SERIES “GENETICS OF ASTHMA AND COPD IN THE POSTGENOME ERA”

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Pharmacogenetics and asthma: false hope or new dawn?

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ABSTRACT: Pharmacogenetic approaches provide the opportunity to improve prescribing for asthmatic patients in order to optimise efficacy and prevent side-effects. Currently, there are comprehensive data available on the extent of genetic variation in the human genome that will further this area of research. However, less is known about the functional consequences of many of the identified polymorphisms in genes whose products may predict drug efficacy. In addition, there is a shortage of well-designed, adequately powered clinical studies in this area. This series summarises the current state of knowledge and identifies areas for further research.

KEYWORDS: Asthma genetics

One interesting observation made by physicians dealing with asthmatic patients is that different individuals with apparently similar degrees of disease severity respond differently to treatment. There are a number of potential explanations for this observation, including the influence of environmental factors, compliance, comorbidity and psychological factors, but even when these are allowed for there remains significant interindividual variability in treatment response which needs to be explained. One potential explanation for this variability in treatment response is that it is due to genetic factors. The study of the contribution of genetic factors to treatment response is termed pharmacogenetics. The great hope for pharmacogenetics is that it can be used to design individualised treatment for patients [1]. In principle, this approach could prevent adverse drug reactions, predict efficacy and be used to tailor the best combination of therapies for an individual. However, despite the wealth of knowledge regarding genetic variability which is currently available, there are relatively few clinical studies addressing the applicability of this approach; hence the value of using genetic information to help in prescribing remains to be

fully established. The aim of the present series is to describe the genetic variability in key targets for drugs used in the treatment of asthma and to assess, where available, clinical data examining the contribution of these genetic factors to treatment response.

VARIABILITY IN THE HUMAN GENOME

Variability in the human genome can take several different forms. The most common form of polymorphism is due to an alteration at a single locus affecting a single base. This is called a single nucleotide polymorphism (SNP). For example, one individual may have a region of DNA with a sequence ACGTT whilst another individual may have ACTTT: this would be an example of an SNP (G-T). SNPs occur with a frequency of ~1:500 bases in noncoding regions and 1:1,000 bases in coding regions [2]. However, there is marked variability in the rates of polymorphism between regions, as will become clear when variation in airway targets is discussed hereafter.

Polymorphisms may also be due to the insertion or deletion of single bases or longer regions of DNA. These are less common than SNPs but, when they occur in coding regions, are more likely to produce functional effects. This is

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because a single base deletion or insertion will cause a frame shift within the coding sequence. Each codon that provides the genetic information for a single amino acid is composed of three base pairs and a single base insertion or deletion on the coding strand will result in a change in the amino acid sequence downstream from the polymorphism. These frame shifts may also introduce premature stop codons into proteins resulting in the formation of a truncated protein product. A good example of this form of mutation is the CC chemokine receptor (CCR)5 Δ 32 polymorphism. CCR5 is a chemokine receptor expressed on macrophages and some T-cell subsets [3]. The Δ 32 mutation is a result of the deletion of 32 bases in the coding region of CCR5 [4]. This results in a frame shift and a premature stop codon being introduced downstream of the deletion: the net result is a truncated protein product which is not expressed in the cell membrane. As CCR5 is a co-receptor for HIV entry into macrophages, individuals who are homozygous for the CCR5 Δ 32 variant lack one of the key cell surface HIV receptors and thus are relatively resistant to the development of AIDS following HIV exposure [4].

The remaining common form of polymorphism occurs as a result of variable numbers of repeats of di-, tri- or tetranucleotide sequences. Such repeat sequences are common within the human genome: these "variable number tandem repeats" are inherently less stable than the surrounding regions and hence are prone to amplification or deletion. The net result is that the number of repeats at a given locus may be quite variable between individuals, resulting in the presence of multiple alleles within the population. These rarely occur in coding regions but are relatively common in noncoding regions, where they may alter transcriptional activity at a given locus or change secondary structure within the DNA, e.g. the 5-lipoxygenase (ALOX5) gene [5].

With any polymorphism present on an autosome it is important to remember that individuals can potentially be homozygous or heterozygous for the given polymorphism. These may produce difficulty in the interpretation of clinical studies where the phenotype of heterozygous individuals may be unclear. The exception to this rule is where the gene of interest is only present on the unique regions of the X chromosome: in this situation all males will have a single copy of the gene and hence will only have a single allele. This applies, for example, to the cysteinyl leukotriene (LT)-1 receptor (CYSLTR1).

