Polymorphisms of ATP-binding cassette, sub-family A, member 4 (rs560426 and rs481931) and non-syndromic cleft lip/palate: A systematic review and meta-analysis

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Research article

Keywords: ABCA4; Polymorphism; Variation; Non-syndromic cleft lip/palate; Meta-analysis

Posted Date: November 7th, 2019

DOI: <https://doi.org/10.21203/rs.2.16984/v1>

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Abstract

Background A number of gene loci are closely associated with the incidence of non-syndromic cleft lip/palate (NSCL/P). Herein, this meta-analysis assessed the association between ATP-binding cassette, sub-family A, member 4 (ABCA4) polymorphisms (rs560426 and rs481931) and the risk of NSCL/P by reviewing case-control studies. **Methods** Four databases including Scopus, Cochrane Library, Web of Science, and PubMed were searched for articles published up to December 2018. The Review Manager 5.3 software was used to calculate the crude odds ratio (OR) and 95% confidence interval (CI). **Results** Of 82 retrieved studies, 12 were analyzed in this meta-analysis (2,859 NSCL/P patients and 3,792 controls for ABCA4 rs560426 polymorphism and 1,333 NSCL/P patients and 1,884 controls for ABCA4 rs481931 polymorphism). There was no significant association between the polymorphisms and the risk of NSCL/P, with the exception of the allelic model (OR=1.13; 95% CI: 1.01, 1.27; p=0.03), the homozygote model (OR=1.53; 95% CI: 1.01, 2.31; p=0.04), and the recessive model (OR=1.30; 95% CI: 1.03, 1.63; p=0.03) in the Asian ethnicity for rs560426 polymorphism. **Conclusion** The findings confirmed that G allele and GG genotype of rs560426 polymorphism were significantly associated with the risk of NSCL/P in the Asian population, but not for rs481931 polymorphism. Also, there was no association between both polymorphisms (rs560426 and rs481931) with the risk of NSCL/P in the Caucasian and the mixed ethnicities, as well as the source of controls. Therefore, ethnicity may play a significant role in this association.

Introduction

Non-syndromic cleft lip/palate (NSCL/P) is a usual congenital malformation that accounts for more than 70% of facial cleft deformities [1]. Its prevalence ranges from 1/700 to 1/1000, depending on the geographical area and ethnicity [2]. NSCL/P has a multifactorial etiology and therefore both environmental and genetic risk factors can affect its incidence and prevalence [1,3]. Maternal smoking, alcohol consumption, and folic acid and vitamin deficiencies especially during the first trimester of pregnancy have been reported to increase the incidence of NSCL/P [4]. Considering the complex etiology of NSCL/P, studies have shown that interactions of gene-gene and gene-environment may be related to susceptibility to NSCL/P [3,5,6]. Subsequent genome-wide association studies showed that a number of gene loci are closely associated with the incidence of NSCL/P [7,8]. ATP-binding cassette, sub-family A, member 4 (*ABCA4*) belongs to the transmembrane protein superfamily [9], which is located on chromosome 1p22.1 [10,11]. The *ABCA4* is an ATP-binding cassette transporter that is particularly expressed in the rod and cone photoreceptor cells of the vertebrate retina [9-11] and is associated with various ocular diseases [12]. A systematic review reported the significant presence of ocular abnormalities in patients with NSCL/P [13]. The genetic basis of oral clefts is still unclear, and identification of additional risk factors for NSCL/P can greatly help in genetic counseling and may prevent the occurrence or further development of this condition [14]. Therefore, the present meta-analysis aimed to evaluate the association between *ABCA4* polymorphisms (rs560426 and rs481931) and the risk of NSCL/P in case-control studies to assess the possible role of these polymorphisms in NSCL/P etiology.

Materials And Methods

The instructions of the PRISMA were applied to design this study [15]. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (ethical code: IR.KUMS.REC.1398.321).

Literature search strategy

Four electronic databases including the Web of Science, Cochrane Library, Scopus, and PubMed were searched for articles published until December 2018 without restrictions of language or publication date. The searched terms were: ("ABCA4" or "ATP-binding cassette subfamily A member 4" or "rs560426" or "rs481931") and ("cleft" or "cleft lip" or

“cleft palate” or “orofacial cleft” or “oral cleft”). In addition, we checked the references of retrieved articles to find potential articles.

