odds ratio for the females.
[d] Based on logistic regression under the additive model.

Table 6. Missense variants identified in the *ARHGAP29* gene with the use of SNV microarray

			NsCL/P Cases		Controls		MAF			
rs number	Alleles[a]	Amino acid change[b]	Genotypes	MAF	Genotypes	MAF	ExAC[c]	SIFT[d]	PolyPhen[e]	Mutation Assessor[f]
rs1999272	T / C	p.Gly1255Asp	0 / 1 / 268	0.002	0 / 0 / 569	0.000	0.0004	tolerated low confidence	benign	predicted non-functional (neutral)
rs143877998	T / G	p.Gln893Pro	0 / 1 / 268	0.002	0 / 0 / 569	0.000	0.0002	tolerated	benign	predicted functional (medium)
rs147752270	T / C	p.Val875Ile	0 / 1 / 268	0.002	0 / 2 / 567	0.002	0.0016	tolerated	benign	predicted non-functional (low)
rs41311172	T / C	p.Arg798Gln	0 / 0 / 269	0.000	0 / 3 / 566	0.003	0.0025	tolerated	probably damaging	predicted non-functional (low)
rs140877322	A / C	p.Arg348Leu	0 / 1 / 268	0.002	0 / 0 / 569	0.000	5.995E-05	deleterious	probably damaging	predicted functional (medium)
rs183410431	T / C	p.Arg84His	0 / 2 / 267	0.004	0 / 0 / 569	0.000	4.505E-05	deleterious	benign	predicted non-functional (neutral)

[a] Underline denotes the minor allele.
[b] ENST00000260526.11.
[c] Exome Aggregation Consortium (ExAC), European (Non-Finnish).
[d] http://genetics.bwh.harvard.edu/pph2/.
[e] http://sift.jcvi.org/.
[f] http://mutationassessor.org/r3/.

Missense variants

Six out of twenty-two (27.3%) genotyped missense *ARHGAP29* variants were identified in the tested samples. Four of them were found only in cleft patients, and one was found exclusively in controls (**Table 6**). One of the cleft specific variants (rs140877322, p. Arg348Leu) was predicted to be deleterious and functional by all prediction tools used in this study. In a non-Finnish European population of ExAC, the frequency of rs140877322 was 5.995E-05.

Discussion

Numerous genes and several polymorphic variants have been detected to confer an increased risk of nsCL/P [22,23]. Beaty et al. characterised four significant loci in GWAS for nsCL/P: *IRF6*, 8q24, *MAFB* (OMIM:*608968), and *ABCA4* [11], with multiple follow-up studies in different populations successfully confirming these loci [24–31]. A critical role for *IRF6* and the 8q24 region in craniofacial development has been previously identified, while the roles of the loci in the genes *ABCA4* and *MAFB* remains largely unclear [32–34]. The most significant SNVs strongly associated with nsCL/P have been identified within the introns of *ABCA4* [35]. However, *ABCA4* is not a good candidate as the etiologic gene for nsCL/P at the 1p22 locus because of its lack of expression in the developing lip or palate in mice [11]. Additionally, there were no reported defects in the craniofacial structure in mice homozygous for targeted loss-of-function mutations in *ABCA4* [36]. Moreover, despite identifying several missense mutations in *ABCA4* in humans, none of them showed suggestive evidence of causing craniofacial malformations [15,37]. However, a recent study suggests a role for *ARHGAP29* (a neighbouring gene of *ABCA4*) in nsCL/P based on expression in craniofacial development using a murine model. The *ARHGAP29* transcript was detected in the medial and lateral nasal processes, and expression was also observed in the mandibular and maxillary processes of developing mouse embryo at E10.5 and the shelves of the secondary palate at E13.5. [38]. Furthermore, its expression depends on *IRF6* [38], one of the pivotal contributors to the underlying genetics of human nsCLP/P [39]. *ARHGAP29* is located 47 kb centromeric to *ABCA4* and encodes Rho GTPase activating protein (GAP) 29, which is involved in many functions related to cellular shape, movement, proliferation, all essential for craniofacial development [12]. Rho is downstream of Tgfb and Wnt signalling pathways [40,41], which have also been implicated in craniofacial development. These are suggestive evidence that *ARHGAP29* is the etiologic gene at this locus and may play a role in nsCL/P. During the last few years, about twelve potentially pathogenic missense variants in *ARHGAP29* have been reported in nsCL/P cases. [17,19,28,35,42]. However, it is not clear if these possibly pathogenic rare variants contribute to the phenotype. Therefore, the purpose of this study was to evaluate the association between common and rare missense SNVs located within the *ARHGAP29* locus and the risk of nsCL/P in the Polish population. Patients with non-syndromic forms of cleft lip with cleft palate and cleft lip only were recruited, with patients with a diagnosis of non-syndromic cleft palate only were excluded from the molecular analyses due to the distinct aetiology of this subtype of oral clefts [43].

