Asthma is a debilitating respiratory disease affecting almost 300 million people worldwide, a statistic that appears to be increasing steadily from year to year[31]. In a survey conducted in 2009, it was shown that 8.2% of the United States population suffer from asthma, a percentage replicated in many other countries[1]. Caused by the inflammation of the lung mucosa and an overproduction of mucus in the airways, asthma can lead to severe coughing, wheezing, and chest tightness. Asthma is one of the costliest healthcare burdens globally, consuming a high amount of resources due to prevalence and severity of the illness. Affecting both adults and children alike, asthma has also been shown to be one of the leading causes of school and work absenteeism[4], with 10.5 million school days and 14.2 million work days missed in 2009 due to asthma[1].

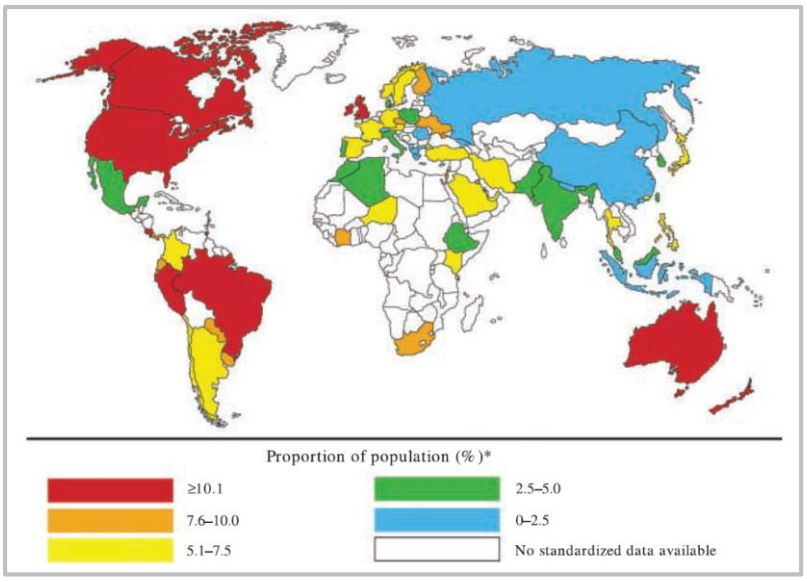

***Figure 1**: Map showing proportion of the world with asthma[31]*

Until the 1960s, the treatment of asthma had been inconsistent and mostly ineffective, due to the general misclassification and misunderstanding of the illness. Ancient Egypt provided the earliest recorded cases of asthma, which was treated at the time with inhalation of herbal incense fumes[9]. The term ‘asthma’ was coined by Hippocrates around 400 BC to refer to the symptom of wheezing and coughing, rather than as the name of the inflammatory disease. In his Ancient Greece, the illness was treated by drinking owl’s blood in wine[9]. The 1800s saw a variety of peculiar treatments, including rubbing the chest with chloroform liniment[16] and

applying intravenous doses of pilocarpine[6], and through the early 1900s, asthma was largely considered a psychosomatic disease related to depression and "cured" by psychoanalysis[9]. Asthma was finally realized as a serious, lung-inflammatory disease in the 1960s, when beta-2-agonists and glucocorticoids first became the consistent prescription to combat asthma.

While mostly effective in tamping down symptoms of asthma, current treatment with B2-adrenergic-agonists, glucocorticoids, and leukotriene modifiers have shown significant response heterogeneity in the asthmatic population[11], with around 60-80% of the variability attributed to genetic factors influencing individual metabolism of the drug, adverse effects from the drug, and drug target-pathway variations[36]. As such, recent research in asthma therapy has shifted focus towards a pharmacogenomics approach, with the end goal of establishing a future where asthma is treated through individually-tailored prescriptions based upon an understanding of each person's genetic responses to drug types and dosages.

With huge impacts and huge therapeutic variability, asthma is a highly-relevant medical topic with huge pharmacogenomics potential. To that end, this paper will address: 1) current asthma treatments and their side effects, 2) research in asthma pharmacogenetics for the development of genetically-appropriate therapies for asthma, in hopes of offering an in-depth look into the status of medical solutions for one of most prevalent illnesses in the world.