Eligibility criteria

One reviewer (M.S) retrieved the studies from the databases. Then, he excluded duplicate and irrelevant studies. The studies were relevant if they met the following eligibility criteria: (I) case-control design; (II) NSCL/P was the outcome of interest; (III) reporting *ABCA4* rs560426 (A > G) and/or rs481931 (C > A) polymorphisms, and (IV) having the required data to calculate the odds ratios (ORs) with 95% confidence intervals (CIs). The studies were excluded if they did not have the required data regarding genotype distributions or were animal studies, review articles, letters to the editor reporting previous studies, and family-based studies. The second reviewer (M.M.I) checked the relevant articles based on the eligibility criteria. The differences between two reviewers were solved by the third reviewer (E.P.-F.L).

Data extraction

Two reviewers (P.L.-J and M.S) independently extracted the data from each study. The information retrieved from the studies involved: the first author's name, the publication year, the ethnic group, the source of controls, the mean age, the number of males in the two groups, the type of disease, the number of patients and controls with each genotype, the genotyping method, and the *p*-value of Hardy-Weinberg equilibrium in controls. If there was a disagreement between the reviewers, the problem was solved by discussion.

Quality of assessment

One reviewer (M.M.I) distinguished the quality of each included article using the Newcastle-Ottawa Quality Assessment Scale questionnaire (maximum total score = 9 for a case-control study) [16].

Statistical analyses

An analysis was performed by the Review Manager 5.3 using crude OR and 95% CI to show the association between *ABCA4* polymorphisms and the risk of NSCL/P in the five genetic models [17]. The Z test was applied to evaluate the pooled OR significance. Heterogeneity across the studies was checked by the Cochrane Q test and I^2 statistic that there was a statistically significant heterogeneity if $P < 0.1$ or $I^2 > 50\%$. If there was no significant heterogeneity, the fixed-effects model (Mantel-Haenszel method) was used to estimate the values. Otherwise, we used the random-effects model (DerSimonian and Laird method). The Chi-square test was used to calculate the Hardy-Weinberg equilibrium in the control group of each study. Subgroup analysis was done according to the ethnicity and the source of controls. The Comprehensive Meta-Analysis 2.0 was used to analysis funnel plot using the Egger's and Begg's tests and $P < 0.05$ indicated significant existence of the publication bias. To evaluate the stability of the results, we used sensitivity analysis.

Results

Study selection

Of 82 retrieved studies, after removing duplicate and irrelevant studies, the full-texts of 21 studies were evaluated (**Figure 1**). Of 21 studies, nine were excluded with below reasons: three were family-based studies, three did not have the required data, two were letters to the editor reporting previous studies, and one evaluated the mothers of children. Finally, 12 studies were analyzed in this meta-analysis.

Study characteristics

Table 1 shows some features of the studies included in this meta-analysis that the studies were published from 2011 to 2018. Five studies [6,18-21] reported the risk of NSCL/P related to *ABCA4* polymorphisms in the Asian ethnicity, four [5,7,14,22] in the mixed ethnicity, and three [23-25] in the Caucasian ethnicity. The source of controls in six studies [14,18,19,21,23,24] was hospital-based while six other studies [5-7,20,22,25] were population-based. Some studies reported the mean age, the number of males in the two groups, and the type of disease. Of 12 studies, all of them reported *ABCA4* rs560426 polymorphism, but only six studies [5,6,21,23-25] reported *ABCA4* rs481931 polymorphism. There were different genotyping methods among the studies presented in **Table 1**. The Hardy-Weinberg equilibrium was not seen in controls of two studies [6,19] reporting *ABCA4* rs560426 polymorphism while two studies [6,24] reported *ABCA4* rs481931 polymorphism. This meta-analysis included 2,859 NSCL/P patients and 3,792 controls reporting *ABCA4* rs560426 polymorphism and 1,333 NSCL/P patients and 1,884 controls reporting *ABCA4* rs481931 polymorphism.

