



## Association between *PTCH1* and *RAD54B* Single-Nucleotide Polymorphisms and Non-syndromic Orofacial Clefts in the Northeast Population of Iran

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### Abstract

**Background:** Non-Syndromic Cleft Lip with or without cleft Palate (NSCL/P) is a common developmental disorder of the head and neck with a multifactorial etiology. The current study aimed to evaluate the potential association of *PTCH1* (rs10512248) and *RAD54B* (rs12681366) polymorphisms with NSCL/P in the Northeast Iranian population.

**Methods:** In the present study, blood samples were taken from 122 subjects with NSCL/P and 161 healthy controls. Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP) were used to conduct genotyping of single-nucleotide polymorphisms.

**Results:** Although differences were observed between cases and controls in rs10512248 and rs12681366, our data did not support a significant association of these polymorphisms with NSCL/P in our population.

**Conclusion:** Our findings suggest that polymorphisms of rs10512248 and rs12681366 may not be potential risk factors for NSCL/P in the Northeast Iranian population due to the multifactorial and multiethnicity characteristics of some genes.

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### Introduction

Disregarding the different forms of heart deformities, orofacial clefts are the most frequent congenital anomalies in humans <sup>1,2</sup>. Whether the patients have other anomalies or malformations, these clefts can be classified into syndromic and non-syndromic forms <sup>3</sup>. However, the incidence and presentation of these congenital disorders vary broadly by ethnic origin, geographical position, and socioeconomic status <sup>4,5</sup>. According to previous reports, the prevalence of Non-Syndromic Cleft Lip with or without cleft Palate (NSCL/P) was much higher in some ethnic groups, including Asians and Native Americans, followed by Caucasians and Africans <sup>6</sup>. Although there is no precise

evaluation of the NSCL/P population in Iranians, the approximate incidence of NSCL/P is about 1 per 1000 live births <sup>7</sup>.

NSCL/P has a multifactorial etiology that both genetic and environmental factors contribute to disease susceptibility <sup>8</sup>. Over the recent years, using genetic association studies, several susceptible genes have been identified to have a role in the complex etiology of NSCL/P <sup>9</sup>. In recent years, Genome-Wide Association Studies (GWAS) and Linkage Disequilibrium analysis have identified several pathogenic genes such as *MTHFR*, *FGF1*, *MSX1*, *IRF6*, *VAX1*, and *SUMO1* to associate with NSCL/P <sup>10</sup>. Recently, GWAS has con-

firming that the SNPs of *PTCH1* (rs10512248) and *RAD54B* (rs12681366) are associated with NSCL/P in different populations, including Chinese, Africans, and Irish<sup>11-13</sup>.

*PTCH1* is located on chromosome 9q22.3 and is responsible for producing the patched-1 protein, a receptor for the sonic hedgehog ligand<sup>14</sup>. Patched-1 and Sonic Hedgehog are part of a complex process that defines the form of different parts of the developing human body and craniofacial morphogenesis<sup>10</sup>. Previous research on mice has displayed that *PTCH1* might influence the fusion of facial processes when the orofacial region is developing to its normal form<sup>15,16</sup>. Metzis V *et al* showed that deletion of *PTCH1* in the mouse embryos alters cell morphology and causes defective nasal pit epithelium invagination and cleft lip<sup>17</sup>. Furthermore, *PTCH1* mutations can cause Gorlin–Goltz Syndrome, in which orofacial clefts are frequently observed. These findings indicate the essential role of *PTCH1* in forming calvaria and bone homeostasis<sup>18,19</sup>.

*RAD54B* is a protein that in humans is encoded by the *RAD54B* gene. This protein plays a vital role in the DNA damage repair<sup>20</sup>. The human *RAD54B* protein has been associated with NSCL/P<sup>13,14</sup>. The results of the GWAS done by X Liu *et al* suggested that the rs12681366 in *RAD54B* could decrease the risk of NSCL/P in a Northern Chinese population<sup>13</sup>. Qiao W *et al* found that the knockdown of *RAD54B* increased the sensitivity of Primary Mouse Embryonic Palatal Mesenchymal cells (MEPMs) to DNA double-strand break inducers<sup>21</sup>. On the other hand, overexpression of *RAD54B* could increase cell apoptosis and diminish cell proliferation<sup>22</sup>. Overall, these reports propose that *RAD54B* could have an essential regulatory role in NSCL/P incidence; although previous studies have found an association between *PTCH1* and *RAD54B* with NSCL/P in Irish, Africans, northern, and southern Chinese. It is necessary to figure out if these associations can also be detected in different populations. Therefore, the purpose of the present retrospective case-control study was to evaluate the relationship between the rs10512248 and rs12681366 with NSCL/P in the northeast of Iran.