Current Asthma Treatments

There are three main prescribed medication for asthma currently in use: $beta_2$-adrenergic agonists, glucocorticoids, and leukotriene modifiers. To analyze how pharmacogenetics can help in prescribing these medications appropriately, it is necessary to first understand the mechanisms and rationale underlying these drugs.

β_2-adrenergic agonists are bronchodilators aimed at relieving bronchoconstriction – the tightening of muscles surrounding the bronchioles – during an asthma attack. These bronchodilators bind to beta-adrenergic receptors on the smooth muscles surrounding the airways and activates the enzyme adenylate cyclase to increase the amount of intracellular cyclic AMP[3]. The binding of these agonists also cause the opening of Ca^+-activated potassium channels, resulting in the rapid hyperpolarization of the membrane potential in these muscle cells[33]. Current research suggests that the mechanism for β_2-adrenergic agonists-triggered airway muscle relaxation is attributed to a combination of the inhibition of myosin-light chains by cyclic AMP and the disruption of stable membrane potential from opened K-channels[3, 33]. Unlike glucocorticoids, β_2-adrenergic agonists are not effective in alleviating the inflammation caused by asthma; rather, it is usually prescribed as a form of short-term relief from the discomforts of asthma symptoms. Commonly prescribed medication include albuterol and terbutaline, both of which are short-acting β_2-adrenergic agonists (SABA). While longer-acting β_2-adrenergic agonists (LABA) – such as salmeterol and formoterol – do exist, they are usually only prescribed with accompanying glucocorticoids for patients with more severe asthma[3].

Glucocorticoids are currently the most effective anti-inflammatory medication for asthmatics. Available in both oral and inhaled forms, these steroid molecules bind to receptors in airway epithelial cells and disrupt the transcription of cytokines. The marked decrease in cytokines lead to an overall reduction in the number of circulating inflammatory cells – mast cells, macrophages, T-lymphocytes, and eosinophils[5]. In addition to reducing inflammation in the lungs, glucocorticoids have also shown to slow down the decline of lung function in asthmatic patients[10] Currently, prescribed glucocorticoids in the United States include beclomethasone dipropionate, flunisolide, and triamcinolone[5].

A third, and lesser-prescribed, therapeutic option for asthma involves the use of leukotriene modifiers. Leukotrienes are molecules formed from arachidonic acid and released by the cells for metabolism by different major pathways[8, 13]. Leukotrienes can cause increased mucus secretions and are known to attract inflammatory leukocytes to the lungs. As such, leukotriene modifiers aim to either inhibit biosynthesis of the leukotrienes or block interactions between leukotrienes and their receptors[20]. There are 4 current leukotriene-modifying drugs approved for medical use: montelukast, zafirlukast, pranlukast and zileuton[47].

Side Effects of Current Treatments

Studies dating back to the 1960s have shown that long-period usage or higher dosage of β_2-adrenergic agonists may actually increase the risk of death from asthma. In a 1994 study from Canada[39]. it was shown that patients who use one canister of β_2-adrenergic agonists more than other similar asthmatics have *double* the risk of fatal asthma. In a separate 2-year study on usage of albuterol, it was shown that regular usage of β_2-adrenergic agonists actually led to faster decline of lung function compared to intermittent usage[46].

The usage of glucocorticoids likewise present the risk of many systemic side effects due to its prescription as a consistent, long-term treatment[23]. Side effects range from the development of cataracts to the marked decrease of bone mass and bone mineral density[21], though the most concerning side effect from the usage of glucocorticoids is the suppression of the hypothalamic-pituitary-adrenal (HPA) axis function, which in severe cases, may lead to the onset of acute adrenal crisis[52].

With the emergence of data suggesting an underlying genetic reason for heterogeneity in treatment response and severity of side effects, it becomes apparent that improvement to asthma

therapy can be achieved with research on genetic factors contributing to drug-response – an emerging field with infinite potential known as pharmacogenomics.

