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Meta-analysis and systematic review for the genetic basis of cleft lip and palate

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ABSTRACT

Cleft lip and palate (CLP) are a usually inherited anomaly described as a gap in the oral cavity's upper lip and/or roof. The etiology of CLP involves both genetic and environmental factors. The current study aimed to examine the genetic basis of nonsyndromic (NS) CLP (NSCL/P) and its association with specific genetic polymorphisms. We conducted a meta-analysis and systematic review of seven articles, which provided information on the correlation between genes and NSCL/P risk.

Our results proved that the MTHFR c.677C > T polymorphism was correlated with the risk of NSCL/P, favoring the control group in the CC genotype and the cases group in the CT genotype. The TT genotype favored the control group. Additionally, the MTHFD1 1958G > A polymorphism was correlated with the high NSCL/P risk in children. However, the MTHFR C677T polymorphism did not show a significant correlation with NSCL/P risk in the analysis, although it was correlated with the high risk in specific populations.

These results contribute to our knowledge about the genetic causes of NSCL/P and highlight the importance of specific genetic polymorphisms in its development. Further research is needed to explore the genetic mechanisms underlying NSCL/P in different populations and to elucidate its implications for diagnosis, treatment, and prevention strategies.

1. Introduction

Cleft lip and palate (CLP) are common birth anomalies described by a separation or opening in the upper lip and/or the roof of the oral cavity.[1,2] These conditions can have significant impacts on affected individuals including difficulties with feeding, speech, hearing, and social interactions. CLP occurs with a frequency of around 1 in 700 live births making it one of the most established congenital anomalies worldwide.[3] The origin of CLP is complex and involves both environmental and genetic factors.[4,5] Nonsyndromic (NS) cleft palate (NSCP) is a prevalent congenital malformation affecting the upper lip and roof or the oral cavity.[6] Its etiology is multifaceted, implying both ecological and genetic factors.[7] While the genetic reasons for NSCP have been extensively investigated, primarily through studies on syndromic CLP, the causes of NS cases remain largely unidentified with candidate gene mutations accounting for only a small fraction of cases.[8]

Recently, there has been significant advancement in uncovering the genetic basis of CLP. Studies have detected numerous genes that cause the development of syndromic forms of CLP which are associated with additional congenital anomalies.[9] Some of these genes have also been implicated in the development of NSCLP, suggesting overlapping genetic mechanisms between syndromic and NS phenotypes.[10] The genetic landscape of NSCP is intricate with various genes and loci implicated in its development.[11] Recent research has identified several genetic risk factors associated with NSCP including mutations in craniofacial genes concerned with head and neck development such as MSX1, IRF6, and TP63.[12] Additionally, studies have highlighted genetic risk factors associated with variations in facial morphology within the normal range which could contribute to NSCP development.[13] Environmental factors such as smoking, alcohol consumption, and exposure to specific medications during pregnancy have also been implicated in NSCP development.[14] The interplay between genetic and environmental risk factors is complex, potentially synergistic, and may contribute to the occurrence of NSCP.[15]

Identifying the genetic basis of NSCP poses challenges due to the condition's multifactorial etiology and the vast number of candidate

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genes and loci involved.[16] Nevertheless, recent advancements in genetic technology, including next-generation sequencing and the genetic foundations of NSCP have been revealed through new insights obtained from genome-wide association studies.[17] Understanding the genetic basis of NSCP holds significant implications for its diagnosis, treatment, and prevention.[18] Genetic testing can help identify individuals at risk of developing NSCP, enabling early intervention and targeted treatment.[19] Moreover, knowledge of the genetic foundations of NSCP may guide the enhancement of new therapeutic and preventive protocols.[20]

In the current review, we aim to present the latest findings on the etiology of NSCLP. We will deliberate the genetic and environmental risk factors associated with NSCLP, as well as the current understanding of its genetic foundations. Furthermore, we will address the challenges encountered in elucidating the genetic basis of NSCLP and explore the potential implications of this knowledge for disease diagnosis, management, and prevention.

Applying meta-analyses, we integrated the available evidence to quantitatively summarize the etiology of NSCLP in the literature.[21] To address the distribution of studies reporting multiple results, we used a three-level model for pooling results, allowing a more accurate estimation of heterogeneity and its sources. Our aims were 1) systematically review and update existing knowledge by presenting the latest findings on the etiology of NSCL/P, 2) Interpret and correlate the specific genetic polymorphisms and NSCL/P risk, 3) Qualitatively assess the literature, and 4) summarize our findings and determine the related limitations and future directions.