The findings showed that common nucleotide variants of the *ARHGAP29* gene are significantly correlated with the risk of nsCL/P. Statistical analysis of the genotyping results revealed that three common SNVs represent a single cleft association signal since they are located within the same intergenic region of *ARHGAP29/ABCD3* genes. These three risk variants rs11165101, rs11165110 and rs2391467 are strongly associated with the risk of this craniofacial anomaly in a tested group of patients. Our data also demonstrated that the minor allele carriers at rs2391467 have a 1.64-fold increased risk of nsCL/P. Moreover, all these results were statistically significant even after applying Bonferroni correction for multiple comparisons. Two other tested variants, rs3789688 and rs2065971, were close to reaching the study significance level. It is of note that the intronic SNV rs3789688 in families of non-Hispanic white ethnicities also showed a strong association with nsCL/P [29]. Although the intronic variants are unlikely to have effects on gene transcription or the final protein structure, multiple analysis revealed that non-coding variants have a significant role in the genetic causes of nsCL/P [3,35,44]. This suggests that the true casual variants implicated in the risk of nsCL/P might affect the *ARHGAP29* gene expression level rather than the structure of an encoded protein or are in pair-wise LD with an unknown actual pathogenic variant.

We hypothesised that some genetic risk for nsCL/P in Polish populations lies in rare exonic markers, thus, our study also included an analysis of twenty-two missense variants. Our results showed six missense *ARHGAP29* variants in the tested samples. One of the cleft specific variants (rs140877322, p.Arg348Leu) was predicted to be probably damaging and deleterious by multiple *in silico* tools. Furthermore, none of the unaffected individuals carried the variant. However, these results should be interpreted with caution because the functional impact of these variants is unknown. Therefore, functional studies in biological model systems are required to identify pathogenic variants and a possible mechanism contributing to the nsCL/P phenotype. Savastano at al. identified ten rare variants in the *ARHGAP29* gene using next-generation sequencing, of these, five were missense changes and the remaining were predicted to be loss-of-function (LoF). These findings provide evidence that the LoF variants but not missense variants may be an important genetic factor and contribute to the aetiology of nsCL/P [45]. To take this idea further, new coding variants which confer risk to nsCL/P should be identified by sequencing, which is crucial for rare variant discovery.

There was no evidence of a gender-dependent association with any of the SNVs studied. However, Carlson at al. confer that the impact of genetic variants on nsCL/P risk differs for males and females. These results are not surprising because the incidence rates of nsCL/P vary by sex. Carlson at al. used a genome-wide approach to identify the genetic contribution to this phenomenon and examined gene by sex interactions in a group of 2142 nsCL/P cases and 1700 controls recruited from different countries. Their analysis identified three loci that achieved genome-wide significance interaction effect, rs11142081, rs72804706, rs77590619 from the 9q22.1, 10q21.1, and 13q13.3 loci, respectively showed evidence of a higher risk of CL/P for females carrying the minor risk allele, while this trend was not present in males. It is worth noting that many biochemical mechanisms affect gene by sex interactions, hence they suggest that due to the diversity of possible mechanisms, it is challenging to explore or discuss each locus adequately [46].