## Materials and Methods

### Research subjects

This analytic cross-sectional study consisted of 120 cases with NSCL/P and 160 healthy subjects as control group. The study population was recruited between 2019-2020 from the Khayyam Hospital of Neishabur, Khorasan Razavi province, a city in the Northeast of Iran, in which previous reports have shown a high rate of NSCL/P. To exclude the syndromic forms of cleft lip/palate, oral and maxillofacial surgeons and medical geneticists precisely examined all cases to confirm that the cleft lip/palate is the only affecting disorder. The written consent and a questionnaire consisting of demographic information, family background of the spe-

cific disease, and parent's pregnancy history were also collected from all the subjects or their parents for the patients below 18 years old. This process was mandatory to rule out any probable prenatal contributory teratogenic factors that could have led patients to cleft lip/palate development.

The inclusion criteria for the control group subjects were as follows: (a) no cleft lip/palate or other inborn deformities, (b) no family history of congenital malformations, (c) no significant difference with the study group regarding age and sex, (d) no history of multiple miscarriages, cerebrovascular stroke, deep vein thrombosis, and cardiovascular diseases in the mother. In the case group, those NSCL/P patients with a mother's history of lack of iron or folic acid consumption during the pregnancy were excluded from the study.

### Ethical statement

The protocols of this study were approved by the Research Ethics Committee of the Dental Faculty of Shahid Beheshti University of Medical Sciences with the code number IR.SBMU.DRC.REC.1399.027.

### Sample collection

Blood samples were collected from all participants of both groups, or if not adults, by parents or legal guardians. Approximately 3 ml of peripheral venous blood was obtained from each subject. In order to prevent the blood samples from clotting, they were collected into EDTA-contained tubes (200  $\mu$ l 0.5-M EDTA) and stored at -80°C until DNA extraction. DNA was immediately extracted from the leukocytes of blood using the standard salting-out procedures<sup>23</sup>. The quantity and quality of extracted DNA samples were evaluated with the spectrophotometry and loaded in agarose gel, respectively. The concentration of DNA was assessed by measuring the Optical Density (OD) at the absorbance of 260/280 nm. Those samples whose absorbance ratio at 260 nm to 280 nm was 1.7-1.9 were considered pure DNA.

### Genotyping

Genotyping of *PTCH1* (rs10512248) and *RAD54B* (rs12681366) polymorphisms was determined using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The primer sequences are shown in table 1. Briefly, PCR was carried out into a 0.5 ml PCR microtube in a final volume of 25  $\mu$ l comprising 5  $\mu$ mol of each primer, 1  $\mu$ l of dNTPs mix (Bioron, GmbH®, Germany), 30 ng of template DNA, 2.5  $\mu$ l of 10 $\times$ PCR buffer (Bioron, GmbH®, Germany), 1.5 mM MgCl<sub>2</sub>, and 0.5 U of Taq DNA polymerase (CinnaGen, Iran).

The cycling conditions for *PTCH1* and *RAD54B* began with a denaturation step at 95°C for 4 min, followed by 33 cycles of 45 s of denaturation at 94°C, annealing temperature for 30 s at 60°C, and 40 s at 72°C, with a terminal elongation step of 5 min at 72°C. Subsequently, the cycles ended with a chilling phase to 4°C. Restriction digestion of PCR products was per-

## Association between Two Novel SNPs and NSCL/P

Table 1. Primer sequences and their related sizes for each polymorphism

Gene	Primer Sequences (5'→3')	Product Size (bp)	RFLP Fragments (bp)
<i>PTCHI</i> (rs10512248)	F: AGCCTCCAAGATGACCTCC R: CCAATTCCTGTGCATTGCTG	293	T allele: 155+43+95 C allele: 198+95
<i>RAD54B</i> (rs12681366)	F: GCTGGCTGCTTTAGGTTAGC R: TTCACCAGACACCTTCTGTTAG	285	Allele: 285 C allele: 234+51

formed using Eco571(*PTCHI*) and NlaIII (*RAD54B*) enzymes at 37°C overnight. Electrophoresis on 2% agarose gels was used in order to separate DNA fragments (Figures 1 and 2).