2. Materials and methods

The current meta-analysis and systematic review aimed to investigate the genetic causes of NSCL/P. This was followed by the criteria of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) by the consideration for reporting the methods and results.[22]

2.1. Search strategy

To find related articles, a thorough search was done through the databases of PubMed/Medline, Scopus, Google Scholar, and Web of Science. The search encompassed articles distributed between 2014 and 2024. The search terms used were carefully selected to capture the relevant genetic factors associated with NSCL/P. The specific search terms and Boolean operators used are documented in Appendix A. No language restrictions were applied during the search. Additionally, to make sure all pertinent research was included, the reference lists of the retrieved papers were thoroughly checked.

2.2. Study selection

The initial screening of articles was performed by the first author, who assessed the titles and abstracts of the retrieved papers. The complete texts of the papers that appeared to meet the appropriate standards were retrieved and further assessed. The inclusion and exclusion criteria (Fig. 1) were predetermined and documented in Appendix B. Any article that did not show the eligibility principles was excluded, and the basis was recorded. To ensure reliability, the second author independently re-evaluated the selected articles. In cases of dispute between the two authors, a consensus was reached through discussion. The disagreement was commonly due to differences in the selection of control groups. For example, there were two control groups, so that only a single control group was used.

2.3. Eligibility criteria

The appropriate standards for article inclusion in this meta-analysis were as follows:

(1) Studies investigating the genetic factors associated with NSCL/P.
(2) Studies reporting on NS cleft palate and/or lip.
(3) Studies provide sufficient genotype and polymorphism data.
(4) Studies include control groups for comparison.
(5) Studies published between 2014 and 2024.
(6) Studies that were not covered by these criteria were ignored.

2.4. Data extraction

The two authors worked independently to collect the records. The records listed below were taken from every study that was included. The first author, year of publication, study population's race, source of controls, the quantity of NSCL/P patients as well as controls for each genotype and associated polymorphism, the genotyping technique used, a p-value of the Hardy-Weinberg equilibrium (HWE) in controls, and the quality score of the study. The data extraction process was performed using a predefined data extraction form, and any discrepancies were determined through discussion between the two authors.

2.5. Quality assessment

The quality judgment of the entered studies was conducted using the Newcastle-Ottawa Scale (NOS).[23] The NOS assigns the greatest score of nine to every study, with a higher score demonstrating higher methodological quality. One of the authors performed the quality assessment independently. Studies with a score of seven or higher were of high quality.

2.6. Qualitative synthesis

The qualitative synthesis involved a measurement of bias and array in the included studies. It aimed to describe the traits and conclusions of the research that were found using the data that was extracted. For a more comprehensive analysis, the likely effects with confidence intervals (CIs) for every study were registered based on five genetic patterns.[24] However, it is important to acknowledge that this approach

The inclusion criteria were:	**The exclusion criteria were**:
• Utilized data from evidence-based studies.	• Simulation studies.
• The control group consisted of both normal lip and palate.	• Unpublished research studies.
• The goal was the genetic etiology of NSCLP.	• Studies concerned with any form of syndromic cleft lip and palate.
• Written in English.	• Reviews of the literature

Fig. 1. Inclusion and exclusion criteria of the study.

raises the issue of multiple testing, which may require appropriate adjustments to control for false-positive results.

2.7. Statistical analysis

A comprehensive statistical analysis using Review Manager 5.3 software was performed to assess the correlation between MAFB polymorphisms and NSCL/P risk. The analysis involved calculating 95 % confidence intervals and crude odds ratios for each study, evaluating the significance of the pooled odds ratio using the Z-test, estimating assortment between studies using the Chi-square test, Tau2, and I^2 statistic, selecting appropriate models based on heterogeneity, and assessing Hardy-Weinberg Equilibrium (HWE). These methods provided valuable insights into the significance, heterogeneity, and genetic equilibrium of the studies, contributing to our perception of the genetic basis of NSCL/P.

3. Results

3.1. Study selection

All the steps followed for the study retrieval and identification for meta-analysis were tabulated in (Fig. 2) with inclusion and exclusion studies in the systematic review and meta-analysis of the genetic basis of cleft lip and palate following the PRISMA guidelines.