SNPS OR HAPLOTYPES?

Where polymorphisms exist within a short segment of DNA their distribution within the population is frequently nonrandom. This is because any given polymorphism arises in an individual on a particular genetic background (*i.e.* in the presence of a given combination of other polymorphisms). If polymorphisms are close together in genetic terms (*i.e.* within a few megabases), recombination will occur relatively rarely between them. Therefore, given combinations of polymorphisms at the locus will occur more frequently than would be predicted purely by assessing the allelic frequency of the individual polymorphisms in isolation. This phenomenon, which is termed linkage disequilibrium, results in the occurrence of haplotypes (*i.e.* combinations of polymorphisms) across regions that can be used as a genetic signature in populations.

While the presence of linkage disequilibrium can sometimes make genetic analyses in populations easier, it can also cause difficulty. To understand this, it is useful to consider two possible scenarios. In the first scenario, there is one functionally relevant polymorphism but strong linkage disequilibrium with other polymorphisms in the same region. A genotyping study examining the functional polymorphism will be expected to produce the clearest association with the relevant phenotype. However, genotyping studies looking at a non-functional polymorphism that is in linkage disequilibrium with the functional polymorphism will also show association with the relevant phenotype. A haplotype analysis exploring all of the polymorphisms across the locus will also show association, but this will not be stronger than examining the causal polymorphism alone. In the second scenario, there are three polymorphisms all of which produce functional effects. In an association study one would ideally want to examine the three causal polymorphisms; however, the nature of the functional polymorphisms across a given locus may not be known at the time a study is undertaken. In this scenario, if a single polymorphism that has functional effects is studied an association will be seen, although this would not be as strong as if all three functional polymorphisms were studied. Studying other combinations of SNPs across this locus would produce variable strengths of association depending on the degree of linkage disequilibrium. In theory, a haplotype-based approach may provide more useful information in this setting, but because the number of haplotypes at the locus will be increased due to polymorphism at nonfunctional SNPs which are also studied, the efficiency of this approach may be much lower. In practice, this means that very large populations are required before full haplotype-based analyses can usefully be performed. The other difficulty with this approach is that it introduces multiple testing: for example, if there are 12 haplotypes across a given region, the size of the population needed to adequately determine the contribution of these haplotypes will be markedly increased relative to the size of the population required to address the contribution of a single SNP due to the multiple-testing issues that arise.

VARIABILITY IN KEY AIRWAY TARGETS

Since the mid-1990s, the current authors and others have systematically screened the coding regions for the majority of the major airway drug targets. Information on genetic variability in these targets is contained in a number of publications [6] and much of the information is also available *via* online databases such as dbSNP [7]. Table 1 describes polymorphisms in key airway drug targets based on information in the public domain at the time of writing. It can immediately be seen that there is marked variability in the extent of genetic polymorphism. For example, the human β_2 -adrenoceptor gene contains 18 SNPs (four of which result in amino acid substitutions) within its coding region and a rate of variability of $\sim 1:180$ [8, 9], whereas other airway targets, such as the muscarinic M3 receptor, are much less polymorphic [10]. This suggests that the potential contribution of genetic variability in key airway targets to treatment response will vary between different targets.

Rather less is known about variability in the regulatory regions that govern the level of expression of key airway targets. While

TABLE 1 Polymorphisms in genes coding for asthma drug targets

Drug class	Candidate gene	Known polymorphism	Pharmacogenetic effect <i>in vivo</i>
β_2-Adrenoceptor agonists[#]	<i>ADRB2</i>	Arg16Gly, Gln27Glu, Thr164Ile, other SNPs of uncertain functional relevance	Probable for Arg16Gly (see text) and Thr164Ile
Leukotriene modifiers[†]	<i>ALOX5</i>	Promoter (Sp1/Egr1 repeat)	Yes (see text)
	<i>LTC4S</i>	Promoter (-444A/C, -1072G/A)	Probable (see text)
	<i>ALOX5AP</i>	Promoter (-336G/A, CA repeat)	Uncertain
	<i>CYSLT1R</i>	927C/T	Not known
Glucocorticosteroids[‡]	<i>GR</i>	Val641Asp	Not known
		Val729Ile	Not known
		Asp363Ser	Probably yes
		Bcl (G/C), rs33389(G/A), rs33388(A/T)	Seen in one study
		S651F, 231insA	
		rs1876828(A/G)	Not known
Muscarinic antagonists[§]	<i>CRHR1</i>		Seen in one study
	<i>CHRM2</i>	1197(T/C), 1696(T/A), 976(A/C)	Unlikely
		Promoter CA repeat, SNP(G/A)	Possible
	<i>CHRM3</i>	rs2350786(A/G), rs324651(G/T), rs8191992(A/T), rs1378650(C/T)	Unlikely
Theophylline	<i>PDE4</i> family	-708A/G, -627G/C, -513C/A, 492C/T, CTTT repeat, GT repeat	Unlikely
		Multiple SNPs in PDE4D gene family	Not known

