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INVESTIGATING THE STATUS OF SINGLE NUCLEOTIDE POLYMORPHISM RS 2235373 IN IRF 6 GENE IN NON-SYNDROMIC CLEFT LIP AND PALATE

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ABSTRACT

Background: Non syndromic Cleft lip and palate NSCLP is a congenital abnormality that develops during intrauterine life. Interferon regulatory factor IRF 6 is a gene associated with NSCLP. Parental ABO blood group analysis may serve as genetic marker regarding this anomaly.

Objectives: To assess the relationship of Single nucleotide polymorphism SNP rs 2235373 parental blood groups in NSCLP.

Methods: In the current study, 60 NSCLP patients and their 60 unaffected parents were included. DNA isolation, Polymerase Chain Reaction PCR and Sequencing of rs 2235373 of IRF 6 was done on blood samples of NSCLP cases. Blood grouping of the parents was done by Slide agglutination method.

Results: Polymorphism was found in rs 2235373 G > A genotype. A striking feature included Rh Positivity with B and A blood types in father and mother respectively. However, there was an insignificant association among SNP rs 2235373 and parental ABO blood groups.

Conclusion: The findings of the present study exhibited polymorphism rs 2235373 in IRF 6 in NSCLP, however may not be independently associated with parental ABO blood types.

KEYWORDS: NSCLP, IRF 6 gene, maternal ABO blood groups, PCR

INTRODUCTION

Due to its multifactorial etiology, studies on non-syndromic cleft lip and palate are of utmost importance to understand disease susceptibility. CLP is a most common congenital abnormality that develops during intrauterine life. These clefts are classified into Syndromic (when the patient is having other developmental disorders along with CLP) and NSCLP (isolated disorder)(Chowdhury et al., 2025; YEĞİN, USLUKAYA, TAŞKESEN, & YILDIRIM, 2022).

The risk factors associated with NSCLP included genetic as well as environmental basis. IRF 6 is a gene associated with non-syndromic cleft lip and palate NSCLP. Identification of inheritance markers may help improve genetic counseling and adopt preventive measures(Cheng, Du, Long, & Huang, 2023; Slavec et al., 2023).

IFR6 rs 2235373 is located in intron 7 (C/T) as the non-coding region. IRF6 non-coding polymorphisms may correlate with NSCLP (Bezerra et al., 2020; Nasroen, Maskoen, Soedjana, Soemantri, & Hilmanto, 2018).

Carbohydrate molecules, which are typically thought of as red blood cell antigens, determine the ABO blood group types (A, B, and O). Relationship of parental ABO blood group analysis may serve as genetic marker associated with NSCLP.

Additionally, they are expressed on various human tissues, including platelets, sensory neurons, and vascular endothelium and epithelium(Andalibi et al., 2020; Mitra, Mishra, & Rath, 2014; Moon, 2014)

TheABO blood type system is being considered as a possible genetic marker due to its inheritance pattern. genetic interactions contributing to cleft formation and to assess the possibility whether the association can be made a basis of genetic counseling.

METHODOLOGY

This study used blood samples from 60 NSCLP patients for genotyping of rs 2235373 and 60 blood samples from their parents who did not have cleft lip and palate, to find out their blood groups.

Blood was drawn under aseptic conditions by venipuncture method and kept in EDTA containing vials.

Patients were grouped into 3 categories 20 cases each in Cleft lip only CLO, Cleft palate only CPO and Cleft lip and palate CLP. Informed written consent was taken from the parents of the patients for sequencing, and the parents themselves for blood grouping.

DNA isolation, PCR and Sequencing

For checking the polymorphism, first of all DNA was isolated from blood samples of NSCLP patients followed by PCR and sequencing of DNA. Alignment of the sequenced product was done by Geneious Software.

Parental blood grouping

After drawing blood following aseptic protocol from both the parents of the cases of NSCLP, blood group types were analyzed by Agglutination method.

Statistical analysis

For analyzing the data SPSS Version 20 was used. For categorical variables which included SNPs, ABO blood groups, percentage and frequencies were checked. P value ≤ 0.05 was considered as significant.