### Statistical analysis

All statistical analyses of the data were accomplished using SPSS software version 18.0 (SPSS Inc, Chicago IL, USA). A standard chi-square test ( $\chi^2$ ) was performed to compare the frequencies of genotypes and alleles between the case and control groups. The  $p < 0.05$  was considered statistically significant. Odds ratios with 95% confidence intervals by unconditional logistic regression analyses were also utilized to estimate the relative relation between *PTCHI* and *RAD54B* polymorphisms and the NSCL/P. Also, the independent sample t-test was used to compare the quantitative variables between groups.  $p < 0.05$  was considered statistically significant.

### Results

A total of 122 NSCL/P subjects (71 males, 51 females) and 161 healthy controls (94 males, 67 females) were recruited for our study. All patients were from Neyshabur city. The distributions of genotypes among the NSCL/P cases and controls revealed that females and males in case and control groups were in Hardy-Weinberg equilibrium. There were no statistically significant differences between the two groups regarding age and sex ( $p = 0.975$ ). According to the independent t-test results, case and control groups had no significant differences in mean maternal age ( $26.92 \pm 6.12$  and

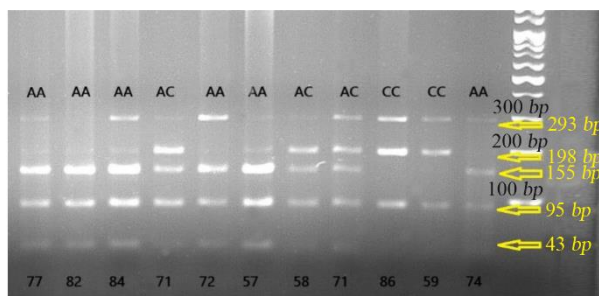


Figure 1. Photograph of RFLP method of *PTCHI* (rs10512248) polymorphism: after digestion of PCR products with the restriction enzyme Eco571 for three genotypes of NSCL/P cases, one specific band of 155 bp was indicated in AA genotype, two specific bands of 198 and 155 bp were revealed in the AC genotype, and one specific band of 198 bp was indicated in CC genotype.

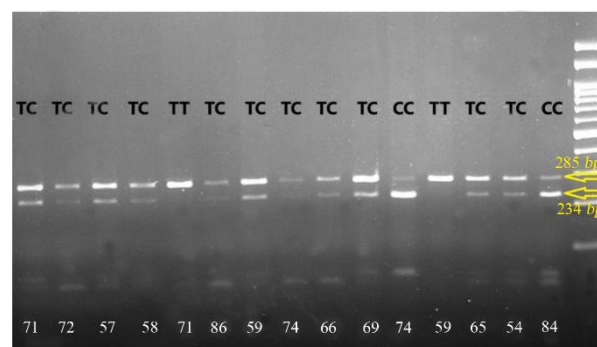


Figure 2. Photograph of RFLP method of *RAD54B* (rs12681366) polymorphism. After digestion of PCR products with the restriction enzyme NlaIII for three genotypes of NSCL/P cases, one specific band of 285 bp was indicated in TT genotype, two specific bands of 285 and 234 bp were revealed in the TC genotype, and one specific band of 234 bp was indicated in CC genotype.

$26.84 \pm 6.32$  yrs, respectively) and participants' age ( $19.71 \pm 9.22$  and  $18.94 \pm 9.182$  yrs, respectively). The distribution of genotypes and allele frequencies of the *PTCHI* and *RAD54B* Polymorphisms in the affected subjects and controls are presented in tables 2 and 3.

The obtained results showed no significant difference between NSCL/P patients and the control group regarding *PTCHI* (rs10512248) and *RAD54B* (rs12681366) polymorphisms in allele and genotype frequencies. In rs10512248, C allele frequency was higher in the control group (17.1%) compared with the case group (15.2%). According to univariate logistic regression analysis, CC and AA genotypes in the case group were higher than the control group. So, the AC genotype could correlate to a lower incidence of NSCL/P in the population than the AA genotype, however, the results were not statistically significant ( $p = 0.615$ ).