Given the impact of rare variants as potential phenotypic modifiers diversity, which has been highlighted by Carlson at al. [47], we analysed cleft type-dependent association with the studied SNVs. However, our sub-phenotype analysis did not reveal any significant genotype-phenotype correlations. Nonetheless, these results should be interpreted with caution due to the small number of patients with nsCLO recruited to our study, therefore, insufficient power to detect significant cleft type differences.

The strength of this study lies in the homogenous study cohort recruited from a single ethnic group. Although the study is limited by a relatively small sample size, the risk variants with the lower allele frequencies may have been missed. Hence, statistical analyses were not well suited to draw reliable associations from low-frequency variants (MAF<0.05), which may be important in explaining nsCL/P susceptibility. Another limitation of our study is that the association analysis focused only on the genetic factors without considering environmental factors that appear to contribute to the aetiology of nsCL/P, like maternal folic acid supplementation or maternal smoking [48,49].

Despite these limitations, the nsCL/P risk loci identified in our research are consistent with previous studies and biological mechanisms, thereby providing further evidence for the role of *ARHGAP29* and new insight into the pathogenesis of the nsCL/P in the Polish population. Functional analyses are required to explore the mechanisms by which nucleotide variants of the *ARHGAP29* gene might increase risk of nsCL/P

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Conflict of interest statement
The authors declare no conflict of interest.

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Supplementary Table 1. Association results for common nucleotide variants at the *ARHGAP29* locusa.

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs743117	chr1: 94441950			T / c	0.37	1.12E-01
rs743113	chr1: 94442739			T / c	0.41	2.20E-01
rs1889547	chr1: 94450131			A / g	0.44	1.31E-01
rs11165057	chr1: 94455420			t / G	0.08	1.72E-01
rs11165058	chr1: 94455721			t / G	0.08	1.42E-01
rs17110736	chr1: 94462769	ABCA4		a / G	0.07	7.88E-01
rs3789375	chr1: 94465132	ABCA4		T / g	0.11	4.69E-01
rs4147871	chr1: 94465461	ABCA4		A / g	0.06	7.72E-01
rs4147869	chr1: 94465677	ABCA4		t / C	0.06	5.75E-01
rs4147868	chr1: 94466066	ABCA4		g / C	0.06	6.62E-01
rs12070273	chr1: 94466088	ABCA4		A / g	0.06	6.62E-01
rs4147864	chr1: 94467238	ABCA4		a / G	0.06	5.70E-01
rs17110761	chr1: 94467407	ABCA4		t / C	0.06	6.62E-01
rs7537325	chr1: 94469631	ABCA4		T / c	0.23	9.20E-01
rs1762114	chr1: 94471075	ABCA4	Synonymous_I2023I	a / G	0.07	9.80E-01
rs4147863	chr1: 94471154	ABCA4		t / C	0.18	6.29E-01
rs4147862	chr1: 94471519	ABCA4		t / C	0.18	8.63E-01
rs4147861	chr1: 94471948	ABCA4		a / C	0.06	5.86E-01
rs557026	chr1: 94472489	ABCA4		t / C	0.11	2.43E-01
rs3789379	chr1: 94472520	ABCA4		A / g	0.18	8.63E-01
rs7531001	chr1: 94472909	ABCA4		a / G	0.18	6.54E-01
rs2275029	chr1: 94473845	ABCA4	Synonymous_P1948P	T / c	0.16	7.95E-01
rs2275031	chr1: 94473896	ABCA4		t / G	0.18	7.89E-01
rs1191234	chr1: 94474020	ABCA4		A / g	0.11	2.21E-01
rs2275032	chr1: 94474185	ABCA4		A / c	0.18	8.95E-01
rs4147857	chr1: 94474328	ABCA4	Synonymous_L1938L	T / c	0.19	5.82E-01
rs4147856	chr1: 94474452	ABCA4		T / g	0.19	5.78E-01
rs17110808	chr1: 94474872	ABCA4		a / G	0.07	2.41E-01
rs2065712	chr1: 94476035	ABCA4		t / C	0.25	6.17E-01
rs11165062	chr1: 94477634	ABCA4		a / G	0.13	5.36E-01
rs945067	chr1: 94477893	ABCA4		t / C	0.20	9.69E-01
rs17391542	chr1: 94477935	ABCA4		a / G	0.11	6.99E-01
rs12085639	chr1: 94478293	ABCA4		a / G	0.16	9.93E-01
rs486879	chr1: 94478425	ABCA4		T / c	0.20	7.67E-01
rs567370	chr1: 94478573	ABCA4		T / c	0.33	9.75E-01
rs12082181	chr1: 94478595	ABCA4		T / c	0.33	8.37E-01
rs17391612	chr1: 94478847	ABCA4		a / G	0.12	5.87E-01
rs3789391	chr1: 94479338	ABCA4		t / C	0.06	6.29E-01
rs12049183	chr1: 94479468	ABCA4		t / C	0.06	5.89E-01
rs2275034	chr1: 94480439	ABCA4		t / C	0.40	5.85E-01
rs3818778	chr1: 94480529	ABCA4		a / C	0.48	1.52E-01
rs914958	chr1: 94481068	ABCA4		A / g	0.21	7.47E-01
rs915201	chr1: 94481596	ABCA4		A / g	0.25	9.13E-01

