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Mendelian randomization analysis reveals a complex genetic interplay among asthma, atopic dermatitis, and GERD

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ABSTRACT

RATIONALE: Gastroesophageal reflux disease (GERD) is commonly associated with atopic disorders, but cause-effect relationships remain unclear.

OBJECTIVES: We applied Mendelian randomization (MR) analysis to explore whether GERD is causally related to atopic disorders of the lung (asthma) and/or skin (atopic dermatitis).

METHODS: We conducted two-sample bidirectional MR to infer the magnitude and direction of causality between asthma and GERD, using summary statistics from the largest genome-wide association studies (GWAS) conducted on asthma (N_{cases}=56,167) and GERD (N_{cases}=71,522). Additionally, we generated instrumental variables (IVs) for atopic dermatitis (AD) from the latest population-level GWAS meta-analysis (N_{cases}=22,474) and assessed their fidelity and confidence of predicting the likely causal pathway(s) leading to asthma and/or GERD.

MEASUREMENTS AND MAIN RESULTS: Applying three different methods, each method found similar magnitude of causal estimates that were directionally consistent across the sensitivity analyses. Using an inverse-variance weighted method, the largest effect size was detected for asthma predisposition to AD (odds ratio [OR], 1.46; 95% confidence interval [CI], 1.34-1.59), followed by AD to asthma (OR, 1.34; CI, 1.24-1.45). A significant association was detected for genetically determined asthma on risk of GERD (OR, 1.06; CI, 1.03-1.09), but not genetically determined AD on GERD. In contrast, GERD equally increased risks of asthma (OR, 1.21; CI, 1.09-1.35) and AD (OR, 1.21; CI, 1.07-1.37).

CONCLUSIONS: This study uncovers previously unrecognized causal pathways that have clinical implications in European-ancestry populations: 1) asthma is a causal risk for AD; and 2) the predisposition to AD, including asthma, can arise from specific pathogenic mechanisms manifested by GERD.

Word count: 250

Key words: Human genetics, epidemiological approach, causal pathways, risk factors

At a Glance Commentary

Scientific Knowledge on the Subject

Numerous clinical and epidemiological studies have reported association between gastroesophageal reflux disease (GERD) and asthma. However, as these observational studies are often limited to cross-sectional design, and susceptible to confounding and reverse causation, whether the presence of GERD causally increase the risk for asthma remains unclear.

What This Study Adds to the Field

A two-sample bidirectional Mendelian randomization (MR) analysis establishes a causal effect of GERD on asthma and identifies a complex genetic interplay between atopic disorders of the skin and lung with GERD in European-ancestry populations. Specifically, our findings uncover previously unrecognized causal pathways that have clinical implications: 1) asthma is a causal risk for atopic dermatitis; and 2) the predisposition to atopic dermatitis, including increased risk of asthma, can arise from specific pathogenic mechanisms manifested by GERD, a disease of the gastrointestinal (GI) tract. Further, our results show that the effect of asthma on GERD is nominal and, hence, provide a basis for reexamining current therapeutic approaches to the management of asthma patients with GERD.

INTRODUCTION

Gastroesophageal reflux disease (GERD), a condition caused by persistent regurgitation of gastric contents leading to the sequelae of esophageal and/or extra-esophageal complications (1), is highly heritable and occurs in ~75% of patients with asthma (2, 3). The frequent coexistence of traits, or GERD comorbidity in patients with asthma (3, 5, 6), may underlie common genetic etiology and/or pathogenic mechanisms shared between the two diseases. Of note, micro-aspiration of endogenous acids can reduce the pH of the airways, and via either reflex mechanisms (7, 8) or direct effects on the airways (8), has been proposed to contribute to the pathophysiology of obstructive lung diseases (9-16).

Subsequently, it was determined that GERD can trigger asthma exacerbation (17). In addition, numerous clinical and epidemiological studies have reported association between asthma and GERD; and, a large body of evidence suggests GERD increases the risk of asthma (18-20). However, as these observational studies are often limited to cross-sectional design, and susceptible to confounding and reverse causation, whether the presence of GERD causally increases the risk for asthma remains unclear.

