Cleft Lip And Depression In Adults: A Multi-Ancestry Mendelian Randomization Study

Anne Lyon Havlik
anne.havlik@yale.edu

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Title
Cleft lip and depression in adults:
A multi-ancestry Mendelian randomization study

Author
Anne Lyon Havlik, BA, BS

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Department: Chronic Disease Epidemiology, School of Public Health
Advisor/Committee Chair: Tormod Rogne, MD, PhD
Committee Members/Secondary Advisor: Russell R. Reid, MD, PhD
ABSTRACT

Background: Clefting of the lip with or without cleft palate (CLP) is the most common congenital craniofacial abnormality globally, affecting one in 700 live births. Traditional observational studies suggest an increased risk of adult depression among subjects with CLP, but these studies are subject to residual confounding.

Objective: We aimed to address residual confounding by evaluating the association between the genetically-predicted risk of CLP on the risk of adult depression in a Mendelian randomization framework.

Methods: We used single-nucleotide polymorphisms strongly associated with CLP as genetic instruments. Genetic associations with adult depression were extracted from separate studies. Three ancestry groups were evaluated: European, East Asian, and African. Two-sample Mendelian randomization inverse-variance weighted analyses were conducted, and sensitivity analyses using weighted median, weighted mode, and MR-Egger regression were performed to assess bias from genetic pleiotropy.

Results: The study included a total of 3,577 CLP cases and 10,345 controls, and 59,406 and 274,957 subjects with and without adult depression. Among subjects of African ancestry, a doubling of the genetically-predicted prevalence of CLP was associated with an odds ratio for adult depression of 1.28 (95% CI 0.94-1.75). Sensitivity analyses supported this finding. There was no clear association between CLP and adult depression in the European or East Asian ancestry groups.

Conclusion: This study may suggest a causal association between cleft lip and the risk of depression among subjects of African ancestry. Our findings warrant further investigation into the role of craniofacial malformations as a cause of depression and an assessment of potential inequity.
ACKNOWLEDGMENTS

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Lastly, thank you to my late grandfather, Dr. Edward Spafford Lyon, for raising me to have a fierce curiosity and encouraging me to observe, explore, and discover the world.
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INTRODUCTION

Orofacial clefting of the lip with or without cleft palate (CLP) is the most common congenital craniofacial abnormality in the world, affecting around one in 700 live births.\textsuperscript{1,2} In the US, one in every 1,600 live births has a cleft lip with a cleft palate.\textsuperscript{3} There exists considerable variation in the prevalence of CLP globally in the axes of ancestry and ethnic origin as well as geographical region.\textsuperscript{4–8} CLP is serious, as it negatively affects an individual’s self-esteem, social skills, and behavior.\textsuperscript{9,10} Significant disadvantages of CLP to psychological health have been documented, such as increased bullying and social exclusion and increased risk of depression and anxiety.\textsuperscript{11,12}

Features of CLP are unlikely to be removed entirely through surgical and medical care.\textsuperscript{13–15} The CLP-affected individual may have a noticeably different appearance even after corrective surgery, such as through scarring. Looking differently from one’s peers can invite unwanted attention in the form of teasing, bullying, and other forms of peer harassment.\textsuperscript{16} Compounding these issues, perceived social stigma and/or acceptance impacts the individual with CLP’s emotional well-being.\textsuperscript{11,17} Studies have supported that the emotional functioning of adult individuals afflicted with CLP (post-surgical correction) is poorer, and these individuals have twice the prevalence of depression when compared to their counterparts in the general population.\textsuperscript{18–21} However, these existing observational studies are susceptible to residual confounding of cleft lip and offspring well-being. Potential confounders in these studies include factors associated with both an increased risk of cleft lip and an increased risk of offspring depression, such as exposure to certain substances during pregnancy (e.g., smoking, alcohol, and certain medications).\textsuperscript{22} One example of this is the maternal use of Topiramate (Topomax) in early pregnancy, which is used off-label for treating depression and has also been shown to increase the risk of orofacial clefting in offspring.\textsuperscript{22,39–41} Two-sample Mendelian randomization (MR) is an approach that allows for large sample
populations, and this design greatly reduces the risk of confounding. Two-sample MR estimates the causal effect of an exposure on an outcome using only summary statistics from genome-wide association studies. This approach can be leveraged as a natural experiment imitating a randomized control trial since genetic variants are determined at random during conception. Where randomized controlled trials use the random assignment of participants to a treatment group as the instrument, genetic variants are used as instruments in two-sample MR.