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs915200	chr1: 94481904	ABCA4		t / C	0.08	7.70E-01
rs915199	chr1: 94481929	ABCA4		t / C	0.23	8.72E-01
rs6681968	chr1: 94484705	ABCA4		t / C	0.42	4.56E-01
rs17110850	chr1: 94485491	ABCA4		A / g	0.11	6.34E-01
rs10493867	chr1: 94486406	ABCA4		T / c	0.25	8.25E-01
rs4147848	chr1: 94486587	ABCA4		t / G	0.29	4.45E-01
rs933073	chr1: 94486667	ABCA4		T / g	0.08	1.62E-01
rs472908	chr1: 94487354	ABCA4		A / g	0.43	1.59E-01
rs2282229	chr1: 94488326	ABCA4		a / T	0.09	1.96E-01
rs1932014	chr1: 94488497	ABCA4		A / g	0.44	3.67E-01
rs1889407	chr1: 94489553	ABCA4		T / g	0.44	3.49E-01
rs2151847	chr1: 94489975	ABCA4		a / C	0.43	4.86E-01
rs11165065	chr1: 94491468	ABCA4		a / G	0.32	5.83E-01
rs3945204	chr1: 94492773	ABCA4		t / C	0.44	5.79E-01
rs4147846	chr1: 94495407	ABCA4		T / c	0.49	6.68E-01
rs4147845	chr1: 94495417	ABCA4		T / c	0.49	6.69E-01
rs4147844	chr1: 94495487	ABCA4		A / g	0.49	6.69E-01
rs2297671	chr1: 94496253	ABCA4		A / g	0.50	6.78E-01
rs4147841	chr1: 94497178	ABCA4		A / g	0.44	4.42E-01
rs3789393	chr1: 94499133	ABCA4		t / C	0.44	4.39E-01
rs3789395	chr1: 94501594	ABCA4		a / C	0.43	3.33E-01
rs1320502	chr1: 94501799	ABCA4		T / c	0.43	3.89E-01
rs1889548	chr1: 94503197	ABCA4		a / C	0.31	7.80E-01
rs11165069	chr1: 94504545	ABCA4		t / C	0.21	8.74E-01
rs2297633	chr1: 94510673	ABCA4		t / G	0.34	6.51E-01
rs3789399	chr1: 94511717	ABCA4		c / G	0.48	2.89E-01
rs544830	chr1: 94512893	ABCA4		T / c	0.48	4.56E-01
rs4147836	chr1: 94516474	ABCA4		t / C	0.22	9.65E-02
rs1191231	chr1: 94516985	ABCA4		a / C	0.48	4.73E-01
rs497511	chr1: 94523113	ABCA4		A / g	0.46	5.19E-01
rs549848	chr1: 94524856	ABCA4		T / c	0.34	4.39E-01
rs521538	chr1: 94525623	ABCA4		a / G	0.22	5.97E-01
rs4147833	chr1: 94528363	ABCA4		t / C	0.21	5.94E-02
rs4847273	chr1: 94529743	ABCA4		A / g	0.26	7.51E-02
rs1007347	chr1: 94530518	ABCA4		T / c	0.26	7.36E-02
rs553608	chr1: 94531013	ABCA4		t / C	0.21	2.17E-01
rs1191232	chr1: 94531192	ABCA4		a / G	0.37	3.28E-01
rs3789405	chr1: 94531324	ABCA4		T / c	0.26	6.85E-02
rs3789407	chr1: 94531606	ABCA4		c / G	0.22	1.08E-01
rs4140392	chr1: 94532013	ABCA4		t / C	0.21	6.51E-02
rs1191228	chr1: 94532562	ABCA4		t / C	0.21	2.17E-01
rs1931575	chr1: 94533014	ABCA4		T / c	0.23	2.76E-01
rs549114	chr1: 94534354	ABCA4		a / G	0.33	7.45E-01
rs2151849	chr1: 94535174	ABCA4		A / g	0.24	3.28E-01