Based on Mendel's laws of inheritance [*random segregation* and *independent assortment* of genes], Mendelian randomization (MR) collects genetic variants (i.e., single nucleotide polymorphisms (SNPs) which are invariant to measured and unmeasured confounding factors or reverse causation) for use in an instrumental variable (IV) analysis to estimate a potential causal effect of a modifiable exposure on a risk outcome (21). Recently, MR methods have been used to clarify our understanding of multiple risk factors (biomarkers) that may simply be correlated from those that are causally related to various health outcomes (22-27). For instance, with respect to asthma, studies have established a positive causal effect of obesity and related traits on disease susceptibility (28-31). Conversely, Freuer and colleagues (32) reported, using one-sample MR within one study cohort, that genetically determined childhood-onset asthma, and not adult-onset asthma, is on the causal pathway leading to a number of gastrointestinal (GI)

disorders, including GERD. The latter study, however, is prone to sample overlap (33) which may limit power and induce some biases, thus motivating additional work to quantify the causal relationship between GERD and asthma.

Here, we used summary-level datasets of the two largest genome-wide association studies (GWAS) conducted on asthma (34) and GERD (35) in European ancestry, and performed two-sample bidirectional MR analysis to infer the magnitude and direction of causality between the two diseases. Additionally, we generated independent genetic IVs for atopic dermatitis (AD) from the latest population-level GWAS meta-analysis (36), and assessed their exposure commonality with genetically determined asthma on the predisposition to GERD. We conducted these studies because AD, an inflammatory disease of the skin, is frequently present in patients with asthma (37), shares a strong genetic etiology with asthma (38), and is believed to be an important early causal factor in the ultimate development of atopic asthma ("atopic march") and asthma severity (39, 40).

MATERIALS AND METHODS

Data Sources: The genetic variants were, all or partially, identified from the UK Biobank. Genetic IVs for asthma was obtained from GWAS in the UK Biobank on a broad asthma definition (56,167 cases and 352,255 controls) (34). The GWAS summary data included results from association test of 35,270,583 SNPs with asthma in a cohort of White British ancestry. Genetic IVs for GERD and atopic dermatitis were obtained from two of the latest and largest population-level GWAS meta-analysis on GERD (71,522 cases and 261,079 controls) (35) and AD (22,474 cases and 774,187 controls) (36), respectively. The participants were primarily of White European ancestry from the UK and Australia for GERD; and from the UK, Finland, and Republic of Estonia for atopic dermatitis. Detailed information on data sources is shown in **Supplementary Table 1**.

Genetic Correlations: The shared genetic architectures of the study traits (asthma, GERD, and AD) were calculated in a pairwise comparison using a linkage disequilibrium score regression (LDSC) method on HapMap3 SNPs (41). Computed genetic correlations were corrected for multiple testing based on the total number of correlations by applying a Bonferroni-corrected threshold of P=0.017 (0.05/3 traits).

Genetic Instrumental Variable (IV) Selection: First, we extracted SNPs associated with each trait at a genome-wide level of significance ($P<5 \times 10^{-8}$) in the respective studies (34-36). To ensure that SNPs were independent, IVs were clumped using a stringent linkage disequilibrium (LD) threshold of $r^2=0.001$ within a genetic window of 10 Mb based on the 1000 Genomes European reference panel. The effect estimates of both exposure and outcome variants were harmonized, expressed per effect allele increase, and possible palindromic SNPs were excluded. F-statistics were performed to assess the strength of genetically determined IVs ($F > 10$ is sufficient for the first MR assumption and does not suffer from weak instrument bias) (21, 42). **Supplementary Table 2** summarizes F-statistics of final instruments.

Mendelian Randomization (MR) Analyses: Figure 1 depicts the workflow of our instrumental variable analyses. As the primary analysis, we used a random-effect inverse-variance weighted (IVW) method, which allows for heterogeneity for the SNPs used in the instruments (43). Before running the IVW method, we first conducted sensitivity analyses to assess the directional pleiotropy (horizontal pleiotropy) using MR-Egger regression (44). Further, we applied MR-PRESSO (pleiotropy residual sum and outlier) to detect any horizontal pleiotropic outlier (45). **Supplementary Table 3** shows the final number of IVs used in the IVW method. To ascertain the robustness of the primary analysis, we also conduced IV analyses using the MR-Egger

method and the weighted median regression method (**Figure 1**). Finally, we performed leave-one-out analysis to test if the effect estimates were influenced by any one variant.

