Understanding the Genetic Causes behind Gastroesophageal Reflux Disease (GERD) for Future Exploration of Targeted Treatments

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Abstract

Genetic influences play a key role in the diagnosis of GERD and the presentation of its typical symptoms. This paper reviews risk factors associated with GERD, focusing heavily on genetic risk factors. The common risk factors associated with GERD, especially those associated with genetic influences, will be discussed. Current research on four genes, GNβ3, ADRB2, BARX1, and ADAMTS17 suggests that GERD can be associated with SNP (single base pair) mutations. While environmental factors such as diet and physical activity also play an important role in GERD development, this paper will target genetic causes and focus on how their interactions with the outside environment determine GERD diagnosis. Considering these influences alone and in combination can help us further understand GERD pathogenesis. Selective treatments in managing gene-specific GERD are important to advancing targeted and personalized management of the disease. Future research expanding on genetic patterns and gene-expressed pathways can be used to advance knowledge of GERD risk and improve treatment.

Introduction

What is GERD?

More than 3 million Americans are diagnosed with gastroesophageal reflux disease every year [1]. As one of the most common digestive disorders, gastroesophageal reflux disease (GERD) is an important health concern with a wealth of studies conducted on its presentation and symptoms. The Montreal Consensus, adopted in 2006, provided researchers and physicians with a globally acceptable definition that defines GERD as “a condition that develops when the reflux of stomach contents into the esophagus causes troublesome symptoms and/or complications” [2]. Typical symptoms observed in common presentations of GERD include heartburn and acid regurgitation [3]. Less commonly, but still notable, is the manifestation of extraesophageal symptoms such as chest pain, chronic cough, or asthma in some patients with GERD [3]. These extraesophageal symptoms are thought to occur due to reflux into the larynx while typical symptoms such as heartburn are directly caused by acid reflux into the esophagus.

Epidemiology and Significance of GERD

As stated above, GERD is an increasingly common gastrointestinal disease; the recorded uptick in patients diagnosed with GERD makes this a cause for concern amongst patients, physicians, and researchers. In this section, we will examine the prevalence of GERD diagnoses across time periods and locations in order to better understand the genetic factors behind skewed or even distributions of the disease. GERD is increasing in prevalence worldwide and has been

estimated to affect up to 20% of the global population [4]. However, the Western world appears to have higher rates of GERD diagnosis compared to the Eastern world; a 2018 study found a reported prevalence of 30% in adults within Western populations compared to a prevalence of below 10% in East Asia [4]. A 2015 survey of two populations in the United States reported that almost one-third of those surveyed had symptoms of gastroesophageal reflux in the past week and acknowledged a 50% increase in GERD prevalence [4]. Europe displays similar prevalence statistics; in the same study, the authors recorded a 25.9% prevalence of GERD in Europe based on epidemiological data [4]. In contrast, the Eastern world displays significantly reduced percentages of those affected by gastrointestinal disease. One 1998 study reviewed reflux esophagitis around the world and noted the condition was much lower in Asia and Africa; a 2019 study estimated that 7.8% of the population in East Asia is diagnosed with GERD [4]. These locational discrepancies can be due to genetic factors or lifestyle and health habits; genetic influences will be explored in further sections of this paper.

Genetic Causes

GNβ3

The GNB3 gene encodes for the beta 3 subunit of a guanine nucleotide-binding protein, or a G protein [5]. G proteins play an essential role in signal transduction systems, receiving signals from cellular receptors and delivering them to effector proteins inside the cell [6]. Various single-nucleotide polymorphisms (SNPs) on the GNB3 gene have been associated with hypertension and obesity and have also been linked to digestive disorders such as functional dyspepsia (chronic indigestion), irritable bowel syndrome, and GERD [5], [7].

A 2017 cohort study by Patel, et al. found that rs5443, a SNP on the GNB3 gene, may influence the severity of GERD symptoms in studied patients [8]. The association between the presence of rs5443 and GERD symptoms was strengthened amongst patients who had a heightened perception of the amounts of reflux produced. This finding helps support the hypothesis that the presence of the rs5443 SNP causes increased pain perception in the esophageal area. This hypothesis can be backed by the fact that activation of the GNB3 gene causes increased signal transduction within the brain-gut axis. Overactivation of the GNB3 pathways could lower the activation threshold in pain receptors, which is likely to cause hypersensitivity to acid reflux. Patel, et al. also found that patients who carried T alleles on the GNB3 gene had an overall worse symptom burden, which is supported by the finding that TT alleles have been associated with increased signal transduction [8]. This study concludes that the perception of the severity of GERD symptoms is partially genetically influenced, specifically by the rs5443 mutation on the secondary messenger gene GNB3.