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs3789411	chr1: 94535546	ABCA4		a / G	0.33	8.01E-01
rs4612636	chr1: 94535689	ABCA4		A / c	0.06	7.91E-01
rs3789412	chr1: 94536067	ABCA4		t / C	0.24	2.06E-01
rs12758774	chr1: 94537295	ABCA4		t / C	0.20	9.00E-01
rs12759306	chr1: 94537642	ABCA4		a / C	0.20	9.17E-01
rs1761375	chr1: 94538011	ABCA4		a / G	0.31	9.04E-01
rs492220	chr1: 94542569	ABCA4		T / c	0.29	6.85E-01
rs3120133	chr1: 94542770	ABCA4		T / g	0.08	5.64E-01
rs4147831	chr1: 94544233	ABCA4	Synonymous_H423H	a / G	0.07	3.45E-01
rs4147828	chr1: 94547889	ABCA4		A / g	0.18	8.39E-02
rs4147827	chr1: 94548080	ABCA4		G / c	0.23	1.50E-02
rs574741	chr1: 94549083	ABCA4		t / C	0.22	6.14E-01
rs546550	chr1: 94550555	ABCA4		A / g	0.29	7.35E-01
rs17461953	chr1: 94551450	ABCA4		A / c	0.23	1.89E-02
rs563429	chr1: 94553866	ABCA4		A / g	0.29	6.51E-01
rs4847196	chr1: 94554453	ABCA4		a / G	0.22	1.06E-01
rs1191238	chr1: 94556894	ABCA4		a / G	0.09	8.84E-01
rs554931	chr1: 94557357	ABCA4		t / C	0.23	7.85E-01
rs483904	chr1: 94557434	ABCA4		t / C	0.23	7.85E-01
rs952499	chr1: 94558425	ABCA4		T / c	0.47	2.95E-01
rs538880	chr1: 94558774	ABCA4		g / C	0.23	8.19E-01
rs2068334	chr1: 94559715	ABCA4		a / G	0.17	2.25E-01
rs4147825	chr1: 94560938	ABCA4		a / G	0.38	8.42E-01
rs4147823	chr1: 94561272	ABCA4		A / c	0.22	6.95E-01
rs4147820	chr1: 94562084	ABCA4		t / C	0.21	6.05E-01
rs12088309	chr1: 94563916	ABCA4		T / c	0.30	1.14E-01
rs3789421	chr1: 94565577	ABCA4		a / G	0.19	8.61E-01
rs950283	chr1: 94567223	ABCA4		T / c	0.36	6.74E-03
rs4147819	chr1: 94569504	ABCA4		a / G	0.08	2.29E-01
rs481931	chr1: 94570016	ABCA4		t / G	0.39	1.25E-03
rs570926	chr1: 94570218	ABCA4		T / c	0.39	1.02E-03
rs570878	chr1: 94570234	ABCA4		t / G	0.48	1.93E-02
rs1211213	chr1: 94571420	ABCA4		A / g	0.36	1.90E-03
rs4147816	chr1: 94574780	ABCA4		t / C	0.40	2.56E-03
rs4147812	chr1: 94575043	ABCA4		A / c	0.40	2.50E-03
rs3827712	chr1: 94575171	ABCA4		T / c	0.40	3.37E-03
rs3789433	chr1: 94575440	ABCA4		a / G	0.26	2.87E-01
rs3789434	chr1: 94575978	ABCA4		T / c	0.08	2.52E-01
rs3789435	chr1: 94576360	ABCA4		A / g	0.48	2.09E-02
rs3827713	chr1: 94576524	ABCA4		c / G	0.48	2.09E-02
rs4147810	chr1: 94576664	ABCA4		A / g	0.08	2.52E-01
rs2297635	chr1: 94576893	ABCA4		a / G	0.08	3.08E-01
rs2297634	chr1: 94576968	ABCA4		T / c	0.48	2.09E-02
rs1889405	chr1: 94577410	ABCA4		t / C	0.24	3.26E-01