As for *RAD54B* (rs12681366), the frequency of the T allele (69.3) compared with C (30.7) was higher within the case group. Also, the C allele was higher in the control group (32.3%) compared with the case group (30.7%). About the genotypes, in the case group, the frequency of homozygote genotypes (TT, CC) was higher than in the control group. However, the heterozygote genotype (TC) of the case group (45.1%) was lower than the control (53.4%). These results were not significant ( $p = 0.334$ ).

Table 2. Allele frequency and genotype distribution of the *PTCH1* polymorphisms in case and control groups

Polymorphism	Case group n (%)	Control group n (%)	OR (95%CI)	p-value
<b>PTCH1 (rs10512248)</b>				
Allele				
A	267 (82.9%)	207 (84.8%)	1	-
C	55 (17.1%)	37 (15.2%)	1.15 (0.7-1.8)	0.541
<b>Genotype</b>				
AA	113 (70.2%)	91 (74.6%)	1	-
AC	41 (25.5%)	25 (20.5%)	0.87 (0.47-1.61)	0.669
CC	7 (4.3%)	6 (4.9%)	1.41 (0.42-4.71)	0.572
<b>Dominant</b>				
AA	113 (70.2%)	91 (74.6%)	1	0.628
AC+CC	48 (29.8%)	31 (25.4%)	0.9 (0.5- 1.5)	
<b>Recessive</b>				
CC	7 (4.3%)	6 (4.9%)	1	0.820
AC+AA	154 (95.7%)	116 (95.1%)	0.9 (0.3- 2.7)	

Table 3. Allele frequency and genotype distribution of the *RAD54B* polymorphisms in case and control groups

Polymorphism	Case group n (%)	Control group n (%)	OR (95%CI)	p-value
<b>RAD54B (rs12681366)</b>				
Allele				
T	218 (67.7%)	169 (69.3%)	1	-
C	104 (32.3%)	75 (30.7%)	1.1 (0.75-1.5)	0.693
<b>Genotype</b>				
TT	66 (41.0%)	57 (46.7%)	1	-
TC	86 (53.4%)	55 (45.1%)	0.64 (0.37-1.09)	0.102
CC	9 (5.6%)	10 (8.2%)	1.69 (0.61-4.67)	0.309
<b>Dominant</b>				
TT	66 (41%)	57 (46.7%)	1	0.335
TC+ CC	95 (59%)	65 (53.3%)	1.26 (0.78- 2)	
<b>Recessive</b>				
CC	9 (5.6%)	10 (8.2%)	1	0.385
TC+ TT	152 (94.4%)	112 (91.8%)	1.5 (0.6- 4)	

## Discussion

Although the cleft lip and palate are among the most common congenital malformation in the craniofacial region, their prevalence differs among various geographical origins and ethnic groups<sup>24</sup>. Both genetics and environmental factors are essential for assuming the main risk factors of NSCL/P. Previous studies have noted the association between *BMP4* (rs17563), *RFC1* (rs1051266), *MTHFR* (C677T), *MSX1* (rs12532), *FGF1* (rs34010), *CDH1* (rs16260), and *DHFR 19-bp* insertion/deletion polymorphisms with NSCL/P in the southeast of Iran<sup>25-30</sup>. The current study was the first

investigation of the association between *PTCH1* (rs-10512248) and *RAD54B* (rs12681366) in the northeast Iranian population with NSCL/P. The results of the previous studies on the Irish, northern, and southern Chinese populations revealed the potential association between these two SNPs and NSCL/P<sup>11-14</sup>. According to our results, no significant differences were found between the genetic polymorphism of rs10512248 and rs12681366 with NSCL/P in our population.

*PTCH1* is a receptor for sonic hedgehog, a signaling molecule with an essential regulatory role in craniofacial morphogenesis, including palatal or labial clefts<sup>31</sup>.