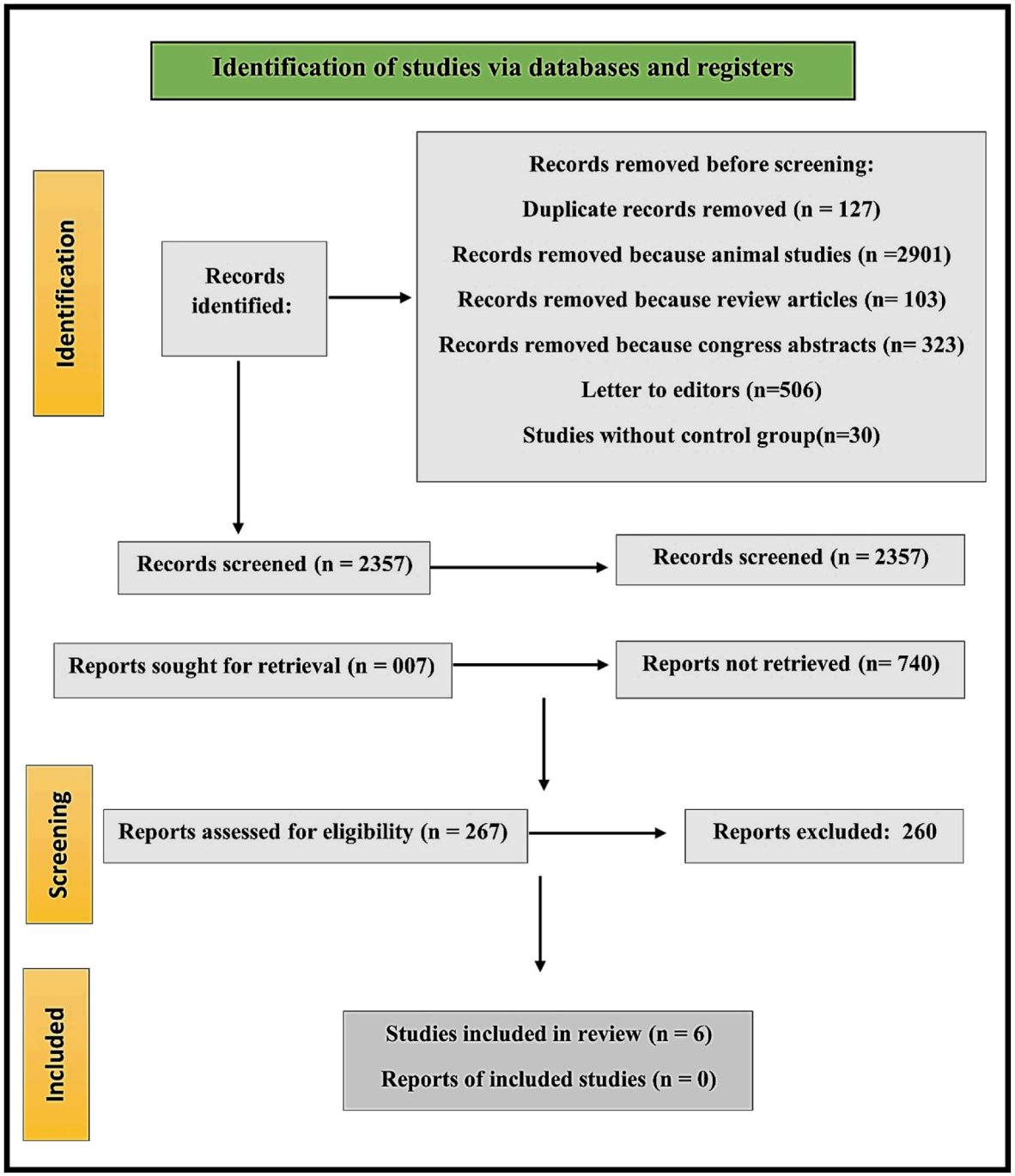

Fig. 2. Study retrieval and identification for meta-analysis. Flow diagram displaying the process for study inclusion and exclusion in the systematic review and meta-analysis of the genetic basis of cleft lip and palate following the PRISMA guidelines.

3.2. Meta-analysis results

The current study encompassed seven studies for the analysis, providing information on the correlation between specific genetic polymorphisms and NSCL/P risk. The subsequent results were obtained.

3.2.1. MTHFR c.677C > T polymorphism

- Genotype: CC

Genotype-CC in cleft lip and palate at the last period was reported in six studies with heterogeneity (p = 0.0005 = 77 %) and the odd ratio (OR) between cases and control = 2.14 (1.25–3.65). p-value: <0.0. Favorable toward the control group as shown in Fig. 3.