A 2009 editorial by Jankowski, et al. summarized and commented on a then-recently published study by De Vries, et al. about the association between GERD and a GNB3 polymorphism. In the De Vries paper, the authors performed a medium-sized control study genotyping participants

identified as having GERD and healthy control patients free of any symptoms [9]. They found that the CT genotype of the C825T polymorphism on the GNB3 gene was more prevalent in GERD patients relative to healthy controls [9]. The C825T SNP gives rise to three possible genotypes: CC, CT, and TT. When the TT or CT genotypes are selected on the 825T allele, the GNB3 gene is alternatively spliced and a shorter, yet still functional, splice variant is produced. High amounts of this variant enhance G-protein activation, while the CC genotype on the 825C allele forms minute amounts of the spliced variant, leading to diminished activation and signal transduction [10]. So, those with the 825T allele of the GNB3 gene may have an increased response to acid neurotransmitters. Jankowski, et al. also commented on an interesting finding from the De Vries study; patients who exhibited more severe reflux symptoms were more likely to have the CT allele rather than the TT genotype [10]. While the TT genotype on GNB3 has been found to increase susceptibility to GERD, further research is necessary to understand the relation between the CT genotype and other gastrointestinal diseases such as functional dyspepsia.

ADRB2

ADRB2 is a gene that codes for the beta-2-adrenergic receptor, a member of the G protein-coupled receptor superfamily [11]. These encoded adrenoreceptors are primarily located in the central nervous system, heart, kidney, and muscle where they are involved in the relaxation of smooth muscles. As mentioned above, G-protein coupled receptors (GPCRs) are transmembrane signaling proteins involved in critical functions around the body, usually regulating these functions through the activation of adenylate cyclase [12]. GPCRs in the gastrointestinal tract are involved in critical functions such as controlling digestion and coordinating repair and growth [13]. Hence, the genes that encode them, such as ADRB2, are crucial targets for digestive diseases like GERD. ADRB2 is also expressed in the nervous tissue responsible for pain perception, implicating it in pain and fatigue disorders as well [14].

A 2013 article by Kushnir, et al. analyzed the relationship between ADRB2 and gastrointestinal diseases by evaluating a cohort of 398 subjects through data collection methods such as bowel symptom assessment, a survey on symptoms and their severity, and DNA sample collection and genotyping [15]. Their experiments allowed them to successfully identify the rs1042714 ADRB2 polymorphism as a risk allele for the development of functional GI (FGID) disorders. For example, amongst a group of subjects suffering from IBS, Kushnir, et al. found those who carried the G allele on the rs1042714 SNP had a higher gastrointestinal symptom severity (such as increased bowel symptom severity), bother, and frequency when compared to subjects who had the homozygous genotype of CC [8], [15]. Carriers of the G allele on the rs1042714 SNP also reported a lower health-related quality of life, specifically within the mental and physical health sections of the survey. These findings allowed the study authors to conclude that G allele carrier status is correlated with more severe phenotypes (symptoms) of gastrointestinal diseases.

The findings in the Kushnir, et al. study have allowed for the establishment of a link between gastrointestinal diseases and the rs1042714 mutation on the ADRB2 gene [15]. The increased severity of the phenotypes amongst the study subjects who carried the G allele on the rs1042714 SNP can be explained by the functioning of the receptor encoded by ADRB2. The beta-2-adrenoreceptor is activated by norepinephrine and epinephrine, the hormones behind the human “fight or flight” response, explaining its association with poorer mental health and increased pain perception [12]. The findings of Kushnir, et al. study are generalizable for all gastrointestinal diseases, including GERD and IBS, allowing for a wide range of applications. Further research that focuses independently on GERD and only its association with SNPs on ADRB2 would allow for a deeper understanding of the gene and the specific pathways it targets in digestive disease presentations.

BARX1

The BARX1 gene codes for a member of the Bar subclass of homeobox transcription factors [16]. Mouse and chick modeling studies have indicated that the encoded transcription factor may play a role in teeth development and the growth of complex structures in the skull and face (craniofacial development) [16]. In a purpose relevant to GERD, the protein has also been associated with the differentiation of epithelial cells in the stomach [16].