In our study, the AA and CC genotypes frequency of *PTCH1* was higher in the case group (74.6%-4.9%) than in the control group (70.2%-4.3%). On the other hand, the heterozygote genotype (AC) had more frequency in the control group (25.5%) than in the case (20.5%). Although these findings were not statistically significant in the present study ( $p=0.615$ ), our results are in line with the research of Liu X *et al*<sup>13</sup>. Their study suggested that the rs10512248 AC genotype could decrease the risk of NSCL/P ( $p=0.020$ ) compared to the AA genotype in the northern Chinese population. However, it needs to be stated that after applying the Bonferroni correction in the mentioned study, the AC genotype did not remain a significant risk factor for NSCL/P. When evaluating differences between case-control and phenotypes, several hypothetical tests are often arranged. Multiple comparison tests can lead to a misunderstanding. Even in the absence of a significant result, there is a 64% chance of seeing a significant result, even if the tests themselves are insignificant. One method of reducing the chances of witnessing a significant result ( $p<0.05$ ) is Bonferroni Correction<sup>32</sup>.

*RAD54B*, an essential DNA damage repair protein, is located on 8p22.1 and belongs to the SWI2/SNF2 helicase superfamily, which modulates the DNA damage checkpoint response and homologous recombination repair pathway<sup>33,34</sup>. Abnormal expression of *RAD54B* has been shown to be related to different types of cancers, such as colorectal cancer, lymphoma<sup>35</sup>, and lung adenocarcinoma<sup>36</sup>. Therefore, understanding the probable effect of *RAD54B* polymorphism on NSCL/P is critical. For rs12681366, the alleles and genotypes frequencies in the case and control subjects were not significantly different in the present study. The frequencies of the TT and CC genotypes among the cases (46.7%-8.2%) were higher than the controls (41.0%-5.6%). Also, the control group's CT genotype (53.4%) had more frequency than the case group (45.1%). In the Liu X *et al* study, the CT genotype in controls (54%) was significantly higher ( $p=0.001$ ) than in cases (46%). This result could suggest that the rs-12681366 polymorphism might decrease NSCL/P risk in the northern Chinese population. Though, this cannot be applicable to the Iranians, based on our study.

The difference observed in our results compared to Liu X *et al*<sup>13</sup> may be due to the different sample sizes (122 patients and 161 healthy members in the present study vs. 596 patients and 466 healthy members) and possible environmental and geographical diversity. Because as mentioned before, NSCL/P is a multifactorial disorder which means that besides the several gene polymorphisms, environmental factors can also alter the risk of incidence<sup>4</sup>. Furthermore, previous studies have shown that the same genetic markers in different ethnic groups do not necessarily lead to the same phenotypic results in the head and neck<sup>37,38</sup>. Therefore, the difference seen in the Chinese and Iranian populations in the *PTCH1* (rs10512248) and *RAD54B* (rs126813-

66) can suggest that *PTCH1* and *RAD54B* may be a multiethnic marker in NSCL/P.

Our current study has some limitations. First, our study's sample size could be a reason why our results were not significantly different in our population. Second, only one variant of the *PTCH1* (rs10512248) and *RAD54B* (rs12681366) was evaluated in this study, other variants of *PTCH1* and *RAD54B* should be considered for further assessment. Third, we assessed only one region and one ethnic group of patients, so the effect of ethnicity could not be evaluated. Future multi-center research with larger samples from different ethnicities is required.

### Conclusion

In conclusion, we performed this study to assess the probable association of *PTCH1* and *RAD54B* gene polymorphisms with NSCL/P in the northeast Iranian population. Our results did not support this hypothesis. Although the genetic component of NSCL/P etiology has received widespread attention in recent years, most genetic variants contributing to this congenital craniofacial malformation are yet to be discovered. Our study is the first report evaluating the relation between rs-10512248 and rs12681366 with NSCL/P in our population. Further research with larger sample sizes and different methods such as genome-wide association studies should be considered.

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### Ethical Approval

The survey was approved by the Ethics Committee of the Dental Faculty of Shahid Beheshti University of Medical Sciences (Code No. IR.SBMU.DRC.REC.1399.027). The study was also preceded by completing written consent forms by all participants or their parents.