-Genotype: CT

Genotype-CT in cleft lip and palate at the last period was reported in three studies with heterogeneity (p = 0.04 = 70 %) and the odd ratio between cases and control = 0.60 (0.31–1.18). P-value: 0.14. Favorable towards the case group as shown in Fig. 4.

-Genotype: TT

Genotype-TT in cleft lip and palate at the last period was reported in four studies with heterogeneity (p = 0.43 = 0 %) and the odd ratio between cases and control = 0.77 (0.62–0.97). p-value: 0.02. Favorable toward the control group as shown in Fig. 5.

3.2.2. MTHFD1 1958G > A polymorphism

- **Genotype: 1958 GA (heterozygous)**
  - Odds Ratio: 2.44
    -p-value: 0.020
    -Correlated with high NSCL/P risk in children
- **Genotype: 1958AA (homozygous)**
  - Odds Ratio: 2.45
    -p-value: 0.012
    -Correlated with high NSCL/P risk in children
- **Dominant Model (AG + AA vs. GG):**
    -Odds Ratio: 2.44
    -p-value: 0.002
  - Correlated with high NSCL/P risk

3.2.3. MTHFR C677T polymorphism

- **Genotype: C/T (heterozygous)**
  - Frequency in controls: 40.7 %
  - Frequency in patients: 15.4 %
- **Allele: T**
  - Frequency in controls: 26 %
  - Frequency in patients: N/A
  - The C677T polymorphism of the MTHFR gene showed low correlation and NSCL/P in the Moroccan population (OR = 0.24, p-value = 0.0005).

3.2.4. MTHFR 1298A > C polymorphism

- Meta-analysis including 22 case-control studies:
- Overall, there is no significant correlation between MTHFR 1298A > C polymorphism and NSCL/P risk.
- Subgroup analysis:
- Significant correlation observed in Asian and Iranian populations.
- No significant association between Caucasians, mixed populations, and Chinese populations.

The meta-analysis and systematic review depicted that the MTHFR c.677C > T polymorphism was correlated with the risk of NSCL/P, favoring the control group in the CC genotype and the cases group in the CT genotype. The TT genotype favored the control group. Additionally, the MTHFD1 1958G > A polymorphism was correlated with high NSCL/P risk in children. However, the MTHFR C677T polymorphism did not show a significant correlation with NSCL/P risk in the analysis, although it was associated with a high risk in specific populations. Thus,