SNP: single-nucleotide polymorphism. [#]: e.g. salbutamol, salmeterol; [†]: e.g. montelukast, zafirlukast, zileuton; [‡]: e.g. prednisolone, beclomethasone; [§]: e.g. ipratropium bromide.

there is some information on databases about promoters for the majority of G-protein coupled receptors (GPCRs), the current authors have found some of this information to be unreliable. Therefore, the current authors' group, in conjunction with others, has systematically set out to define the promoters responsible for transcriptional regulation of key GPCRs. While this work is still in progress it is clear that there is at least as much genetic variability in regulatory regions governing transcription as in the coding regions of the target themselves. Less is known about the functional relevance of polymorphisms in these regulatory regions of DNA, although there is reasonable evidence supporting functional roles for polymorphisms in the promoter of the human β_2 -adrenoceptor [11–13] and the muscarinic M2 receptor [14].

PHARMACOKINETIC FACTORS

The best examples of pharmacogenetic effects in clinical practice at present are due to variability in drug metabolising pathways for drugs that have narrow therapeutic indices. For example, toxicity to azathioprine can be predicted to a large extent by thiopurine methyl transferase status [15], with poor metabolisers having a much higher incidence of bone marrow suppression following administration of the drug. Other examples include cytochrome P450 2 C9 status and warfarin metabolism [16], and cytochrome P450 2 D6 status and antidepressant drug metabolism [17]. However, the majority of drugs used in the treatment of airway disease do not have narrow therapeutic indices (with the exception of theophylline) and hence pharmacokinetic factors are less important in determining the variability in treatment response. This, however, still remains a relatively under-studied area of asthma pharmacogenetics.

DOWNSTREAM PATHWAYS AND HOMEOSTATIC MECHANISMS

While it might be predicted that genetic variability in the key targets for drugs or in the key metabolising pathways affecting drug levels might be most important in determining variability in treatment response, genetic variability in downstream signalling cascades or homeostatic mechanisms that are activated by exposure to drugs would also be expected to contribute to interindividual variability. One might expect the contribution of these factors to be less than the contribution due to variability in the primary drug target or drug metabolising pathways. Far less is known about the contribution of variability in these downstream pathways. Ideally, obtaining a genetic profile of the series of genes influenced by a given drug would provide the opportunity to fully explain the genetic contribution to drug responsiveness but, in practice, the number of targets which would need to be studied and the size of the populations required to adequately assess this approach will prove limiting to its applicability to clinical practice.

CLINICAL STUDIES

While there are a number of studies suggesting functional effects of polymorphisms which have been identified in key airway drug targets, there have been relatively few prospective clinical studies addressing the potential contribution of these variants to treatment response. The majority of studies which have been undertaken have tended to be retrospective and to involve relatively small numbers of patients. While these have been of interest and in general indicate that there may be clinically relevant effects for some polymorphisms, the true assessment of the value of using genetic testing to predict

treatment response will be critically dependent upon large-scale, randomised clinical trials where individuals are randomised to treatment by genotype. To date there has only been one efficacy study using this approach in the asthma genetic field [18], although others are currently in progress or planned.

ALOX5 AND TREATMENT RESPONSE

One of the first studies examining pharmacogenetic contribution to treatment response used retrospective genotyping of a promoter polymorphism in *ALOX5*. Transcriptional regulation of *ALOX5* was shown to be dependent on the number of repeats of an SP1 motif in the promoter for the *ALOX5* gene: all genotypes with nonwild-type (*i.e.* five) repeat numbers were shown to have reduced levels of transcriptional activity, which might be expected to translate to lower levels of production of 5-lipoxygenase *in vivo* [5, 19]. The *ALOX5* promoter polymorphism does not show an association with asthma susceptibility [20], but individuals homozygous for the nonwild-type alleles failed to respond to the drug ABT-761, an inhibitor of 5-lipoxygenase, suggesting that treatment response to 5-lipoxygenase inhibitors is determined by this polymorphic locus [21]. This drug did not proceed into clinical practice and hence there have been no further studies examining 5-lipoxygenase inhibitor responses in asthmatics. However, response of individuals given cysteinyl LT-1 receptor antagonists has been examined with respect to *ALOX5* polymorphism. As homozygous individuals for nonwild-type alleles are relatively rare, studies have been in general underpowered. In one study, the current authors found no contribution of *ALOX5* polymorphism to treatment response in individuals heterozygous for nonwild-type alleles, but there were insufficient numbers to determine whether homozygous individuals responded differently [22].