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs1889404	chr1: 94577423	ABCA4		t / C	0.24	2.67E-01
rs3789438	chr1: 94577462	ABCA4		t / G	0.08	2.98E-01
rs4147807	chr1: 94579053	ABCA4		A / g	0.22	4.89E-01
rs3789439	chr1: 94579426	ABCA4		T / c	0.22	2.56E-01
rs10782976	chr1: 94581125	ABCA4		a / G	0.31	3.96E-01
rs3789441	chr1: 94581384	ABCA4		t / C	0.27	2.56E-01
rs3789442	chr1: 94581456	ABCA4		c / G	0.22	2.14E-01
rs3789443	chr1: 94581529	ABCA4		A / g	0.23	2.06E-01
rs3789444	chr1: 94581540	ABCA4		t / C	0.23	1.84E-01
rs7535005	chr1: 94581905	ABCA4		t / C	0.25	3.63E-01
rs3789445	chr1: 94582249	ABCA4		T / g	0.25	1.08E-01
rs4147803	chr1: 94582293	ABCA4		g / C	0.43	9.24E-02
rs4147798	chr1: 94585009	ABCA4		t / C	0.23	1.60E-01
rs3761906	chr1: 94587362			t / G	0.05	7.37E-01
rs2151846	chr1: 94587687			T / g	0.43	1.06E-01
rs1931572	chr1: 94588992			T / c	0.40	5.48E-02
rs10874835	chr1: 94591481			a / G	0.23	2.55E-01
rs1105123	chr1: 94592290			T / c	0.28	1.40E-01
rs12071152	chr1: 94593399			a / G	0.05	9.04E-01
rs11802196	chr1: 94594043			A / c	0.41	8.50E-02
rs11165081	chr1: 94594080			A / c	0.41	8.00E-02
rs17111122	chr1: 94596464			A / g	0.25	1.59E-01
rs6686599	chr1: 94596831			a / G	0.39	2.89E-02
rs1931565	chr1: 94596867			a / G	0.33	2.00E-01
rs11581939	chr1: 94607234			T / c	0.35	7.70E-02
rs2022378	chr1: 94607607			a / C	0.24	9.07E-01
rs4847286	chr1: 94607848			T / g	0.41	8.22E-02
rs871664	chr1: 94609478			t / C	0.49	3.78E-02
rs2774920	chr1: 94611300			A / g	0.09	5.60E-01
rs12742802	chr1: 94629643			a / C	0.25	5.39E-01
rs1411701	chr1: 94635028	ARHGAP29		a / G	0.38	2.75E-02
rs12044374	chr1: 94635986	ARHGAP29		t / C	0.43	6.87E-02
rs10874840	chr1: 94636836	ARHGAP29		A / g	0.36	1.63E-02
rs12752790	chr1: 94637058	ARHGAP29		T / c	0.43	6.87E-02
rs1048866	chr1: 94638711	ARHGAP29		t / C	0.37	2.91E-02
rs1048854	chr1: 94643531	ARHGAP29	Synonymous_Q891Q	T / c	0.27	5.92E-02
rs11577575	chr1: 94646514	ARHGAP29		a / G	0.23	7.50E-01
rs4847294	chr1: 94657769	ARHGAP29		A / g	0.43	7.99E-02
rs1541098	chr1: 94667970	ARHGAP29		T / c	0.27	9.07E-02
rs2274788	chr1: 94674726	ARHGAP29		T / c	0.24	2.36E-01
rs3789689	chr1: 94685585	ARHGAP29		T / g	0.07	2.75E-01
rs6541343	chr1: 94689027	ARHGAP29		a / G	0.08	1.16E-01
rs12724116	chr1: 94689734	ARHGAP29		A / g	0.15	7.11E-01
rs3789688	chr1: 94691240	ARHGAP29		t / C	0.47	8.34E-04