Statistical Analysis: All statistical analyses were performed using the TwoSampleMR (47) and MR-PRESSO (45) packages in R Software 4.1.0. To correct for multiple testing, we applied Bonferroni-correction imposing a significance threshold of 0.017 (i.e. 0.05/3 tests).

RESULTS

Genetic Correlations: Prior to performing MR analysis, we first evaluated the genetic correlation between the study traits in the GWAS summary statistics (34-36). Using LDSC (41), we detected the highest genetic correlation between atopic dermatitis and asthma (r_g=0.710, SE=0.165, P=1.72 x 10^{-5}), followed by the correlation between asthma and GERD (r_g=0.362, SE=0.051, P=2.04 x 10^{-12}), and then between GERD and atopic dermatitis (r_g=0.2, SE=0.049, P=3.90 x 10^{-5}).

Asthma and Atopic Dermatitis: Based on the ranked order in genetic correlations, and the well-recognized link between atopic disorders and asthma (37, 38), we first conducted MR analysis of atopic dermatitis (AD) and asthma. We conducted these studies because AD is believed to be on the causal pathway along the progression of asthma ("atopic march") (39, 40).

Using the GWAS summary statistics conducted on asthma (N_{cases}=56,167) (34) and atopic dermatitis (N_{cases}=22,474) (36), we derived 48 genetic variants for asthma and 11 genetic variants for AD. Of note, 11 AD-associated SNPs (F-statistics of SNPs range from 32 to 62) were equally strong instruments as that of 48 asthma-associated SNPs (F-statistics of SNPs range from 30 to 247) (**Supplementary Table 2**).

As expected, genetically determined AD was associated with increased risk of asthma (**Figure 2**), increasing the risk by 34% using the IVW method (OR, 1.34; 95% CI, 1.24-1.45; $P_{IVW}=1.32 \times 10^{-14}$). Similar effect estimates of AD on risk of asthma were detected using the weighted median regression method (OR, 1.32; 95% CI, 1.21-1.44; $P=5.77 \times 10^{-10}$) as well as the MR-Egger method (OR, 1.54; 95% CI, 1.08-2.20; $P=4.14 \times 10^{-2}$) (**Supplementary Table 4**). Leave-one-out analysis showed that the effect estimates were not influenced by any one variant (**Supplementary Figure 1**). In addition, the MR-Egger regression intercept did not significantly deviate from zero (**Supplementary Table 5**), suggesting no evidence of 'horizontal pleiotropy' or violation of the second MR assumption (45). These results collectively established that atopic dermatitis is a causal risk factor for development of asthma.

Surprisingly, when exposure and outcome were reversed, 48 genetic variants for asthma were also causally associated with increased risk of atopic dermatitis, increasing the risk by 46% (OR, 1.46; 95% CI, 1.34-1.59; $P_{IVW}=1.67 \times 10^{-18}$) (**Figure 2**). The effect estimates were directionally consistent across the sensitivity analyses (**Supplementary Table 4**) with no evidence of horizontal pleiotropy (**Supplementary Table 5**); and, this causal direction was not driven by a single outlying variant (**Supplementary Figure 2**). As our study entailed partially overlapping sets of participants that can affect the causal estimates, we calculated the magnitude of potential bias, including Type 1 error rate inflation due to sample overlap, using an online tool described in Burgess and colleagues (46). Across the entire range of possible sample overlap (**Supplementary Table 6**), we did not detect evidence for sample overlap in GWAS summary statistics (34-36). Hence, two-sample bidirectional MR analyses not only confirmed a well-recognized causal pathway in the progression of asthma, but also identified a previously unrecognized association that is suggestive of asthma as a plausible risk factor for developing atopic dermatitis.