Recognizing the psychological challenges that individuals born with orofacial clefting experience, this study seeks to enhance our knowledge of the relationship between the congenital anomalies of cleft lip and adult depression across several global populations. The Mendelian randomization approach of this thesis provides a novel approach to evaluating this issue and unique evidence about the causal effects of cleft lips on adult depression. We hypothesize that there exists an effect of a cleft lip on the risk of adult depression.
METHODS

Research Design

Two-sample MR analyses were performed using data from large genetic studies of the risk of cleft lip and the risk of adult depression, evaluating three distinct genetic ancestry groups. The statistical methodology for MR is based on instrumental variable analysis. The three general assumptions for a valid instrument in MR are that there is an association between the genetic instrument and exposure (relevance assumption), the genetic instrument has no association with confounders of the exposure-outcome pathway (independence assumption), and the genetic instrument is not associated with the outcome besides through the exposure pathway (exclusion restriction assumption). The instrumental variables in this study are single-nucleotide polymorphisms (SNPs) associated with cleft lip, which serve as a proxy/predictor for cleft lip. Two-sample MR uses summary-level data from genome-wide association studies (GWAS) to evaluate the association of these instrumental variables representing the genetically-predicted exposure of CLP and genetic susceptibility to adult depression. Figure 1 shows a schematic representation of the two-sample Mendelian randomization study performed.
**Figure 1. Conceptual framework of Mendelian randomization study performed.** This graphic is a schematic representation of the two-sample Mendelian randomization study conducted. The figure illustrates the assumptions of the instrumental variable analysis using genetic instruments: arrow 1 depicts the relevance assumption, arrow 2 depicts the independence assumption, and arrow 3 depicts the exclusion restriction assumption. See text for details.

**Data Sources and Study Populations**

This study utilized publicly available data from GWAS with appropriate ethical approvals (Table 1). Single-nucleotide polymorphisms strongly associated with cleft lip were extracted from GWAS and used as genetic instruments. We sought to evaluate all genetic ancestries with available data on the genetic risk of both CLP and adult depression, and three distinct ancestry groups were available. European, East Asian, and African ancestry groups were evaluated in this study. The European and African ancestry phenotypic exposure definitions were both ‘cleft lip with or without cleft palate’ (CLP). In contrast, the East Asian ancestry phenotypic exposure definition was ‘cleft lip only’ (Table 1). SNPs that were determined to be strongly associated with CLP to have a p-value <5e-8 in both the European and East Asian ancestries were included. Due to a paucity of data in the African ancestry group for genetic
CLP studies, SNPs strongly associated with CLP in African ancestry used a less stringent p-value cut-off of p-value <5e-6. SNPs used in analyses for each ancestry were identified that were independent of each other (R^2 < 0.01), keeping SNPs with the lowest p-value. Explained variance for each ancestry was estimated by summing absolute R^2 values from each independent SNP. Superpopulations were taken from the 1000 Genomes Project as references for R^2 values between SNPs (‘EUR’ was used for European ancestry, ‘EAS’ for East Asian ancestry, and ‘AFR’ for African ancestry). Genetic associations with adult depression were extracted from separate studies (Table 1).
<table>
<thead>
<tr>
<th>Ancestry group</th>
<th>Study</th>
<th>Countries</th>
<th>Phenotype definition</th>
<th>Total number of participants</th>
<th>Cases/Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXPOSURE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td><em>Dardani et al. 2020</em>&lt;sup&gt;25&lt;/sup&gt;</td>
<td>US</td>
<td>cleft lip with or without cleft palate</td>
<td>5,951</td>
<td>1,692/4,259</td>
</tr>
<tr>
<td>East Asian</td>
<td><em>Huang et al. 2019</em>&lt;sup&gt;26&lt;/sup&gt;</td>
<td>China</td>
<td>cleft lip only</td>
<td>6,933</td>
<td>1,883/5,050</td>
</tr>
<tr>
<td>African</td>
<td><em>Butali et al. 2019</em>&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Ghana, Nigeria, Ethiopia</td>
<td>cleft lip with or without cleft palate</td>
<td>3,002</td>
<td>479/2,523</td>
</tr>
<tr>
<td><strong>OUTCOME</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td><em>Howard et al. 2019</em>&lt;sup&gt;28&lt;/sup&gt;</td>
<td>UK, Germany, Denmark, Iceland</td>
<td>self-reported clinical diagnosis of depression</td>
<td>138,884</td>
<td>43,204/95,680</td>
</tr>
<tr>
<td>East Asian</td>
<td><em>Giannokoupoulou et al. 2021</em>&lt;sup&gt;29&lt;/sup&gt;</td>
<td>China</td>
<td>depression defined from health records and self-report</td>
<td>194,548</td>
<td>15,771/178,777</td>
</tr>
<tr>
<td>African</td>
<td><em>Pan-UK Biobank Team 2020</em>&lt;sup&gt;30&lt;/sup&gt;</td>
<td>UK</td>
<td>if the participant ever had prolonged feelings of sadness or depression</td>
<td>931</td>
<td>431/500</td>
</tr>
</tbody>
</table>

GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; UK, United Kingdom; US, United States of America.
Analyses

Inverse-variance weighted (IVW) analysis was used as the main analysis, leveraging the genetic variants (SNPs) extracted from each ancestry group separately. The causal estimate of each SNP was determined by the Wald ratio, which is the SNP-outcome association divided by the SNP-exposure association. The IVW method combines the Wald ratio estimates together, where the weight of each ratio included is the inverse of the variance of the selected genetic variant for cleft lip-adult depression association. This relies on the assumptions of MR described previously (Figure 1). Therefore, the estimates used could be biased by a genetic variant having direct effects on other pathways than the exposure. This phenomenon is known as horizontal pleiotropy. Due to these factors, bias from pleiotropy can occur in the standard MR analysis of inverse-variance weighted. Resultingly, pleiotropy was investigated through the sensitivity analyses of weighted mode, weighted median, and Mendelian randomization-Egger (MR-Egger) regression. The weighted mode-based estimation assumes the most common causal effect of MR is consistent with the true causal effect. The weighted median estimation assumes 50% or greater of the instrument’s total weight is derived from valid instruments. MR-Egger loosens the assumptions of MR and permits directional pleiotropic effects.

The p-value cut-off used for statistical significance in these analyses was 0.05. In order to have more interpretable results, the causal estimates returned by MR analyses were multiplied by 0.693 ($\log_2{2}$) and then log transformed to represent the average change in the outcome of adult depression per 2-fold increase in the exposure prevalence of CLP in that ancestry group. MR analyses were conducted using the TwoSampleMR package (version 0.5.6) in R (version 4.1.1).
RESULTS
This study included a total of 3,577 CLP cases and 10,345 controls, and 59,406 and 274,957 subjects with and without adult depression, respectively. In the European ancestry group, there were nine SNPs representing 1.5% explained variance of cleft lip. Seven SNPs were used in the East Asian ancestry group, representing 1.0% explained variance. Four SNPs representing 0.6% explained variance of cleft lip were used from the African ancestry group.