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs6673491	chr1: 94693145	ARHGAP29		t / C	0.08	1.18E-01
rs12750249	chr1: 94721660			T / c	0.29	3.39E-03
rs11165101	chr1: 94729088			a / C	0.40	1.71E-04
rs17396055	chr1: 94730954			a / G	0.32	3.47E-03
rs2391472	chr1: 94737583			T / c	0.08	9.28E-02
rs11165110	chr1: 94752469			a / G	0.40	2.19E-04
rs1330855	chr1: 94760885			A / g	0.20	3.21E-02
rs11580391	chr1: 94769368			t / C	0.29	6.70E-03
rs16928	chr1: 94786514			t / C	0.08	1.14E-01
rs17111408	chr1: 94788623			t / C	0.08	1.28E-01
rs12027548	chr1: 94788768			A / g	0.08	1.07E-01
rs11584317	chr1: 94792449			t / C	0.15	1.93E-01
rs2065971	chr1: 94807102			A / c	0.42	2.91E-04
rs2391467	chr1: 94850443			A / g	0.50	2.56E-06
rs1572575	chr1: 94852474			A / g	0.06	1.59E-01
rs12037634	chr1: 94867056			T / g	0.07	3.36E-01
rs11165135	chr1: 94870535			T / g	0.48	1.20E-02
rs6681849	chr1: 94874521			t / G	0.48	1.14E-02
rs10399785	chr1: 94877801			T / c	0.48	1.21E-02
rs4148060	chr1: 94881143			A / g	0.44	1.03E-01
rs10493872	chr1: 94890418	ABCD3		t / G	0.28	6.61E-02
rs12750904	chr1: 94893928	ABCD3		A / g	0.36	1.55E-02

[a]*ARHGAP* + / - 200kb.
[b]NCBI build 37 / hg19.
[c]Lowercase letter denotes the minor allele.
[d]MAF, minor allele frequency based on the entire sample frequencies.
[e]The p_{trend} values below 2.54E-04 (0.05 / 197 SNVs) were interpreted statistically significant.
Significant p-values are highlighted in bold font.