Asthma and GERD: For MR analysis of asthma and GERD, we identified 62 asthma-associated SNPs and 21 GERD-associated SNPs in the GWAS summary statistics (34, 35) that were sufficiently strong independent genetic instrumental variables (**Supplementary Table 2-3**). On the one hand, we detected small, but significant effect estimate of genetically determined asthma on increased risk of GERD (OR, 1.06; 95% CI, 1.03-1.09; $P_{IVW}=4.94 \times 10^{-4}$) (**Figure 3** and **Supplementary Table 4** and **Supplementary Figure 3**). On the other hand, we found strong and large causal effect estimate of genetically determined GERD on increased risk of asthma (**Figure 3** and **Supplementary Table 4** and **Supplementary Figure 4**), increasing the risk by 21% (OR, 1.21; 95% CI, 1.09-1.35; $P_{IVW}=5.63 \times 10^{-4}$).

Interestingly, 21 genetic variants associated with GERD also increased risk of atopic dermatitis (OR, 1.21; 95% CI, 1.07-1.37; $P_{IVW}=3.32 \times 10^{-3}$); the effect size of GERD on atopic dermatitis was similar to that of GERD on asthma (**Figure 3** and **Supplementary Table 4** and **Supplementary Figure 5**). There was no evidence of a causal relationship in the opposite direction (i.e., AD on risk of GERD) (**Supplementary Table 4** and **Supplementary Figure 6**). These results, taken together, established a complex genetic interplay between the inflammatory diseases of the lung and skin with GERD in European-ancestry population and suggested that the predisposition to asthma, including atopic dermatitis, can arise from specific pathogenic mechanisms manifested by GERD.

DISCUSSION

In this study, we set out to test whether asthma, an inflammatory disorder of the airways, is causally related to GERD, a condition that is perpetuated by chronic regurgitation of gastric contents. Applying three different MR methods, we found similar magnitude of causal estimates with an appreciably larger effect size for genetically determined GERD predisposing to asthma (increasing asthma risk by 21%) than genetically determined asthma on risk of GERD (6%

increase) in European-ancestry populations. The effect size was directionally consistent across the sensitivity analyses with no evidence of weak instrument bias or possible sample overlap in GWAS summary statistics. Further, consistent with computed genetic correlations, and a widely held notion that atopic dermatitis, an inflammatory disease of the skin, is an important early risk for the subsequent development and progression to asthma, genetically determined AD increased risk of asthma by 34%. Of note, the largest effect size was detected for asthma predisposition to AD (increase AD risk by 46%). Interestingly, while AD did not increase GERD, GERD increased risk of AD (21% increase) as it did for asthma. Thus, our studies reveal a complex genetic interplay between inflammatory diseases of the lung and skin with GERD and, moreover, suggest that the predisposition to asthma, including atopic dermatitis, can arise from specific pathogenic mechanisms manifested by GERD.

The frequent coexistence of traits, or GERD comorbidity in patients with asthma, may underlie common genetic etiology and/or pathogenic mechanisms shared between the two diseases. Of note, the lung and gut arise from the foregut which, in time and space, undergoes a divergent developmental program that is requisite to each organogenesis and permissive for distinct physiological functions (41, 42). Anatomically, the upper respiratory and GI tracts are in close proximity, separated by a short-lived tracheoesophageal septum, and the stimulatory and inhibitory reflex mechanisms of the upper GI tracts (i.e. esophagus) are designed to ensure protection against aspiration of ingested and gastric contents (reflux) into the airways (50, 51). Micro-aspiration of endogenous acids, due to altered reflex and/or reflux mechanisms (7, 8), can reduce the pH of the airways and has been proposed to contribute to the pathophysiology of obstructive lung diseases (13-16). Although numerous studies have suggested that GERD increases the risk of asthma (18, 19), most, if not all, are limited to retrospective clinical and epidemiological observations. Hence, whether the presence of GERD causally increases the risk for asthma and/or whether patients with asthma are genetically susceptible to develop GERD remain unclear. Of note, randomized controlled trials of acid-suppressive therapies in asthma

patients with GERD (adults and children) have shown limited therapeutic improvements on asthma symptoms and/or pulmonary function (2, 52-54). These issues have led us to explore whether there exists a true cause-effect relationship between these two highly heritable diseases.