A 2019 cohort study conducted by Argyrou, et al. screened groups of GERD patients and healthy control subjects for BARX1 polymorphisms and found a considerable association between the expression of the gene and GERD [17]. Specifically in this study, the rs11789015 polymorphism of BARX1 was studied. After extracting genomic DNA from blood samples taken from the study participants, allele-specific PCR was conducted in order to identify and replicate the polymorphism. Analysis of results in the rs11789015 polymorphism showed that the frequencies of both the AG and GG genotypes as well as that of the minor allele G were significantly increased in GERD patients compared to healthy controls [17]. This result helped researchers conclude that the study participants exhibited an association between the presence of the transcription factor expressed by BARX1 and susceptibility to GERD development [17].

Based on the current understanding of the function of BARX1 and the results of the study mentioned above, it is possible to conclude that the relationship of GERD with the presence of BARX1 may be related to the role its transcription factor plays in esophageal differentiation in the stomach mesenchyme tissue. The dysregulation of this gene involved in esophageal development would lead to a reduction in anatomical antireflux mechanisms, explaining the susceptibility link between BARX1 and GERD [17].

ADAMTS17

ADAMTS17 is a gene that encodes a member of the ADAMTS protein family, specifically the ADAM Metallopeptidase With Thrombospondin Type 1 Motif 17 protein [18]. ADAMTS family

members are cell-surface proteins containing sheddase functionalities that allow them to regulate the shedding of important molecules in cells such as membrane-bound proteins or growth factors [19]. These protein functions may promote breast cancer cell growth and survival, pathological tissue remodeling, and the autosomal-recessive Weill-Marchesani syndrome, a rare genetic disorder in which patients develop eye and skeletal abnormalities [18], [20]. ADAMTS17 belongs to a subgroup of orphan enzymes, meaning that its physiological substrate and functions have yet to be clearly defined, but its unique structure containing both potential adhesion and protease domains as well as its involvement in numerous biological processes could be of some importance to GERD causing mechanisms [20].

A 2016 GWAS meta-analysis conducted by Bonfiglio, et al. identified 30 GERD-suggestive risk loci and concluded that the ADAMTS17 gene would be the best candidate on which to conduct further research [21]. The researchers arrived at this conclusion after testing GERD loci for the presence of eQTL effects, which are genomic loci that explain variations in the expression levels of mRNAs. After conducting analysis on cis-eQTLs, which are focused on transcriptional regulation, the researchers observed that specific GERD risk regions are enriched with significant eQTLs, with esophagus muscularis, the outer muscle layer of the esophagus, containing the highest number of eQTLs [21]. The ADAMTS17 gene was associated with the presence of eQTL effects in this tissue, hence the researchers concluded that the ADAMTS17 gene should be a primary candidate for further investigation.

The 2019 Argyrou, et al. study discussed previously also investigated a possible correlation between ADAMTS17 polymorphisms and the development of GERD. In this study, the rs4965272 polymorphism was studied since the meta-analysis by Bonfiglio et al. mentioned above identified this SNP as a possible pathogenetic factor for GERD development [17]. Genotypic analysis was conducted on blood samples taken from a cohort of Greek patients at three Athens hospitals. Some of the study participants suffered from GERD while others displayed no symptoms or pain responses. The Argyrou et al. study showed that patients with the homozygous GG genotype of the rs4965272 polymorphism of ADAMTS17 had 3.42-fold increased odds of developing GERD compared to those carrying the TT genotype of the SNP. They also concluded that a homozygous GG genotype was the only genotype that statistically increased the probability of GERD development in the studied cohort; the presence of only one G allele alone was not sufficient to increase the odds [17].

Understanding the functions of the ADAMTS17 gene and the proteins it expresses are key to uncovering the biological reasoning behind this correlation between the presence of GERD in a patient and the ADAMTS17 polymorphism. While it is speculated that the unique structure of the ADAMTS17 protein as well as its ability to confer different regulatory influences on microfibrils in the cell environment are the factors behind this correlation, further research is necessary in order to confirm and thoroughly understand the role of the ADAMTS17 gene in GERD development.

Further investigation into the function of ADAMTS17 can also lead to discoveries about the functions of other ADAMTS protein-coding genes such as ADAM10, an established candidate gene for GERD that controls e-cadherin proteolytic cleavage, a biological mechanism that occurs at a higher rate than normal in GERD patients [22]. E-cadherin cleavage leads to increased permeability due to a loss of cell-to-cell adhesion, which is a pathway that has been implicated in GERD pathology.