### References

1. Mangold E, Ludwig KU, Nöthen MM. Breakthroughs in the genetics of orofacial clefting. *Trends Mol Med* 2011; 17(12):725-33.
2. Machado RA, Martelli-Junior H, Reis SRdA, Küchler EC, Scariot R, das Neves LT, et al. Identification of novel variants in cleft palate-associated genes in Brazilian patients with non-syndromic cleft palate only. *Front Cell Dev Biol* 2021;9:638522.
3. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. *Lancet* 2009;374(9703):1773-85.
4. van Rooij IA, Ludwig KU, Welzenbach J, Ishorst N, Thonissen M, Galesloot TE, et al. Non-syndromic cleft

- lip with or without cleft palate: genome-wide association study in europeans identifies a suggestive risk locus at 16p12.1 and supports SH3PXD2A as a clefting susceptibility gene. *Genes* 2019;10(12):1023.
5. Auerkari EI, Bilynov Y, Yuniastuti M, Listyowati L, Sulistyani LD. Association of a polymorphism in the gene encoding methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) 1958G> A with orofacial cleft. *Pesqui Bras Odontopediatria Clin Integr* 2021;21:e5896.
  6. Murthy J, Bhaskar L. Current concepts in genetics of nonsyndromic clefts. *Indian J Plast Surg* 2009;42(1):68-81.
  7. Saber K, Amir Mansour S, Mozafar K, Farid N. Incidence of cleft lip and palate in Iran. A meta-analysis. *Saudi Med J* 2011;32:390-3.
  8. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet* 2011;12(3):167-78.
  9. Indencleef K, Roosenboom J, Hoskens H, White JD, Shriver MD, Richmond S, et al. Six NSCL/P loci show associations with normal-range craniofacial variation. *Front Genet* 2018;9:502.
  10. Reynolds K, Zhang S, Sun B, Garland MA, Ji Y, Zhou CJ. Genetics and signaling mechanisms of orofacial clefts. *Birth Defects Res* 2020;112(19):1588-634.
  11. Butali A, Mossey PA, Adeyemo WL, Eshete MA, Gowans LJ, Busch TD, et al. Genomic analyses in African populations identify novel risk loci for cleft palate. *Hum Mol Genet* 2019;28(6):1038-51.
  12. Carter TC, Molloy AM, Pangilinan F, Troendle JF, Kirke PN, Conley MR, et al. Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. *Birth Defects Res A Clin Mol Teratol* 2010; 88(2):84-93.
  13. Liu X, Yang S, Meng L, Chen C, Hui X, Jiang Y, et al. Association between PTCH1 and RAD54B single-nucleotide polymorphisms and non-syndromic orofacial clefts in a northern Chinese population. *J Gene Med* 2018;20(12):e3055.
  14. Yu Y, Zuo X, He M, Gao J, Fu Y, Qin C, et al. Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. *Nat Commun* 2017;8:14364.
  15. Juriloff DM, Harris MJ, Mager DL, Gagnier L. Epigenetic mechanism causes Wnt9b deficiency and non-syndromic cleft lip and palate in the A/WySn mouse strain. *Birth Defects Res A Clin Mol Teratol* 2014;100 (10):772-88.
  16. Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, et al. Impaired FGF signaling contributes to cleft lip and palate. *Proc Natl Acad Sci USA* 2007;104(11):4512-7.
  17. Metzis V, Courtney AD, Kerr MC, Ferguson C, Rondón Galeano MC, Parton RG, et al. Patched1 is required in neural crest cells for the prevention of orofacial clefts. *Hum Mol Genet* 2013;22(24):5026-35.
  18. Larsen AK, Mikkelsen DB, Hertz JM, Bygum A. Manifestations of Gorlin-Goltz syndrome. *Dan Med J* 2014; 61(5):A4829.
  19. Muzio LL. Nevoid basal cell carcinoma syndrome (Gorlin syndrome). *Orphanet J Rare Dis* 2008;3:32.
  20. Nagai Y, Yamamoto Y, Yasuhara T, Hata K, Nishikawa T, Tanaka T, et al. High RAD54B expression: an independent predictor of postoperative distant recurrence in colorectal cancer patients. *Oncotarget* 2015;6(25): 21064-73.
  21. Qiao W, Huang P, Wang X, Meng L. Susceptibility to DNA damage caused by abrogation of Rad54 homolog B: A putative mechanism for chemically induced cleft palate. *Toxicology* 2021;456:152772.
  22. Wang R, Li Y, Chen Y, Wang L, Wu Q, Guo Y, et al. Inhibition of RAD54B suppresses proliferation and promotes apoptosis in hepatoma cells. *Oncol Rep* 2018;40 (3):1233-42.
  23. Rivero ER, Neves AC, Silva-Valenzuela MG, Sousa SO, Nunes FD. Simple salting-out method for DNA extraction from formalin-fixed, paraffin-embedded tissues. *Pathol Res Pract* 2006;202(7):523-9.
  24. Elahi MM, Jackson IT, Elahi O, Khan AH, Mubarak F, Tariq GB, et al. Epidemiology of cleft lip and cleft palate in Pakistan. *Plast Reconstr Surg* 2004;113(6):1548-55.
  25. Rafiqdoost H, Hashemi M, Danesh H, Bizhani F, Bahari G, Taheri M. Association of single nucleotide polymorphisms in AXIN2, BMP4, and IRF6 with Non-Syndromic Cleft Lip with or without Cleft Palate in a sample of the southeast Iranian population. *J Appl Oral Sci* 2017;25:650-6.
  26. Soghani B, Ebadifar A, Khorshid HRK, Kamali K, Hamedi R, Moghadam FA. The study of association between reduced folate carrier 1 (RFC1) polymorphism and non-syndromic cleft lip/palate in Iranian population. *BioImpacts* 2017;7(4):263-8.
  27. Ebadifar A, Ameli N, Khorramkhorshid HR, Zeinabadi MS, Kamali K, Khoshbakht T. Incidence assessment of MTHFR C677T and A1298C polymorphisms in Iranian non-syndromic cleft lip and/or palate patients. *J Dent Res Dent Clin Dent Prospects* 2015;9(2):101-4.
  28. Rafiqdoost H, Hashemi M, Narouei A, Eskandari-Nasab E, Dashti-Khadivaki G, Taheri M. Association between CDH1 and MSX1 gene polymorphisms and the risk of nonsyndromic cleft lip and/or cleft palate in a southeast Iranian population. *Cleft Palate Craniofac J* 2013;50(5): 98-104.
  29. Rafiqdoost Z, Rafiqdoost A, Rafiqdoost H, Hashemi M, Khayatzadeh J, Eskandari-Nasab E. Investigation of FGF1 and FGFR gene polymorphisms in a group of Iranian patients with nonsyndromic cleft lip with or without cleft palate. *Int J Pediatr Otorhinolaryngol* 2014;78(5):731-6.
  30. Rafiqdoost F, Rafiqdoost A, Rafiqdoost H, Rigi-Ladez M-A, Hashemi M, Eskandari-Nasab E. The 19-bp deletion polymorphism of dihydrofolate reductase (DHFR) and nonsyndromic cleft lip with or without cleft palate: evidence for a protective role. *J Appl Oral Sci* 2015;23:272-8.
  31. Cui D, Li L, Lou H, Sun H, Ngai S, Shao G, et al. The ribosomal protein S26 regulates p53 activity in response to DNA damage. *Oncogene* 2014;33(17):2225-35.