Similar to randomized controlled trials, Mendelian randomization applies a universal concept in human genetics [*random segregation* and *independent assortment* of genes] as proxies to infer whether a modifiable risk factor is causally linked to a health outcome (21). Recently, with an increasing availability of large GWAS summary statistics, MR methods have been used broadly to establish causal relationships between commonly associated traits/diseases and, in so doing, opened new avenues for cost-effective, well-rationalized study designs for clinical trials, including the basis for further hypothesis-driven mechanistic studies. For instance, with respect to asthma, studies have recently established a positive, unidirectional causal effect of obesity and related adiposity traits (i.e., the weighted allele score for body mass index [BMI]) on risk of asthma (28-31). A higher BMI was also identified as a causal risk for atopic dermatitis (55). Of note, Green and colleagues (56) found that central fat distribution (i.e., a higher waist-hip ratio), and not BMI, is causally associated with GERD. Because obesity is a potential risk for multiple chronic disorders (24), and there is already emergence of new MR approaches to this space (29, 57-59), we did not perform MR analysis of obesity and/or obesity-related traits with our study traits (asthma, AD, and GERD) or additional multivariable MR, including genetic associations with potential confounders (i.e., BMI, sex, smoking status) within the respective patients cohorts of the non-disclosive summary-level GWAS (34-36).

Recently, Freuer and colleagues (32) reported that the presence of asthma onset in childhood, but not in adulthood, is positively associated with GERD later in life. For this study, they collected genetic IVs for childhood-onset and adult-onset asthma, as well as genetic IVs for GERD within one study cohort in UK Biobank (33); and ran one-sample MR in one causal pathway, which may suffer weak instrument bias and some predicted power. On the one hand, a longitudinal study in UK general population reported that patients with asthma are at increased

risk of developing GERD and not vice versa (20). On the other hand, two longitudinal follow-up studies in Korean children (60) and adults (61) detected a reciprocal causality between GERD and asthma. Based on these observational studies, here we performed two-sample bidirectional MR analysis to infer the magnitude and direction of causality between asthma and GERD. Interestingly, Zhu and colleagues found that shared genetic loci between asthma and allergic diseases (hay fever/allergic rhinitis or atopic dermatitis) were enriched in immune/inflammatory systems and localized to several epithelial tissues, including the skin, lung, and esophageal tissues (38). Accordingly, we also generated genetic IVs for atopic dermatitis, and assessed their exposure commonality with genetically determined asthma on the predisposition to GERD.

In this study, we detected a small, but significant effect size for genetically determined asthma on risk of GERD (OR, 1.06; 95% CI, 1.03-1.09; $P=4.94 \times 10^{-4}$) in European ancestry population. For our analysis, asthma SNPs were identified from GWAS in the UK Biobank on a broad asthma definition (34) and may have inflated the nominal causal effect due to an unaccounted natural history (time and course) of patient-reported asthma symptoms. In addition, the genetic variants (asthma, GERD, and AD) were, all or partially, identified from participants in the UK Biobank (34-36) and, as such, may suffer from sample overlap–one of the limitations of MR studies using publicly available GWAS summary statistics. To this end, we simulated IV bias across the entire range of possible sample overlap (**Supplementary Table 6**) and performed F-statistics of final instruments (**Supplementary Table 2**), but found no evidence of sample overlap in the GWAS summary statistics or weak instrument bias. Further replication studies are needed in non-European populations for the generalizability of our findings, however.

Most strikingly, as opposed to the findings of Freuer et al. (32), we detected the presence of a reciprocal causal effect of GERD on asthma (genetically determined GERD increased the risk for asthma by 21%). Although MR analysis does not provide or suggest the underlying biology, here we speculated different mechanisms, including those involving acid reflux effects on the lung. Barbas et al. (62) showed that chronic aspiration of gastric fluid evokes a shift of immune