Implications of Pharmacogenetics to Asthma Treatment

The terms 'pharmacogenetics' and 'pharmacogenomics' are often used interchangeably, although the two concepts are actually slightly different, with the first term looking at specific genetic influences on a single drug's metabolism in the body (e.g. how variations in one or two genes cause differences in the response of individuals to β_2-adrenergic agonists) and the latter term describing a more complex, overarching study of all genes and all gene interactions in the genome with the potential of affecting drug response (e.g. looking at the correlation between variations in cytochrome P450s and general drug metabolism). In pharmacogenetic studies, research generally begins with the identification of candidate genes with Genome-Wide Association Studies (GWAS), followed by analysis of gene variants on pharmacodynamic or pharmacokinetic properties. With respect to asthma, most pharmacogenetic studies focus on important pharmacodynamic parameters including: a) Forced Expiratory Volume 1 or FEV_1 – the percentage of a person's vital capacity released in the first second of breathing out, and b) Peak Expiratory Flow Rate or PEFR – the maximum speed at which a person can breathe out air. It is important to note that many studies report percentage improvement in FEV_1 as a separate value known as acute bronchodilator response or BDR.

β_2-adrenergic agonists

As the most commonly prescribed asthma medication, β_2-adrenergic agonists are the main targets of many GWAS. As such, it was easily found that the enzyme most relevant to β_2-adrenergic agonists is the β_2-adrenergic receptor, coded by *ADRB2*, an intronless gene located on chromosome 5q31.32[15] with over 80 identified single nucleotide polymorphisms (SNPs)[23]. Of

these SNPs, two (at amino acid positions 16[23,32] and 27[23]) have been identified to have an impact on determining receptor function, ligand binding, and signal transduction while also showing promising association with BDR. The most studied polymorphism with implication in asthma therapy is SNP16 (rs1042713)[32, 34] which usually codes for a glycine or an arginine. *In vitro* studies show that the glycine variant increases receptor down-regulation in the presence of a β_2-adrenergic agonist[32, 17]. Population comparison show an equal distribution of SNP16 variants through Caucasian and Hispanic, male and female subjects, suggesting that variation may not be ethnic or gender-dependent[15].

The exact association between variations in SNP16 with individual response to intermittent β_2-adrenergic agonists usage is undetermined due to findings that are unable to establish such associations or studies with conflicting results. For example, an early study on a sample of 269 Caucasian and Hispanic children have shown that the homozygous ^{16}Arg/Arg residues in *ADRB2* is associated (OR:1.77, p =0.029)[15, 32,34] with positive BDR after usage of SABAs [23, 32, 30], showing 2.3% more recovery in FEV_1 in ^{16}Arg/Arg asthmatic children (15.3% increase in FEV_1 after use) compared to ^{16}Gly/Gly asthmatic (13% increase in FEV_1) subjects[23]. The study further showed that ^{16}Arg/Arg subjects were 5.3 times more likely and ^{16}Arg/Gly heterozygote subject 2.3 times more likely to show a positive response to albuterol treatment compared to the ^{16}Gly/Gly

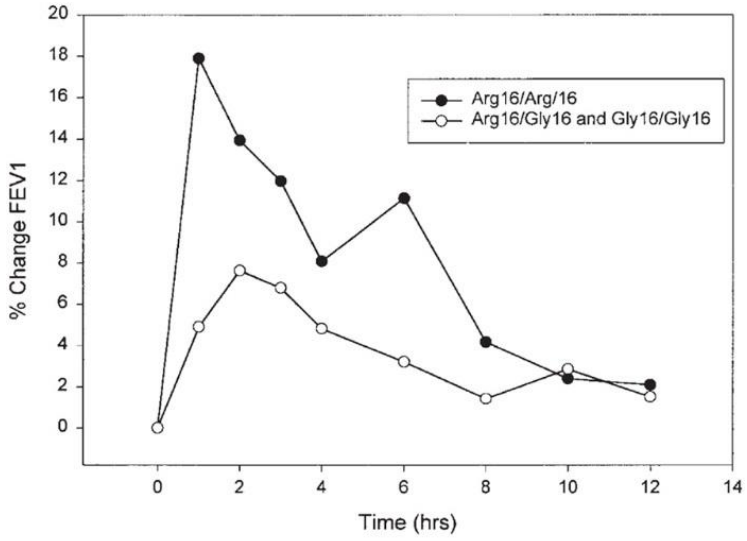

***Figure 2**: Homozygous 16Arg individuals show greater improvement in FEV1 with intermittent albuterol use[30]*

homozygous children[30]. These findings were later repeated in other cohort studies[25, 26] involving both adults and children.