LEUKOTRIENE C₄ SYNTHASE

An interesting group of subjects are those with aspirin-sensitive asthma, in that one might predict that genetic variability in LT pathways might well predict aspirin sensitivity [23]. Perhaps surprisingly, no single genetic variant has yet been identified that explains aspirin sensitivity or response to cysteinyl LT receptor antagonists in these pathways. As mentioned previously, there are data regarding *ALOX5* polymorphism and cysteinyl LT receptor responsiveness. In addition, one study identified an LTC₄ synthase (*LTC4S*) promoter SNP which predicted aspirin sensitivity, although the overall contribution of this SNP was relatively small [24]. Multiple *LTC4S* promoter SNPs have now been identified, but their contribution to asthma susceptibility seems limited [25]. Several studies have also addressed the potential contribution of the *LTC4S* -444A/C SNP to cysteinyl LT receptor-1 antagonist response. These studies have been able to demonstrate functionally relevant effects in *in vitro* systems [26] and clinical associations with treatment response, although the studies performed to date have been small [27, 28].

β₂-ADRENOCEPTOR POLYMORPHISM AND TREATMENT RESPONSE

By far the most studied airway drug target is the β₂-adrenoceptor. As already mentioned, this is a highly polymorphic locus and there are good data suggesting altered functional responses (downregulation profiles) for the

Arg-Gly16 and the Gln-Glu27 polymorphisms [29, 30]. In addition, a rare polymorphism (Thr-Ile164) was found to alter agonist binding properties and adenylyl cyclase activation in one study using transformed cell populations [31]. Individuals with the Ile164 polymorphism failed to respond as effectively to catechol ligands and displayed altered duration of action of the long-acting β₂-agonist drug salmeterol, probably due to altered binding of the long lipophilic tail of this molecule to regions of transmembrane domain 4 [32].

As the Ile164 polymorphism is rare (allelic frequency 3% in the Caucasian population) homozygous individuals are very uncommon: the current authors have failed to identify any such individuals despite screening >1,000 Caucasian subjects. Therefore, the clinical phenotype of individuals expressing this polymorphism in homozygous configuration remains uncertain. There are no good studies examining the airway responses of individuals heterozygous for this polymorphism, although there are reports of altered survival in patients with heart failure and altered vascular responses [33, 34].

The majority of studies have focussed on the common Arg16 polymorphism, for which the best clinical data exist. Both this polymorphism and the codon 27 polymorphism have been examined as potential risk factors for asthma and asthma-related phenotypes: a large effect of these polymorphisms has been excluded [35], although the studies performed to date have been inadequately powered to exclude a small contribution. There are reasonable data suggesting individuals with the Arg16 allele have greater initial bronchodilator responses to β₂-agonists but following prolonged exposure demonstrate greater downregulation [36, 37]. This resulted in a reduced bronchodilator response in a large retrospective study of individuals with asthma exposed to salbutamol *q.i.d.* [37]. These observations led to the only large-scale randomised study in the field of airway pharmacogenetics, in which individuals either homozygous for Arg16 or Gly16 were randomised to regular salbutamol or placebo in a crossover design [18]. Interestingly, it was shown that individuals homozygous for the Arg16 form of the receptor failed to maintain responses to salbutamol. In addition, there were clinically relevant differences in relief bronchodilator usage and symptom scores between the groups. While short-acting β₂-agonists *q.i.d.* are not routinely used in Europe, the data suggest that in the setting where these drugs are used on a regular basis individuals homozygous for the Arg16 allele will fail to respond effectively in the longer term.