responses (from T helper 1 (Th1) to Th2) in a mouse model of asthma. Using isolated human airway smooth muscle (ASM) cells in culture as a physiological model, we have reported that small reductions in extracellular pH evoke contraction via an ovarian cancer G protein-coupled receptor 1 (OGR1 or GPR68) expressed on ASM (13), suggesting that alterations in extracellular pH (i.e., airway acidification caused by exogenous and/or endogenous acids, or as a consequence of airway inflammation) has direct effects on the contractility of an end-effector cell of acute airway narrowing in asthma (63). In addition, reflex-mediated increases in ASM contraction and airway resistance (64), mediated by autonomic nerves innervating the airways (65), or by mechanical perturbation to the mucosa of the upper airways and esophagus (7, 66), can contribute to asthma pathogenesis. Together these studies highlight an importance of characterizing reflex and/or reflux mechanisms regulating ASM tone and contractility in asthma patients with GERD (65). Our results also warrant further investigation into the diagnosis and treatment of GERD comorbidity in patients with asthma.

Among the three traits studied, we detected the largest effect size for asthma predisposition to atopic dermatitis (increase AD risk by 46%). This is a marked contrast to the widely-held notion that atopic dermatitis is an early causal factor in the ultimate development of atopic asthma (39, 40). While the causal mechanisms for this atopic march remain subject to debate (40), one plausible mechanism is the loss-of-function mutations in the filaggrin gene (*FLG*) in patients with atopic dermatitis that lead to dysregulation of epidermal skin barrier function and consequent induction of allergic sensitization and airway hyperresponsiveness through an antigen-specific Th2 responses (67). In addition to variants in *FLG* that define the shared genetic pathways in atopic dermatitis and asthma (67-69), Sliz and colleauges (36) have also identified other novel missense variants, including *DSC1* (desmocollin 1) and *SERPINB7* (serpin family B member 7), that may contribute to altered mechanical stability and epidermal barrier properties in European ancestry. Accordingly, our results not only confirmed a plausible causal effect of atopic

dermatitis on asthma, but also identified a previously unrecognized association that is suggestive of asthma may be a causal risk factor for atopic dermatitis.

Interestingly, GERD equally increased the risks for asthma (OR, 1.21; 95% CI, 1.09-1.35; $P=5.63 \times 10^{-4}$) and AD (OR, 1.21; 95% CI, 1.07-1.37; $P=3.32 \times 10^{-3}$): AD did not increase risk of GERD. These results suggested that the predisposition to atopic dermatitis, including increased risk of asthma, can arise from specific pathogenic mechanisms manifested by GERD, a disease of the GI tract. To the best of our knowledge, a causal effect of GERD on atopic dermatitis has not been established. While further studies are warranted to establish the specific mechanisms, we speculate the intestinal neuro-immune axis (70), including inter-kingdom microbial crosstalks (71), constitute possible homeostatic mechanisms regulating inflammatory responses in the lung and skin, and shaping the ultimate development of atopic disorders–asthma and atopic dermatitis. We believe further insights into the causal pathway to these complex traits may come from advances in MR that incorporate tissue-specific microbiome, metabolome, transcriptome, and phenome-wide analyses (25, 72-75). Such findings would not only provide a greater granularity to disease etiology, but also discover new biomarker(s) and development of new targeted intervention strategies.

Taken together, this study established a complex genetic interplay among asthma, atopic dermatitis, and GERD in European-ancestry populations. Importantly, our findings not only substantiated the association between asthma and GERD, but also uncovered previously unrecognized associations that have clinical implications: 1) asthma is a causal risk factor for atopic dermatitis; and 2) the predisposition to atopic dermatitis, including increased risk of asthma, can arise from specific pathogenic mechanisms manifested by GERD. Further studies are needed for the identification and characterization of the gut-lung-skin axis.

FIGURE LEGENDS

Fig. 1. The workflow of instrumental variable analysis to estimate a potential causal effect of a modifiable exposure on a risk outcome.

Fig. 2. Causal relationships between asthma and atopic dermatitis. IVW method was used to estimate the magnitude and direction of effect sizes and presented as odds ratios (ORs) and 95% confidence intervals.

Fig. 3. Causal relationships between GERD and atopic disorders (asthma or eczema). IVW method was used to estimate the magnitude and direction of effect sizes and presented as odds ratios (ORs) and 95% confidence intervals.

Supplementary Materials

Supplementary Figures:

Supplementary Tables:

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