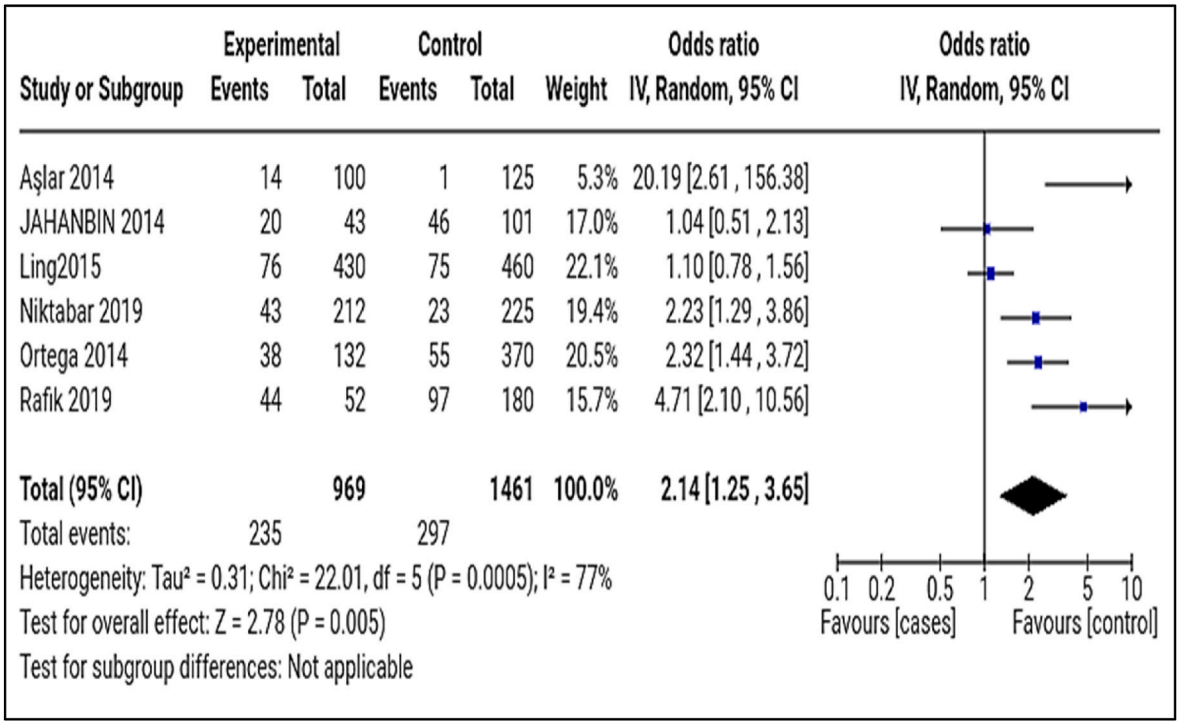

Fig. 3. Forest plot of odds ratio with confidence intervals for Genotype -CC.

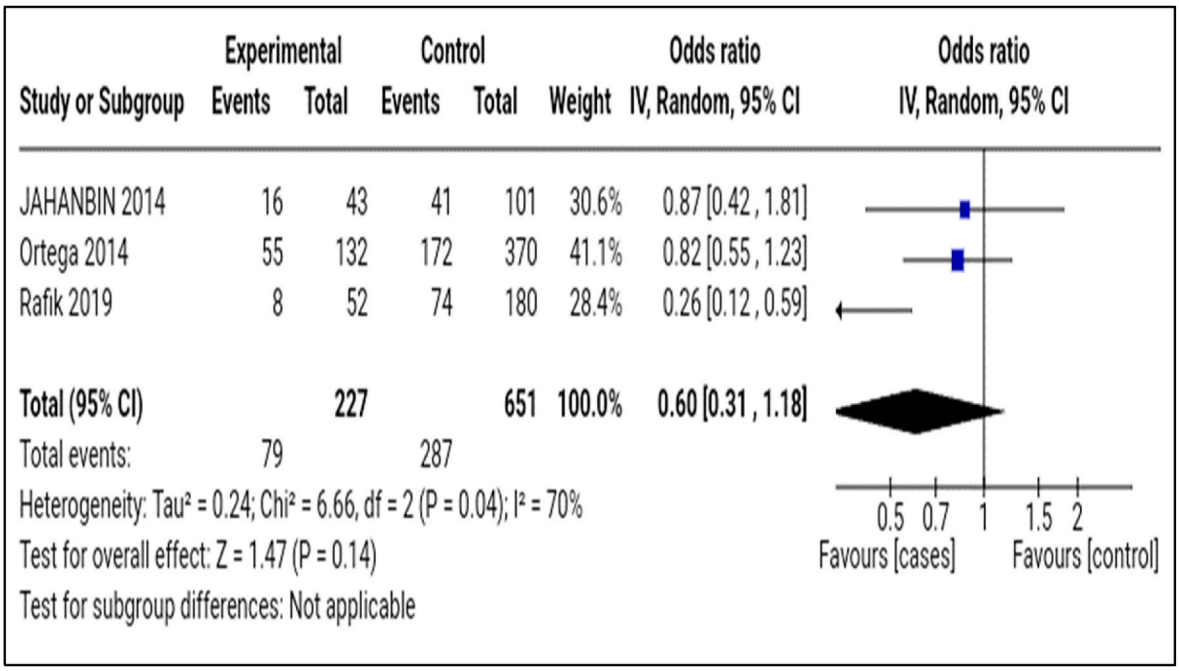

Fig. 4. Forest plot of odds ratio with confidence intervals for Genotype -CT.