The critical questions regarding this locus still remain to be answered. These are whether individuals homozygous for the Arg16 allele should be given an alternative bronchodilator (*e.g.* an anticholinergic) and whether these individuals respond normally to long-acting β₂-agonists. One recent retrospective study suggests that Arg16 patients taking combination therapy with inhaled corticosteroid and a long-acting β-agonist may respond equally well to those with the Gly16 variant, suggesting that inhaled steroids may protect from the potential pharmacogenetic effect driven by this locus [38]. Prospective randomised trials are urgently required to address these issues. Another important question is whether all of the effects at this locus are driven by the codon 16 polymorphism or whether this is just a marker for a haplotype-driven treatment

response. Because linkage disequilibrium is strong across this region, the majority of Caucasian individuals with the Arg16 allele have a single haplotype when other SNPs at this locus are examined [39]. However, this does not apply to other racial groups where the allelic frequency of SNPs has been shown to be different (e.g. in the Afro-Caribbean population) [40].

There have been a number of other small studies, in general retrospective, which have examined the possibility that responses to long-acting β_2 -agonists may be different according to genotype at this locus and, in general, these have supported the idea that Arg16 homozygous individuals may respond less well in terms of either bronchodilator or bronchoprotective responses [41–44]. However, as mentioned previously, a large-scale prospective study is urgently needed to address this question fully, particularly in light of a retrospective analysis showing that the Arg16 polymorphism may also predict poor efficacy with these agents in asthmatics regardless of inhaled steroid usage [45]. The potential implication for guidelines on asthma management would be that alternative treatment strategies may be better than long-acting β_2 -agonists for this group of patients: however, this approach would also require a formal prospective study to be undertaken before it could be broadly recommended.

MUSCARINIC RECEPTORS

Anticholinergic agents are effective as bronchodilators in asthma, although they are more extensively used in chronic obstructive pulmonary disease. Ipratropium bromide is a nonselective antimuscarinic agent which acts as an antagonist at M1, M2 and M3 receptors in the airways. Tiotropium is relatively selective for M3 receptors. The airway effects of acetylcholine on smooth muscle are predominantly due to effects driven by M2 and M3 receptor stimulation. As mentioned previously, the coding region for the M2 and M3 receptor genes (*CHRM2* and *CHRM3*) have been screened [10]: while both coding regions contain SNPs, polymorphism is present at a much lower rate than is seen in some other targets (e.g. the β_2 -adrenoceptor). The level of expression of the M2 and M3 receptor in the airways is partly dependent upon transcriptional controlling mechanisms: both the *CHRM2* and *CHRM3* promoters have been defined and are known to contain a range of polymorphic variants [14, 46]. The current authors have recently shown that different alleles of a dinucleotide repeat in the M2 promoter alter levels of reporter gene expression [14]: this may, therefore, result in altered M2 receptor expression *in vivo*. The effects of these polymorphisms on treatment response (if any) remain to be established.

GLUCOCORTICOID RESPONSIVENESS

A proportion of asthmatic patients are relatively resistant to the effects of glucocorticoids and extensive attempts have been made to identify the molecular bases for this steroid resistance. Glucocorticoids mediate their effects at the intracellular level by binding to the intracellular glucocorticoid receptor. This exists as two splice variants (GR α and GR β) which differ in their ninth exon due to alternate splicing [47]. Several groups have sequenced the glucocorticoid receptor and a number of SNPs, some of which cause amino acid substitutions, have been identified. Rare polymorphisms have been identified that cause familial glucocorticoid resistance [48, 49]. There are also

some functional data looking in particular at the Asn 363 Ser polymorphism, which suggest altered responses in, for example, dexamethasone suppression tests in subjects carrying the serine 363 form of the receptor [50]. However, studies in asthma have, to date, not revealed an obvious correlation between any specific polymorphism in the glucocorticoid receptor gene and a response to steroids. In view of this, groups have looked for alteration in downstream signalling pathways or other related genes. In a recent study examining a series of potential candidate genes, TANTISIRA *et al.* [51] identified the *CRHR1* gene as a potential marker for steroid responsiveness. Further work is required to define the relative importance of genetic factors as diagnostic markers for steroid responsiveness and a number of studies are ongoing which address this issue.

CONCLUSION

There are now extensive data describing the degree of polymorphism within the coding region of the genes for major airway drug targets. Less is known about regulatory regions, although this information will become available over the next couple of years. With the clinical studies now underway (including some prospective trials), the next 5 yrs will allow a full assessment of the value of pharmacogenetic data to be made in terms of the value of genetic testing for prediction of efficacy and/or side-effects. There remains an extensive barrier in translating these approaches into everyday healthcare, which will require a considerable investment in education and service development if it is to be successful. The cost effectiveness of these approaches will need to be carefully assessed. In addition, novel markers for treatment response will be generated by high-throughput techniques, including expression profiling, and potentially by genome-wide single-nucleotide polymorphism analysis in large cohorts.

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