32. Sham PC, Purcell SM. Statistical power and significance testing in large-scale genetic studies. *Nat Rev Genet* 2014;15(5):335-46.
33. Yasuhara T, Suzuki T, Katsura M, Miyagawa K. Rad54B serves as a scaffold in the DNA damage response that limits checkpoint strength. *Nat Commun* 2014;5:5426.
34. Feng S, Liu J, Hailiang L, Wen J, Zhao Y, Li X, et al. Amplification of RAD54B promotes progression of hepatocellular carcinoma via activating the Wnt/ $\beta$ -catenin signaling. *Transl Oncol* 2021;14(8):101124.
35. Hiramoto T, Nakanishi T, Sumiyoshi T, Fukuda T, Matsuura S, Tauchi H, et al. Mutations of a novel human RAD54 homologue, RAD54B, in primary cancer. *Oncogene* 1999;18(22):3422-6.
36. Chang JG, Chen CC, Wu YY, Che TF, Huang YS, Yeh KT, et al. Uncovering synthetic lethal interactions for therapeutic targets and predictive markers in lung adenocarcinoma. *Oncotarget* 2016;7(45):73664-80.
37. Dalaie K, Yassaee VR, Behnaz M, Yazdani M, Jafari F, Farimani RM. Relationship of the rs10850110 and rs11611277 polymorphisms of the MYO1H gene with non-syndromic mandibular prognathism in the Iranian population. *Dent Med Probl* 2020;57(4):433-40.
38. Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol* 2009;10(3):241-7.