RESULTS

Genotyping of SNP rs 2235373 was done for all 60 cases of NSCLP. Polymorphism was found with GA genotype. The distribution of genotypes in Father's ABO blood antigens was described in Table 1.

A salient feature of the study included Rh positivity among parental blood groups. B+ and A+ blood groups ranked the most common among father and mother of the cases respectively.

Insignificant association was shown among parental blood types and SNP. The distribution of genotypes in father ABO blood antigens and SNP was mentioned in Table 1. Similarly insignificant relationship was observed among rs 2235373 genotypes and mother's ABO blood groups. Table 2.

Table 1: Distribution of Genotype and Father's ABO blood antigens

Father's ABO Blood group	SNP rs 2235373 Genotypes n (%)		AA	Total	P value
	GG	GA			
A+	7(11.6)	1(1.66)	0(0)	8	0.890
B+	18(30)	8((13.33)	2(3.33)	28	
AB+	2(3.33)	2(2.33)	0(0)	4	
O+	14(0)	4(1.66)	0(0)	18	
A -	0(0)	1(0)	0(0)	1	
B-	0(0)	0(0)	0(0)	0	
AB-	0(0)	0(0)	0(0)	0	
O-	1(1.66)	0(0)	0(0)	1	
Total	42(70)	16(26.6)	2(2.33)	60	

Fisher Exact test

Table 2: Distribution of Genotype and Mother's ABO blood antigens

Mother's ABO Blood group	SNPs rs 2235373 Genotypes n (%)				P value
	GG	GA	AA	Total	
A+	14(26.66)	1(1.66)	1(1.66)	16(3.33%)	0.365
B+	6(10)	5(8.33)	1(1.66)	12(20)	
AB+	6(10)	1(1.66)	0(0)	7((11.66)	
O+	13(21.66)	8(13.33)	0(0)	21(35)	
A -	2(3.33)	0(0)	0(0)	2(3.33)	
B-	0(0)	1(1.66)	0(0)	1(1.66)	
AB-	0(0)	0(0)	0(0)	0(0)	
O	1(1.66)	0(0)	0(0)	1(1.66)	
Total	42(70)	16(26.6)	2(3.33)	60(100)	

DISCUSSION

Although, NSCLP is one of the most common congenital and genetic defects, biological mechanisms and molecular basis of this multifactorial disorder are still unknown. Currently, genetic factors are subject of intense research and considered as gold standard. In order to assess SNP rs2235373 of IRF 6 in Non syndromic cleft lip and palate 60 patients were selected. These were further subdivided into three groups comprising of 20 cases each of CLO, CPO and combined CLP cases. In the present study polymorphism rs 2235373 was commonly found. Our findings are in consistent to study by Phan et al,2023 which revealed association with SNP rs 2235373 of IRF 6 and NSCLP. The results of the current study are also in consistent with multiple studies across different populations including studies conducted in Ughur population in China, Indonesia and other ethnicities combined SNPs (haplotype)involving rs 2235373 showed strong association with NSCLP however the results of the present study are contradictory to an international study by Sahu et al,2026 (Mijiti, Ling, & Moming, 2015; Muruganantham & Veerabathiran, 2026; Nasroen et al., 2018; Phan, Phuong, Dang, Tram, & Vu, 2023).

Additionally this study tends to advance knowledge of genetic predisposition and its possible interaction with inherited blood group patterns ,given the prevalence of NSCLP in the Khyber Pakhtunkhwa region the scarcity of region specific data .It was evaluated in NSCLP in a single studies though it was not related to SNPs (Sivanand et al., 2021). There were no studies found in relation of parental blood groups and SNP rs 2235373 on extensively searching the literature.

CONCLUSION

A prominent finding of Rh-positive cases and predominance of specific parental blood types highlights the novelty of the study. Insignificant relationship among specific SNP and parental ABO blood types implies that these variables act via separate biological processes. Individual polymorphism effect on a lower level however insignificant association does not indicate lack of relevance.

Conflict of Interest: The authors declare no conflict of interest

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