Conclusion

Possible Treatments

Identification of genes that are correlated with a disease can aid in treatment through the development of gene-specific solutions that target the faulty genetic mechanisms associated with the disease. After identifying genes that have a probable connection to GERD, researchers can target the identified genes and their expressed proteins through new treatments, allowing for the targeting of whole genetic pathways through novel drugs or other targeted therapies. Among the different genes discussed earlier in this paper, we identified a strong correlation between the ADAMTS17 gene and the development of GERD. We can examine a specific targeting mechanism for an ADAMTS family protein that can be potentially applied to inhibit GERD symptoms.

The next step after identifying ADAMTS17 as a possible risk locus for GERD is to explore prospective treatments for GERD that specifically target the ADAMTS17 gene and the protein it expresses. In existing literature, there is minimal information on the function of ADAMTS17 specifically, but there is a wealth of research conducted on other ADAMTS enzyme-coding genes. In a recent article published in the Journal of Medicinal Chemistry, Meibom, et al. detail their discovery of the first orally bioavailable ADAMTS7 inhibitor, BAY-9835 [23]. These researchers began by designing an inhibitor of the protein's catalytic domain and then improved selectivity by modifying the chemical structure and properties, eventually resulting in a molecule, BAY-9835, that selectively blocks ADAMTS7 function [23]. A similar type of inhibitor can also be developed for ADAMTS17, with chemical modifications to BAY-9835 to account for the structural differences between expressed proteins. The basic ADAMTS structure is overall conserved throughout, with the majority of ADAMTS enzymes being composed of a pro-domain, a catalytic domain, and an ancillary domain with thrombospondin repeats [20]. So, this similarity in structure will prove to be helpful when exploring a potential small molecule inhibitor for the ADAMTS17 protein.

Future Research

The field of GERD research is an expansive field in which many research questions remain unanswered. One promising avenue for future research is the management of GERD symptoms through the regulation of enzymes that control important biochemical pathways associated with

GERD. Enzymatic regulation of specific protein-coding genes discussed earlier such as ADAMTS17 can be used. We can examine how the identification of small molecule inhibitors that regulate enzyme functionality relevant to GERD can foster the development of novel drugs or biomarkers.

Aside from designing inhibitory molecules for ADAMTS17 proteins, another specific treatment that can be explored is enzymatic regulation. This can be explored through a study conducted on the regulation of ADAM17, a related member of a superfamily that includes ADAMTS17. The main difference between ADAM family proteins and ADAMTS family proteins is that ADAMs are integral membrane proteins that are involved in the process of splitting other membrane proteins, while ADAMTS proteins do not have a membrane anchor and are usually only involved in mechanisms with cell-surface or secreted molecules [24]. However, both of these protein types belong to the same overall family so the regulatory pathways used for one can be modified for use on the other. Now that these similarities have been established, we can explore the ADAM17 regulatory mechanisms discussed by Le Gall et al. in a 2010 research article. In this paper, the authors explain that the ADAM17 protein is activated via a rapid and reversible exposure of its catalytic site [25]. This domain on the protein can be stimulated by a range of molecules including cytokines such as TNFα, EGF growth factor, enzymes such as thrombin (which reacts with cell surface receptors), lysophosphatidic acid lipid (which regulates activity by binding to GPCRs), and other biochemicals. Identifying and understanding molecules that regulate ADAM17 functionality can help in the development of targeted therapies or drugs or the discovery of biomarkers that provide information on disease diagnosis. The 2010 Le Gall et al. research study also conducted experiments with the tight binding active site inhibitor DPC333 on ADAM17 proteins [25]. The researchers found that cells pretreated with DPC333 had very low shedding activity compared to cells that were not treated with DPC333. But, they also discovered that although DPC333 can quickly bind to stimulated forms of ADAM17, irreversibly inactivate them, and block their shedding function, DPC333 is unable to bind to ADAM17 proteins in most unstimulated cells and does not prevent shedding in those quiescent cases. However, Le Gall et al.. also claimed that once DPC333 gained access to ADAM17 in stimulated cells, it could not be washed out and prevented re-activation of ADAM17 [25]. This makes the case for DPC333 as an effective small-molecule inhibitor for ADAM17 in many cases. DPC333 has also been mentioned in various biological experiments and papers as a selective inhibitor of ADAM17, making its target specificity well-documented. It is worth investigating the inhibitory effects of DPC333 on other metalloproteinases such as ADAMTS17, which could be of great value in the study of ADAMTS17 proteins and their link to gastrointestinal disorders, especially GERD.

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