Figure 2 shows a forest plot of all ancestries with the main analysis results and sensitivity analyses performed. Scatter plots and forest plots of the results of each ancestry group separately are shown in Figure 3. Among subjects of African ancestry, a doubling in the genetically predicted prevalence of CLP was associated with an odds ratio of adult depression of 1.28 (95% confidence interval [CI] of 0.94-1.75; Figure 2 and Figure 3). In the European ancestry group, there was no evidence of an association of CLP with the risk of adult depression in the main analysis, resulting in an odds ratio of 1.01 (95% CI 1.00-1.01; Figure 2 and Figure 3) per doubling in the prevalence of cleft lip. Similarly, the East Asian ancestry group had an odds ratio of 1.01 (95% CI 0.98-1.04; Figure 2 and Figure 3) per doubling in the prevalence of cleft lip, where CLP was not significantly associated with the risk of adult depression.

The sensitivity analyses (weighted mode, weighted median, and MR-Egger) shown in Figure 2 generally corroborate the findings of the main analyses. See Supplementary Figure S1 for scatter plots of sensitivity analyses performed by ancestry group. The sensitivity analyses of the African ancestry group show a potential bias in the analysis from pleiotropy. There was no clear association between CLP and adult depression in the European or East Asian ancestry groups.
Figure 2. Forest plot of results and sensitivity analyses of two-sample Mendelian randomization (MR) analyses investigating the association of genetically predicted CLP with the risk of adult depression by ancestry group. The main analysis was IVW, and three sensitivity analyses were used: weighted mode, weighted median, and MR-Egger.

OR, odds ratio; CI, confidence interval; IVW (inverse-variance weighted)
Figure 3. Forest plots of results of two-sample Mendelian randomization (MR) analyses investigating the association of genetically predicted CLP with the risk of adult depression by ancestry group. Forrest plots show each SNP’s effect on adult depression. A black dot represents each SNP, and the horizontal line represents the CI. The red line at the bottom of the plot shows the IVW-summarized result. SNP effects are reported in the log-odds scale.

CLP, clefting of the lip with or without cleft palate; SNP, single nucleotide polymorphism; CI, 95% confidence interval; IVW, inverse-variance weighted.
DISCUSSION

In this MR study of three genetic ancestry groups, genetically predicted CLP had a tendency for effect on the risk of adult depression in African ancestry. A clear association between genetically predicted cleft lip and adult depression in European or East Asian ancestries was not found. These findings may suggest a differing effect of genetically predicted cleft lip on the risk of adult depression between African ancestry and that of European or East Asian ancestries despite the lack of precision in African ancestry findings.

However, results suggesting genetically predicted CLP had a tendency for effect on the risk of adult depression in the African ancestry group had sensitivity analyses suggesting a potential bias due to pleiotropy. In the sensitivity analysis of the relaxed-assumptions MR-Egger specifically for the African population, after accounting for pleiotropy, cleft lip reduces the risk of depression. The MR-Egger finding for the African population also had a wide 95% confidence interval of 0.20-3.29. The confidence interval included the point estimate from the main analysis (odds ratio of 1.28); thus, it did not dispute the main finding. Corroborating this finding, the main analysis alongside the weighted mode and weighted median sensitivity analyses had wide confidence intervals, although not as wide as the MR-Egger analysis.

The results from the different ancestry groups must be interpreted separately. Due to the absence of robust GWAS studies on CLP, different phenotype definitions of CLP occurred in the exposure studies (i.e., European and African ancestry GWAS used the exposure definition of “cleft lip with or without cleft palate,” while the East Asian ancestry GWAS used the exposure definition of “cleft lip only”). Similarly, there was heterogeneity in the definitions of adult depression in the outcome GWAS. The European ancestry group used a self-report of a clinical diagnosis of depression, while the East Asian ancestry group used a measure of depression defined from health records and self-report, and the African ancestry group defined depression by asking if a participant ever had prolonged feelings of sadness or
depression. There exists wide diversity in both the different coding practices of exposure and outcome definitions and completeness of the registries upon which the GWAS used in this study are based. We, therefore, chose not to conduct a meta-analysis of the results across the three ancestry groups.