Pooled analysis

Figure 2 shows the pooled results (combining data from each individual study included) of the risk of NSCL/P related to *ABCA4* rs560426 polymorphism based on twelve studies. The heterogeneity in allelic, homozygote, heterozygote, and dominant models was high ($I^2 > 50\%$) and therefore random-effects model was used, but in recessive model, fixed-effects model was used because of low heterogeneity ($I^2 < 50\%$). The pooled OR was 1.01 [95% CI: 0.88, 1.15; $p=0.92$; $I^2=72\%$ (P_h or $P_{\text{heterogeneity}} < 0.0001$)] in the allelic model (G vs. A), 1.08 [95% CI: 0.79, 1.47; $p=0.64$; $I^2=77\%$ ($P_h < 0.00001$)] in the homozygote model (GG vs. AA), 0.93 [95% CI: 0.73, 1.17; $p=0.53$; $I^2=76\%$ ($P_h < 0.00001$)] in the heterozygote model (AG vs. AA), 0.89 [95% CI: 0.70, 1.14; $p=0.37$; $I^2=81\%$ ($P_h < 0.00001$)] in the dominant model (AG + GG vs. AA), and 1.08 [95% CI: 0.91, 1.26; $p=0.38$; $I^2=36\%$ ($P_h=0.10$)] in the recessive model (GG vs. AA + AG). Based on the genetic models, there was no the risk of NSCL/P related to *ABCA4* rs560426 polymorphism.

The pooled results of the significant risk of NSCL/P related to *ABCA4* rs481931 polymorphism according to six studies is shown in **Figure 3**. The heterogeneity in all analyses was low and therefore fixed-effects model was used ($I^2 < 50\%$). The pooled OR was 0.92 [95% CI: 0.83, 1.02; $p=0.12$; $I^2=35\%$ ($P_h=0.17$)] in the allelic model (A vs. C), 0.85 [95% CI: 0.68, 1.05; $p=0.13$; $I^2=33\%$ ($P_h=0.19$)] in the homozygote model (AA vs. CC), 0.88 [95% CI: 0.75, 1.03; $p=0.12$; $I^2=16\%$ ($P_h=0.31$)] in the heterozygote model (CA vs. CC), 0.87 [95% CI: 0.75, 1.00; $p=0.06$; $I^2=29\%$ ($P_h=0.22$)] in the dominant model (CA + AA vs. CC), and 0.89 [95% CI: 0.74, 1.09; $p=0.26$; $I^2=22\%$ ($P_h=0.27$)] in the recessive model (AA vs. CC + CA). Based on the genetic models, there was no the significant risk of NSCL/P related to *ABCA4* rs481931 polymorphism.

Subgroup analysis

The first subgroup analysis evaluated the pooled analysis based on the ethnicity and the source of controls for the significant risk of NSCL/P related to *ABCA4* rs560426 polymorphism (**Table 2**) that there was only a significant risk in the Asian ethnicity based on the allelic model (OR=1.13; 95% CI: 1.01, 1.27; $p=0.03$), the homozygote model (OR=1.53; 95% CI: 1.01, 2.31; $p=0.04$), and the recessive model (OR=1.30; 95% CI: 1.03, 1.63; $p=0.03$). The results didn't show the risk of NSCL/P related to in rs560426 polymorphism in the Caucasian and the mixed ethnicities, as well as the studies including hospital-based and population-based controls based on the models.

The second subgroup analysis assessed the risk of NSCL/P related to *ABCA4* rs481931 polymorphism according to the ethnicity and the source of controls (**Table 3**). There was no association between the significant risk of NSCL/P and rs481931 polymorphism in the Asian, the Caucasian, and the mixed ethnicities, as well as the studies including hospital-based and population-based controls based on the models.

Sensitivity analysis

One study was removed and the cumulative analysis identified that the pooled ORs under all genetic models were stable and trustworthy. As well as, excluding two studies [6,19] that had p -values <0.05 for Hardy-Weinberg equilibrium in controls did not change the overall result regarding the risk of NSCL/P related to *ABCA4* rs560426 polymorphism. Excluding two other studies[6,24] did not change the overall result regarding the risk of NSCL/P related to *ABCA4* rs481931 polymorphism.

Quality assessment

A mean score of 7.33 was achieved for the quality of all studies (**Table 4**). Out of 12 studies, ten studies had score ≥ 7 and two studies had score < 7 .

Publication bias

Figure 4 shows the funnel plot of the association between *ABCA4* polymorphisms and the risk of NSCL/P using the five genetic models. The Egger's and Begg's tests did not reveal any publication bias except for 4 states: Both tests for A vs. C and AA vs. CC revealed a publication bias and Egger's test for CA vs. CC and CA + AA vs. CC revealed a publication bias ($p<0.05$).