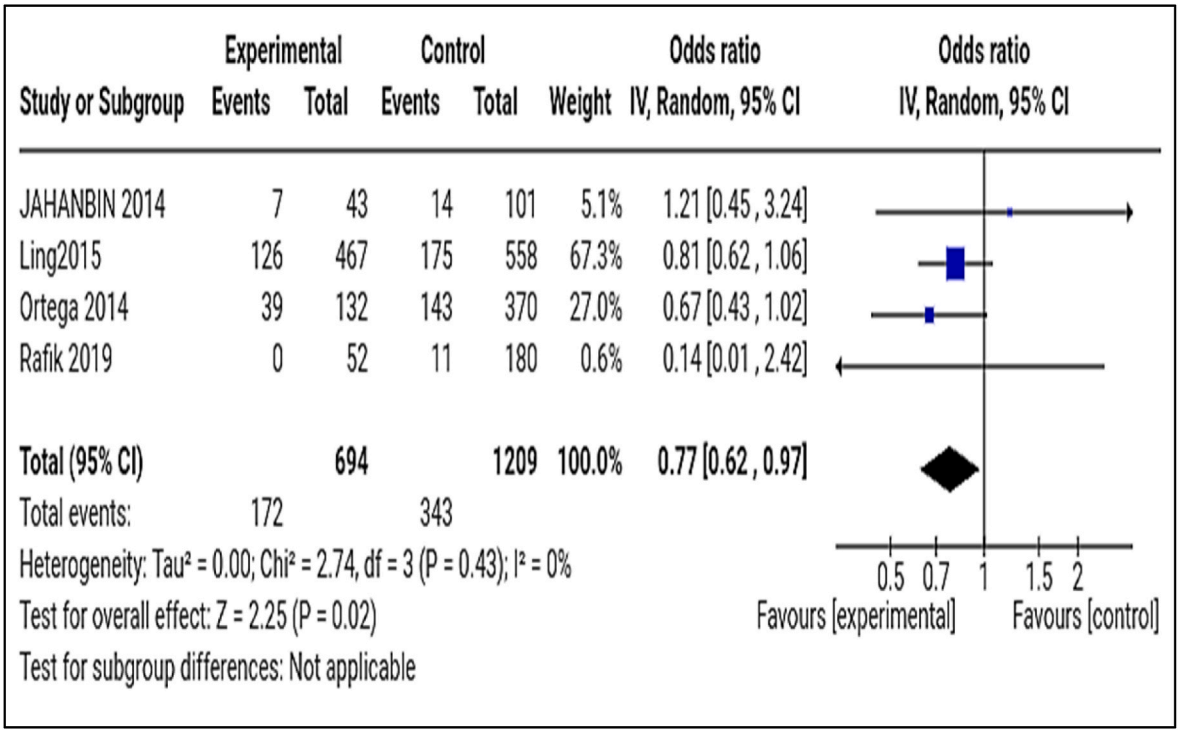

Fig. 5. Forest plot of odds ratio with confidence intervals for Genotype -TT.

additional research is required to explore the genetic basis of NSCL/P and its implications in another population.

4. Discussion

4.1. MTHFR c.677C > T polymorphism

NSCL/P is a common congenital anomaly with a complicated etiology implying both environmental and genetic causes.[24,25] A critical function of the MTHFR gene is in the metabolism of folic acid, and the c.677C > T polymorphism has been extensively studied in NSCL/P risk.[26,27] The meta-analysis results revealed a significant correlation between the CC genotype and increased NSCL/P risk, favoring the control group.[27] In contrast, the CT genotype showed a non-significant correlation with NSCL/P, favoring the case group.[27] The TT genotype was associated with a decreased NSCL/P risk, favoring the control group.[27]

4.2. MTHFD1 1958G > A polymorphism

The MTHFD1 gene is involved in the one-carbon metabolism mediated by folic acid, and the 1958G > A polymorphism has been investigated about NSCL/P risk.[26,28] The meta-analysis findings indicated a significant correlation between the 1958 GA and 1958AA genotypes and increased NSCL/P risk in children.[26] Furthermore, the dominant model (AG + AA vs. GG) also showed a significant correlation with NSCL/P risk.[24]

4.3. MTHFR C677T polymorphism

The MTHFR C677T polymorphism has been extensively studied in various populations, including the Moroccan population.[27] The meta-analysis results did not show a significant correlation between this polymorphism and NSCL/P risk overall.[27] However, within the Moroccan population, a low association was observed between the C677T polymorphism and NSCL/P risk.[27]

4.4. MTHFR 1298A > C polymorphism

The MTHFR 1298A > C polymorphism has been widely investigated for NSCL/P risk. The meta-analysis incorporating 22 case-control studies showed no significant correlation between this polymorphism and NSCL/P risk. However, the Subgroup analysis did not show any significant correlation with Chinese, mixed, or Caucasian populations, but it did show a significant correlation with Iranian and Asian populations.[26]

4.4.1. Interpretation

The meta-analysis and systematic review provide valuable insights into the correlation between specific genetic polymorphisms and NSCL/P risk.[19,22] The findings suggest that the MTHFR c.677C > T polymorphism may influence NSCL/P susceptibility, with the CC genotype potentially conferring a higher risk.[27] The MTHFD1 1958G > A polymorphism was correlated with a high NSCL/P risk, particularly in children.[24] On the other hand, the MTHFR C677T polymorphism did not show a significant correlation with NSCL/P risk overall but exhibited population-specific effects.[27] The MTHFR 1298A > C polymorphism also demonstrated varying associations with NSCL/P risk across different populations [28.]