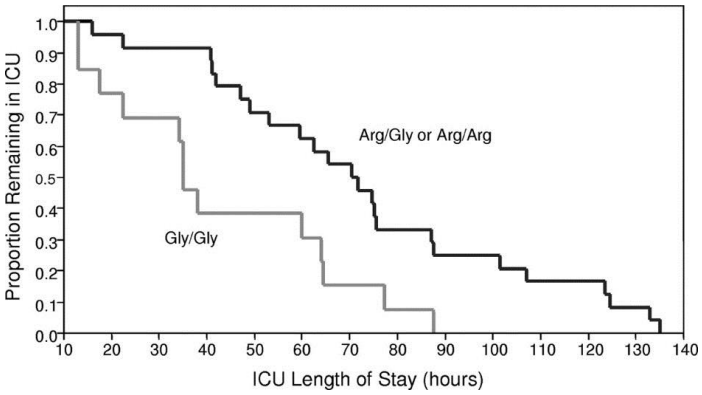

***Figure 3**: Homozygous 16Gly individuals show lower response time to albuterol use, suggesting more positive response to treatment compared to 16Arg homozygous individuals[7]*

However, in a recent 2009 study[7] on the effect of SNP16 variation on response speed to β_2-adrenergic agonist (measured by ICU stay duration), it was shown that ^{16}Gly/Gly homozygous individual conversely needed *shorter* periods of SABA treatment (43 hours) in the ICU to reach the safe-level Modified Pulmonary Index Score (MPIS) of 7 or below, compared to ^{16}Arg/Arg (70 hours) or ^{16}Arg/Gly individuals (75 hours)[7], suggesting a *conflicting* result whereby ^{16}Gly/Gly individuals actually have a more positive result to SABA treatment. As such, it is currently unknown whether the variation in SNP16 has any effect on response in individuals who use SABA treatments intermittently and only under acute asthma exacerbations.

While associations between SNP16 variants and effect on response to intermittent usage of SABA are undetermined and varying, there is strong evidence of an association between SNP16 and <u>regular</u> usage of SABAs or LABAs[23], with ^{16}Arg/Arg individuals showing consistently poorer outcomes compared to ^{16}Gly/Gly patients. In a study of 250 patients, it was shown that ^{16}Arg/Arg patients actually showed decreased PEFR with regular

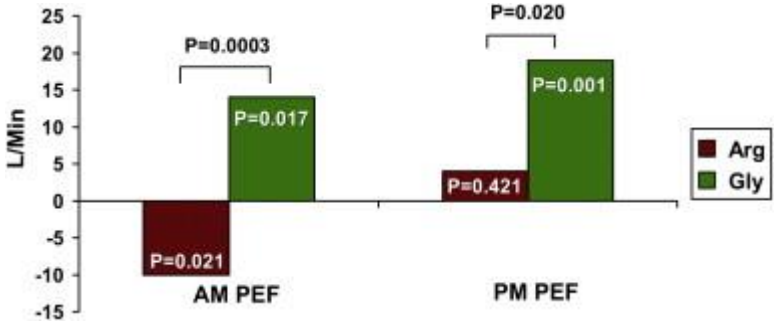

***Figure 4:** Individuals homozygous for the 16Arg allele show decrease in PEFR compared to homozygous 16Gly individuals[23]*

usage of albuterol, recording an A.M. PEFR difference of almost 23.8±9.5 L/min (p = 0.012) and a P.M. PEFR difference of 31.6±10.2 L/min (p = 0.0019) between ^{16}Arg/Arg patients and ^{16}Gly/Gly patients[22]. ^{16}Arg/Arg patients show an average decline of almost 31.1±13.0 L/min (p = 0.0167) in PEFR after regular usage of albuterol. In another study on regular usage of SABAs, it was shown that ^{16}Arg/Arg patients actually had increased frequency of acute asthma exacerbations with regular usage of SABAs (an increase from 0.81 to 1.91 per year)[18, 23], while no effect was observed in ^{16}Gly/Gly individuals.