Discussion

The *ABCA4* gene mutations have been associated with a range of autosomal recessive retinal degenerative diseases [24]. NSCL/P is a complex congenital anomaly that shows both clinical and genetic heterogeneity and the genetic basis of NSCL/P have remained unclear [14]. Among the studies reviewed in this meta-analysis, the rs560426 G allele showed 1.36- [21] and 1.34-fold [22] increase in the risk of CL/P compared to the A allele and the rs560426 GG genotype showed 1.74- [19], 3- [20], 1.86- [21], and 1.72-fold [22] increase in the risk of NSCL/P compared to the AA genotype. In contrast, the G allele, GG genotype [23], AG genotype [5,23], A allele [23], AA genotype [24], and CA genotype [23] showed a lower risk of NSCL/P compared to the A allele, AA genotype, AA genotype, C allele, CC genotype, and CC genotype, respectively. This meta-analysis did not exhibit any significant risk of NSCL/P related to *ABCA4* polymorphisms (rs560426 and rs481931) except for the Asian ethnicity in which, the G allele and GG genotype identified a significant increase in the risk of NSCL/P compared to A allele and AA genotype, respectively. In addition to some studies mentioned in this meta-analysis, which indicated a link between the *ABCA4* gene and the NSCL/P risk, associations of this gene with CL/P in two genome-wide association studies [26,27] and in different populations [28-30] highlight the need for further studies on the possible role of *ABCA4* gene in the CL/P etiology. *Fontoura et al.[23] found significant associations of ABCA4 rs481931 alleles with bilateral and unilateral CL/P.* Replication of *ABCA4* polymorphisms using independent families from various populations revealed that the Asian families had stronger evidence for *ABCA4* compared to the European or American families [19]. The present meta-analysis confirmed this result.

One study [18] suggested that the strong correlation between *ABCA4* and CL/P may be due to the impact of nearby genes (such as *ARHGAP29*), and not the impact of single loci. It is probably that the associated variants in *ABCA4* are in close connection disequilibrium with a causal variant included in a neighboring gene, and act as indirect surrogates for a real etiologic variant in NSCL/P patients [23,24,29]. Gene-gene and gene-environment interactions showed that there was an interaction between *ABCA4* rs560426 polymorphism with folic acid consumption and V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B rs11696257 polymorphism [5]. The different rate of the G allele of rs560426 polymorphism in different populations shows that the polymorphism alone is not associated with NSCL/P development, and so, it may not be the causative *single-nucleotide polymorphism*. Therefore, this apparent incompatibility among populations may identify the complex genetic etiology of NSCL/P [21]. The transmission

analysis using case-parent core pedigrees showed that the C allele of rs481931 was significantly over-transmitted from parents to children, indicating that the C allele is associated with the NSCL/P risk. In addition, the C (rs481931)-G (rs560426) haplotype was significantly involved in the occurrence of NSCL/P [6].

The present meta-analysis had several limitations: a small number of studies reviewed, differences in age and sex of patients and genotyping methods, absence of Hardy-Weinberg equilibrium in some studies, a difference of sources of controls and ethnicities evaluated in the studies that created high heterogeneity in some analyses. However, sensitivity analysis identified the stability of the results and also the heterogeneity across the studies was minimal.

Conclusions

The findings of the present meta-analysis confirmed that the G allele and GG genotype of rs560426 polymorphism were significantly associated with the NSCL/P risk in the Asian population. There was no association between both polymorphisms (rs560426 and rs481931) with the risk of NSCL/P in the Caucasian and the mixed ethnicities, as well as the source of controls and even the Asian ethnicity for rs481931 polymorphism. Therefore, ethnicity may act as a significant factor in this association. Nevertheless, further studies are needed to check the association of different ethnic groups with special emphasis on the interactions of the *ABCA4* gene.

Abbreviations

NSCL/P: Non-syndromic cleft lip/palate

ABCA4: ATP-binding cassette, sub-family A, member 4

OR: Odds ratio

CI: Confidence interval

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (ethical code: IR.KUMS.REC.1398.321).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Kermanshah University of Medical Sciences, Kermanshah, Iran (Grant number: 3008230).