4.4.2. Limitations and future directions

A few limitations should be supposed with the results of this meta-analysis and systematic review.[24,25,27] First, the sample sizes through the selected studies were varied, which may have influenced the statistical power and generalizability.[26,28] The studies involved were conducted in different populations, potentially introducing heterogeneity. Gene-to-gene and gene-to-environment interactions were not thoroughly explored due to the limited available data. Future studies should address these limitations and investigate additional genetic variants and potential interactions to further elucidate the genetic basis of NSCL/P.

5. Conclusion

The meta-analysis and systematic review provide evidence supporting the correlation between specific genetic polymorphisms and NSCL/P risk. The MTHFR c.677C > T and MTHFD1 1958G > polymorphisms were found to be correlated with NSCL/P risk. The MTHFR C677T polymorphism showed population-specific effects. The MTHFR 1298A > C polymorphism demonstrated varying associations across different populations. These findings may help the rapid diagnosis of NSCL/P through genetic testing. Furthermore, they will increase our understanding of the molecular mechanisms that underlie NSCL/P development. Thus, this will assist early intervention and management which have improved the affected individuals' quality of life and overall quality of care. Moreover, the present study may help in the progress of clinical trials for genetic and drug therapies for NSCL/P. However, Further research is needed to explore additional genetic interactions and variants specifically in causing NSCL/P, to identify possible preventive measures.

Authors contributions

All authors have made substantive contributions to this study and/or manuscript, and all have reviewed the final paper before its submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobcr.2024.12.012.

Appendix A

Boolean operator	Function	Example
AND	Provides results that contain **both or all** keywords	Genotypes CC AND CT
OR	Provides results that contain **either** keyword	NSCL OR NSCP
NOT or AND NOT	Provides results that contain **the first** keyword but **not the second**	nonsyndromic cleft NOT syndromic cleft
Quotation marks ""	Provides results with **the exact phrase**	"nonsyndromic"
Parentheses ()	Allows you to **group keywords** and control the order in which the terms will be searched	(**Genetic Basis**) AND NSCL/P
Asterisk *	Provides results that contain a **variation** of the keyword	Development* "development," "developer," and "developing."

Appendix B. as in Fig. 1

The inclusion criteria were:	The exclusion criteria were:
• Utilized data from evidence-based studies. • The control group consisted of both normal lip and palate. • The goal was the genetic etiology of NSCLP. • Written in English.	• Simulation studies. • Unpublished research studies. • Studies concerned with any form of syndromic cleft lip and palate. • Reviews of the literature