ADRB2 SNP27 (rs1042714)[34] has shown significant linkage disequilibrium with SNP16, and hence, has been hypothesized to be likewise associated with bronchodilator response to SABAs and LABAs[15]. This amino acid position has two main variants, glutamic acid and glutamine, with ^{16}Arg commonly found in conjunction with ^{27}Gln. Studies suggest that SNP27 may be correlated to treatment response speed[23].

Aside from the well-studied *ADRB2* gene, there are a variety of other genes that have been identified in Genome-Wide Association Studies (GWAS) to be associated with positive BDR in the presence of β_2-adrenergic agonists, including: arginase 1 and 2 (*ARG1*, *ARG2*), corticotropin-releasing hormone receptor-2 (*CRHR2*), and adenylyl cyclase type 9 (*ADCY9*)[32]. In a cohort study on *ARG1*, three SNPs in the promoter were identified to be significantly associated to positive BDR – rs2781659 (p = 0.048), rs2781663 (p = 0.075), and rs2781665 (p = 0.085)[28], with all three SNPs demonstrating high linkage disequilibrium ($r^2 > 0.95$). This study has been replicated, though the effect of variants have yet to be reported.

Glucocorticoids

Genome-wide association studies have identified over 14 candidate genes associated with BDR in glucocorticoid treatments, with corticotropin-releasing hormone receptor-1 (*CRHR1*)[23,

32, an enzyme that receives steroid ligands and activates the hypothalamic-pituitary-adrenal (HPA) axis, being one of the most well-studied candidates. While the mechanism of the association is unknown, it is likely that glucocorticoids – also steroid in structure – may inhibit this enzyme and hence, lead to the earlier mentioned side effect of adrenal crisis. In a large-scale cohort study[40], it was shown that patients with the SNP rs242941 minor allele (frequency = 30%) was associated with positive response to glucocorticoid treatment in both adults ($p = 0.025$) and children ($p = 0.006$), with an average 13.28%±3.11% increase in adult FEV_1 post-treatment, compared to an increase of only 5.49%±1.40% in adult patients with homozygous wild-type allele. In children, the minor allele was associated with a 17.80%±6.77% increase in FEV_1 compared to 7.57%±1.50% in children with the wild-type allele.

Similar to *ADRB2*, *CRHR1* SNPs significantly associated with BDR also demonstrate high linkage disequilibrium. As such, haplotype association studies for rs1876828, rs242939, and rs242941 were also carried out[40], with a result of haplotype GAT/GAT (frequency = 27%) showing strong association with positive response to glucocorticoid treatment in both adults ($p = 0.02$) and children ($p = 0.01$). Adults with homozygous GAT/GAT haplotypes showed almost double the $\%FEV_1$ improvement observed in patients with non-GAT/GAT haplotypes, and GAT/GAT children showing about three times the FEV_1 improvement observed in children with non-GAT/GAT haplotypes.

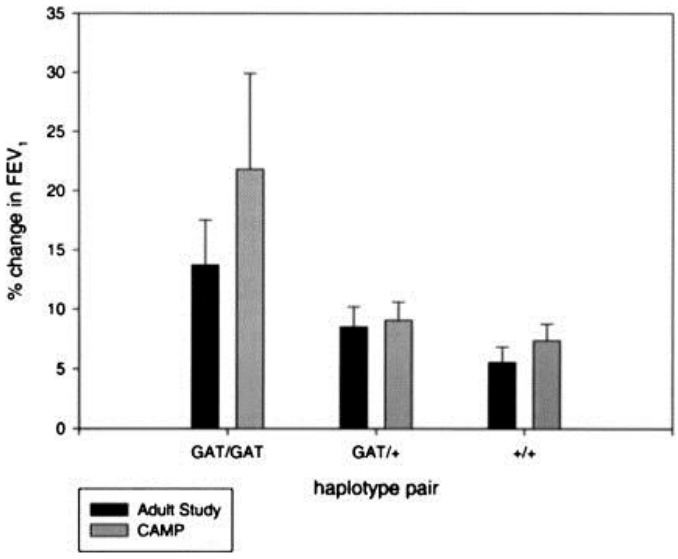

***Figure 5**: Haplotype association studies showing greater FEV1 improvement in GAT/GAT individuals compared to non-GAT/GAT individuals[40]*