This two-sample MR study does not take into consideration surgical interventions of CLP. Surgical capacity and standard of CLP care vary widely globally, including the typical timing and quality of surgical intervention, which may give rise to some variation in results by ancestry group.\textsuperscript{42,43} Two-sample MR analyses also do not take into consideration the socio-cultural aspects of orofacial clefting. The consequences, including social stigmatization, of CLP have been shown to range from social ostracism to death in the contexts of different countries.\textsuperscript{44-46} For example, one study in Zimbabwe found that CLP was associated with stigma and superstition (including parental attribution of CLP to witchcraft).\textsuperscript{44} Differing cultural aspects of social stigmatization associated with CLP may also contribute to the differences by ancestry group shown in the results of this study.

Although the mechanism of the relationship between cleft lip and adult depression remains unknown, previous studies have demonstrated the increased risk of those with cleft lip (and those receiving multiple surgeries for cleft lip repair) on psychosocial outcomes.\textsuperscript{37,38} In one study of Norwegian adults with repaired CLP, adults with complete clefts were compared to their same-age counterparts, finding that anxiety and depression were reported twice as much in the subjects with CLP compared to the controls and concluding that there is an impaired level of psychosocial well-being in those with CLP.\textsuperscript{21}

Dardani et al. 2020 is an MR study that has used CLP as an exposure, studying the outcome of educational attainment in the European population. Similar to the findings of this study, Dardani et al. 2020 found limited evidence in European ancestry for a causal relationship between CLP and educational attainment. Dardani et al. 2020 interpreted their
null finding to mean that common variants are unlikely to predispose individuals born with CLP to low educational attainment.\textsuperscript{25}

This analysis has strengths and some limitations. This study uses the novel method of a causal inference method (two-sample MR) to investigate the effects of genetically predicted CLP on adult depression. Additionally, several sensitivity analyses were used to test the robustness of the main analyses. An important limitation of this MR study is that it only included individuals from three distinct genetic ancestry groups (European, East Asian, and African) due to the availability of GWAS data in both the exposure and outcome domains. The small sample sizes of the GWAS used in the African ancestry group limit the findings of this study. Larger GWAS in the African ancestry group on both CLP and adult depression would provide more precise evidence, such as narrower 95\% CIs, with these analyses. As previously mentioned, the differences in definitions of both exposure and outcome used in each ancestry group are a limitation of this study, preventing meta-analysis. A precise phenotypic definition across all ancestry groups did not exist. Furthermore, exact morphologies of the orofacial clefting (e.g., unilateral, bilateral) were not reported in the case definitions from the GWAS used. It is important to note that summarized GWAS data does not include stratification on many axes of diversity that may be of interest in this relationship. Further research is encouraged to reduce the disparities in genetics and increase representation from ancestry groups that carry a disproportionate share of the cleft lip global disease burden.

Conclusion

The findings from this multi-ancestry MR study may suggest a causal relationship between genetically predicted cleft lip and the risk of depression that could exist in the African ancestry group. Due to the scarcity of genetic instruments in this population, this study should be replicated when larger African CLP GWAS are available. There is no evidence that adult
depression is affected by genetically predicted CLP in the East Asian or European ancestry groups. More research is needed to evaluate whether these findings are replicated in other settings and to further elucidate the fundamental mechanisms of the CLP and adult depression relationship.
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doi:10.3109/02844319509008968


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43. Patel PB, Hoyler M, Maine R, Hughes CD, Hagander L, Meara JG. An opportunity for diagonal


Appendix

Supplementary Figure S1. Scatter plots of results by ancestry group. Two-sample MR results for associations between genetically predicted CLP and adult depression, using the main analysis (IVW) and sensitivity analyses (MR Egger, weighted median, and weighted mode). Along the x-axis is the SNP-exposure effect, and along the y-axis is the SNP-outcome effect. Each black dot represents a single SNP’s effect, and vertical and horizontal lines represent the CIs. Regression lines show estimates of the IVW main analysis and sensitivity analyses. SNP effects are reported in the log-odds scale. SNP, single nucleotide polymorphism; CI, 95% confidence interval; IVW, inverse-variance weighted.