References

1. Alghonemy WY, Hegazy AA, Elmigdadi F, Nasser GA, Helal MB. Potential teratogenic effect of prenatal dexamethasone administration on palate development: experimental study in rats. *Transl Res Anat.* 2024, 100338. Published online.
2. Kohli SS, Kohli VS. A comprehensive review of the genetic basis of cleft lip and palate. *J Oral Maxillofac Pathol.* 2012;16(1):64–72.
3. Chaudhary I, Tripathi AM, Yadav G, Dhinsa K. Cleft lip and palate: a review. *Indian J Contemp Dent.* 2016;4(1):53–58.
4. Filonenko VV, Kaniura OA, Sokolovskiy VA. Treatment of dentognathic anomalies and deformations in children with congenital unilateral cleft lip and palate. *J Int Dent Med Res.* 2024;17(1):357–362.
5. Adinda FR, Putri FM, Setiawan AS. Sociodemographic characteristics and mapping of patients with cleft lip and palate. *J Int Dent Med Res.* 2024;17(1).
6. Khan MI, Prashanth CS, Srinath N, Neela PK, Mohiuddin MK. Genetic analysis of the single-nucleotide polymorphisms rs880810, rs545793, rs80094639, and rs13251901 in nonsyndromic oral clefts: a case–parent trio study. *Glob Med Genet.* 2023;10(1):34–37.
7. Ye X, Ahmed MK. Genetic factors responsible for cleft lip and palate. In: *Surgical Atlas of Cleft Palate and Palatal Fistulae.* Springer; 2022:53–66.
8. Askarian S, Gholami M, Khalili-Tanha G, et al. The genetic factors contributing to the risk of cleft lip-cleft palate and their clinical utility. *Oral Maxillofac Surg.* 2023;27(2):177–186.
9. Dąbrowska J, Biedziak B, Bogdanowicz A, Mostowska A. Identification of novel risk variants of non-syndromic cleft palate by targeted gene panel sequencing. *J Clin Med.* 2023;12(5):2051.
10. Shibano M, Watanabe A, Takano N, et al. Target capture/next-generation sequencing for nonsyndromic cleft lip and palate in the Japanese population. *Cleft Palate-Craniofacial J.* 2020;57(1):80–87.
11. Demeer B, Revencu N, Helaers R, et al. Likely pathogenic variants in one third of non-syndromic discontinuous cleft lip and palate patients. *Genes.* 2019;10(10):833.
12. Avasthi KK, Muthuswamy S, Asim A, Agarwal A, Agarwal S. Identification of novel genomic variations in susceptibility to nonsyndromic cleft lip and palate patients. *Pediatr Rep.* 2021;13(4):650–657.
13. Oner DA, Tastan H. Cleft lip and palate: epidemiology and etiology. *Otorhinolaryngol Head Neck Surg.* 2020;5(4):1–5.
14. Gowans LJJ, Comnick CL, Mossey PA, et al. Genome-wide scan for parent-of-origin effects in a sub-Saharan African Cohort with nonsyndromic cleft lip and/or cleft palate (CL/P). *Cleft Palate-Craniofacial J.* 2022;59(7):841–851.
15. Cui R, Chen D, Li N, et al. PARD3 gene variation as candidate cause of nonsyndromic cleft palate only. *J Cell Mol Med.* 2022;26(15):4292–4304.
16. Vyas T, Gupta P, Kumar S, Gupta R, Gupta T, Singh HP. Cleft of lip and palate: a review. *J Fam Med Prim Care.* 2020;9(6):2621–2625.
17. Paradowska-Stolarz A, Mikulewicz M, Duś-Ilnicka I. Current concepts and challenges in the treatment of cleft lip and palate patients—a comprehensive review. *J Personalized Med.* 2022;12(12):2089.
18. Oboli GO, Chukwuma DI, Fagbule OF, Abe EO, Adisa AO. Molecular genetics of cleft lip and palate: a review. *Ann Ib Postgrad Med.* 2020;18(1):S16–S21.
19. Yusof MS, Mohd Ibrahim H. The impact of cleft lip and palate on the quality of life of young children: a scoping review. *Med J Malaysia.* 2023;78(2):250–258.
20. Moher D, Liberati A, Tetzlaff J. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6. Published online.
21. Damen JAA, Moons KGM, van Smeden M, Hooft L. How to conduct a systematic review and meta-analysis of prognostic model studies. *Clin Microbiol Infection.* 2023;29(4):434–440.
22. Wells GA, Shea B, O'Connell D, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* 2000. Published online.
23. Jahanbin A, Hasanzadeh N, Abdolhoseinpour F, et al. Analysis of MTHFR gene C. 677C> T and C. 1298A> C polymorphisms in Iranian patients with non-syndromic cleft lip and palate. *Iran J Public Health.* 2014;43(6):821.
24. Murthy J, Gurramkonda VB, Lakkakula BVKS. Significant association of MTHFD1 1958G> A single nucleotide polymorphism with nonsyndromic cleft lip and palate in Indian population. *Med Oral Patol Oral Cir Bucal.* 2014;19(6):e616.
25. Estandia-Ortega B, Velázquez-Aragón JA, Alcántara-Ortigoza MA, Reyna-Fabian ME, Villagómez-Martínez S, González-del Angel A. 5, 10-Methylenetetrahydrofolate reductase single nucleotide polymorphisms and gene–environment interaction analysis in non-syndromic cleft lip/palate. *Eur J Oral Sci.* 2014;122(2):109–113.
26. Jin LL, Chen EJ, Wei HOU, Liu XH, Ying HU. The association between folate pathway genes and cleft lip with or without cleft palate in a Chinese population. *Biomed Environ Sci.* 2015;28(2):136–139.
27. Rafik A, Rachad L, Kone A samad, Nadifi S. MTHFR C677T polymorphism and risk of nonsyndromic cleft lip with or without cleft palate in the Moroccan population. *Appl Clin Genet.* 2019:51–54. Published online.
28. Niktabar SM, Aarafi H, Dastgheib SA, et al. Association of MTHFR 1298A> C polymorphism with susceptibility to non-syndromic cleft lip with or without palate: a case-control study and meta-analysis. *Fetal Pediatr Pathol.* 2021;40(1):1–17.