Another promising candidate gene associated with positive response to glucocorticoids is *TBX21*, a gene encoding a T-box transcription factor found in inflammatory T-cells[32, 42]. Since glucocorticoids are anti-inflammatory drugs, it follows logically that such a gene would display association with drug response[42]. The main SNP studied in *TBX21* is rs2240017, which has a histidine to glutamine (minor allele, 4.5% frequency) variability. This SNP has shown high association with positive BDR upon usage of glucocorticoids, recording a p value of 0.0001. The minor allele has shown to be associated with an increase in PC_{20} (a variation on the FEV_1 measurement), doubling in improvement compared to the wild-type allele. Unlike *CRHR1*, *TBX21* is less relevant in determining the effect of the drug on the asthmatic population simply because the occurrence of the minor allele is so low, it is unlikely that studies on this gene would give significant feedback on genetic tailoring of the drug[42].

Very recent studies suggest that minor alleles of three SNPs (rs4804773, rs7249320, rs1042428) in *FECR2*[41], a gene encoding a low-affinity lgE receptor, may be associated with increased risk of asthma exacerbation upon usage of glucorticoids, with the exacerbation OR (95% confidence interval) being 1.80, 1.69, and 1.85 respectively and overall p-value of 0.004. The frequency of these minor alleles range from 17% to 26%, an amount high enough to be of concern when considering glucocorticoids as a long-term treatment for the inflammation[23, 41].

Leukotriene Modifiers

Leukotriene C4 synthase (*LTC4S*) – an enzyme involved in the direct synthesis of leukotrienes – has been shown to be associated with BDR under leukotriene modifier treatment, with the main allelic variation (rs730012)[2, 23, 47] located upstream of the start codon. The minor allele consists of a cytosine instead of an adenine, with an allelic frequency of 32%. It was shown that with the cytosine variation, patients recorded a 14% improvement in FEV_1 compared

to the 3% improvement in patients with the wild type variant. In another study, this same variant was also shown to decrease cases of asthma exacerbations by 76% ($p = 0.023$)[27].

Around 2 SNPs in *ALOX5*, a gene encoding a 5-lipoxygenase involved in the synthesis pathway of leukotrienes[13, 23, 32], have been identified to have strong associations with improvement in FEV_1 values under leukotriene modifier treatment. Unlike the substitution variants mentioned in all cases above, one of the variations (rs2115819) in *ALOX5* is an addition/deletion variation, where the patients with 5-repeat (sequence: GGGCGG) showed reduced numbers of exacerbations compared to patients with the 4-repeat and a 12% increase in FEV_1 improvement[23]. Another study identified that the homozygotes with a mutant promoter allele in *ALOX5* leads to a 1.1% decline in FEV_1, while even heterozygotes show an 18% increase in FEV_1 after leukotriene modifying treatment ($P = 0.0001$)[12]. This mutant allele, however, is only observed in 5% of the population, and hence does not seem to carry heavy impact in variability in treatment.

Conclusion

Asthma treatments began with the inhalation of herbal fumes and have stumbled through thousands of years to finally settle upon bronchodilating and anti-inflammatory drugs, an essential development that saves millions of lives today. Yet, these drugs are still far from perfect, as individuals differ greatly in genetic makeup, and these drugs, while life-saving to some, may create debilitating effects in others. With the advent of pharmacogenomics, however, researchers are one step closer towards perfecting asthma treatment. Through access to high-throughput genome tests and SNP chips, Genome-Wide Association Studies have provided a solution towards increasing individuality of drug treatments. While asthma pharmacogenetics are still *years* from clinical practice[32], significant progress has already been attained in

understanding how variations in genes like *ADRB2* and *CRHR1* lead to heterogeneity in drug response. Perhaps one day, clinicians may be able to read a patient's genetic disposition, prescribe drugs to increase treatment response or avoid toxicity, and save the lives of even more patients worldwide.

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