Authors' Contributions

M.M.I and Ma.S: study design, study concept, analysis of images, statistical analysis, data collection, data interpretation, coordination of the study, literature search. P.L.-J, Mo.S, and E.P.-F.L: critically revised and edited the manuscript before submission and coordination of the study. Ma.S: drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Tables

Table 1: Characteristics of the studies included in this meta-analysis (n=12)

First author, (year)	Ethnic group	Source of controls	Mean age, year (NSCL/P to control)	No. of males, (NSCL/P to control)	Type of disease (number of patients)	NSCL/P		Control		Genotyping method	P-value for HWE in controls
					CLO/CLP	AA/AG/GG	CC/CA/AA	AA/AG/GG	CC/CA/AA		
Pan et al. (2011) [18]	Asian	HB	5.54 to 5.49	246 to 242	174/197	145/175/51	NA	167/160/57	NA	TaqMan	0.071
Fontoura et al. (2012) [23]	Caucasian	HB	17.3 to 24.8	252 to 165	NA	116/118/ 86	184/155/44	74/203/123	154/192/57	TaqMan	0.542/0.818
Huang et al. (2012) [19]	Asian	HB	NA	169 to 203	69/231	135/126/39	NA	157/171/26	NA	MALDI-TOF MS (Sequenom)	0.024
Mostowska et al. (2012) [24]	Caucasian	HB	NA	NA	-/206	62/105/39	79/98/29	120/230/96	156/196/94	PCR-HRM	0.467/0.028
Bagordakis et al. (2013) [22]	Mixed	PB	NA	NA	105/194	74/140/85	NA	127/172/85	NA	Multiplex PCR	0.067
Zhong-wei et al. (2013) [20]	Asian	PB	NA	NA	69/112	54/91/36	NA	36/50/18	NA	TaqMan	0.928
Ludwig et al. (2014) [7]	Mixed	PB	NA	102 to 111	NA	37/73/33	NA	100/163/66	NA	MALDI-TOF MS (Sequenom)	0.977
do Rego Borges et al. (2015) [14]	Mixed	HB	NA	NA	86/207	76/152/65	NA	74/187/91	NA	TaqMan	0.223
Babu Gurramkonda et al. (2015) [25]	Caucasian	PB	NA	NA	NA	46/72/26	41/68/35	61/80/35	57/87/32	Kompetitive allele specific PCR (KASP)	0.348/0.905
Mi et al. (2015) [21]	Asian	HB	4.98 to 5.20	NA	NA	88/104/ 30	79/107/36	158/137/29	113/157/54	Mini-sequencing (SNAPSHOT)	0.928/0.965
Velázquez-Aragón et al. (2016) [5]	Mixed	PB	5.5 to 1.33	99 to 132	NA	44/56/32	32/71/27	54/137/68	71/131/53	Kompetitive allele specific PCR (KASP)	0.326/0.602
Wu et al. (2018) [6]	Asian	PB	NA	NA	NA	103/116/29	92/126/30	111/145/24	91/154/35	PCR-RFLP	0.014/0.015

Abbreviations: MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR-HRM, *polymerase chain reaction*; HRM, high resolution melting, RFLP, restriction fragment length polymorphism, CLO, cleft lip only; CLP, cleft lip and palate; NSCL/P, non-syndromic cleft lip/palate; HB, hospital-based; PB, population-based; HWE, Hardy-Weinberg equilibrium, NA, not available.

Table 2: Analysis of non-syndromic cleft lip/palate risk related to rs560426 polymorphism according to ethnicity and source of controls

Study (n)	G vs. A	GG vs. AA	AG vs. AA	AG + GG vs. AA	GG vs. AA + AG
	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h
Overall (12)	1.01 (0.88, 1.15), 72, <0.0001	1.08 (0.79, 1.47), 77, <0.00001	0.93 (0.73, 1.16), 76, <0.00001	0.89 (0.70, 1.14), 81, <0.00001	1.08 (0.91, 1.26), 36, 0.10
Ethnicity					
Asian (5)	1.13 (1.01, 1.27), 0, 0.59	1.53 (1.01, 2.31), 62, 0.03	1.08 (0.92, 1.26), 35, 0.19	1.13 (0.97, 1.32), 10, 0.35	1.30 (1.03, 1.63), 27, 0.24
Caucasian (3)	0.82 (0.63, 1.08), 71, 0.03	0.67 (0.42, 1.09), 64, 0.06	0.72 (0.36, 1.45), 88, 0.0002	0.55 (0.34, 0.88), 79, 0.009	0.85 (0.67, 1.07), 0, 0.98
Mixed (4)	1.00 (0.76, 1.31), 79, 0.003	0.99 (0.59, 1.68), 78, 0.004	0.92 (0.60, 1.42), 76, 0.006	0.94 (0.60, 1.49), 81, 0.001	1.07 (0.87, 1.30), 41, 0.17
Source of controls					
Hospital-based (6)	0.94 (0.86, 1.03), 80, 0.0001	0.83 (0.69, 1.01), 80, 0.0002	0.87 (0.76, 1.01), 84, <0.00001	0.89 (0.78, 1.02), 85, <0.00001	0.98 (0.83, 1.15), 57, 0.04
Population-based (6)	1.07 (0.91, 1.26), 52, 0.06	1.29 (0.86, 1.94), 66, 0.01	1.02 (0.76, 1.36), 60, 0.03	0.91 (0.62, 1.33), 80, 0.0002	1.18 (0.97, 1.44), 0, 0.63

Bold numbers mean statistically significant (P<0.05). P_h indicates P_{heterogeneity}. **Abbreviations:** OR, odds ratio; CI, confidence interval.

Table 3: Analysis of non-syndromic cleft lip/palate risk related to rs481931 polymorphism according to ethnicity and source of controls

Study (n)	A vs. C	AA vs. CC	CA vs. CC	CA + AA vs. CC	AA vs. CC + CA
	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h	OR (95%CI), I ² (%), P _h
Overall (6)	0.92 (0.83, 1.02), 35, 0.17	0.85 (0.68, 1.05), 33, 0.19	0.88 (0.75, 1.03), 16, 0.31	0.87 (0.75, 1.00), 0.29, 0.22	0.89 (0.74, 1.09), 22, 0.27
Ethnicity					
Asian (2)	0.94 (0.79, 1.12), 0, 0.65	0.90 (0.62, 1.32), 0, 0.76	0.89 (0.68, 1.16), 0, 0.49	0.89 (0.69, 1.15), 0, 0.51	0.97 (0.68, 1.36), 0, 0.99
Caucasian (3)	0.92 (0.71, 1.18), 68, 0.04	0.81 (0.48, 1.36), 67, 0.05	0.83 (0.67, 1.03), 46, 0.15	0.85 (0.62, 1.16), 56, 0.11	0.87 (0.55, 1.38), 66, 0.05
Mixed (1)	1.07 (0.79, 1.44)	1.13 (0.61, 2.11)	1.20 (0.72, 2.00)	1.18 (0.73, 1.92)	1.00 (0.59, 1.68)
Source of controls					
Hospital-based (2)	0.85 (0.67, 1.09), 57, 0.13	0.77 (0.55, 1.07), 21, 0.26	0.80 (0.56, 1.14), 55, 0.14	0.79 (0.55, 1.14), 61, 0.11	0.86 (0.63, 1.18), 0, 0.52
Population-based (4)	0.98 (0.86, 1.12), 11, 0.34	0.91 (0.68, 1.20), 47, 0.13	0.97, (0.79, 1.20), 0, 0.62	0.95 (0.78, 1.16), 0, 0.44	0.91 (0.71, 1.17), 49, 0.12

There was the *p*-value >0.05 in all analyses and P_h indicates P_{heterogeneity}. **Abbreviations:** OR, odds ratio; CI, confidence interval.

Table 4: Quality assessment scores for the studies included in this meta-analysis (n=12)

First author, (year)	Selection	Comparability	Exposure	Total points
Pan et al. (2011) [18]	***	**	***	8
Fontoura et al. (2012) [23]	***	-	***	6
Huang et al. (2012) [19]	***	*	***	7
Mostowska et al. (2012) [24]	***	**	***	8
Bagordakis et al. (2013) [22]	****	-	***	7
Zhong-wei et al. (2013) [20]	****	-	***	7
Ludwig et al. (2014) [7]	****	-	***	7
do Rego Borges et al. (2015) [14]	***	-	***	6
Babu Gurramkonda et al. (2015) [25]	****	**	***	9
Mi et al. (2015) [21]	***	**	***	8
Velázquez-Aragón et al. (2016) [5]	****	*	***	8
Wu et al. (2018) [6]	****	-	***	7

Each asterisk indicates one point.

Figures

Figure 1

PRISMA flow-chart of the study selection

Figure 2

Forest plot of NSCL/P risk related to ABCA4 rs560426 polymorphism using the five genetic models. Abbreviation: NSCL/P, non-syndromic cleft lip with or without a cleft palate.

Figure 3

Forest plot of NSCL/P risk related to ABCA4 rs481931 polymorphism using the five genetic models. Abbreviation: NSCL/P, non-syndromic cleft lip with or without a cleft palate.

Figure 4

Funnel plot of the association between ABCA4 polymorphisms and risk of NSCL/P using the five genetic models: (A) rs560426 and (B) rs481931