Variability in Human Drug Response
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Pharmacogenetics of respiratory system drugs

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ABSTRACT

Genetic factors can affect the pharmacokinetics of respiratory drugs in a number of ways. Defects in drug metabolising enzymes are the best characterised genetic factors: approximately 10% of Caucasians are slow acetylators (ie have a low affinity N-acetyltransferase isozyme) and will therefore (for example) metabolise isoniazid less rapidly and are more susceptible to peripheral neuropathy. Common polymorphisms in drug targets may also affect treatment response.

Of these polymorphisms the treatment modifying effects of the β_2 adrenoceptor polymorphism and 5-LO promoter polymorphism are best described. Individuals homozygous for the Gly 16 β_2 adrenoceptor polymorphism demonstrate bronchodilator tachyphylaxis (Tam *et al.* 1997). This polymorphism has a high allelic frequency in the Caucasian population (Gly 16 homozygotes 37 %, n = 626). 5-LO promoter polymorphisms modify promoter activity and hence can potentially alter the response to 5-LO inhibition (In *et al.* 1997). Thus common genetic variants of genes whose products are targets of respiratory system drugs may alter treatment response in individuals.

Polymorphism are known to exist in the β_2 adrenoceptor (cpdpm 16,27, 164), the H_1 receptor (degenerate polymorphism at codon 356) and the 5-lipoxygenase (5-LO) promoter (alleles 11-5).

Key words: Drug metabolism, polymorphism, pharmacogenetics, β_2 adrenoceptor, 5-lipoxygenase.

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INTRODUCTION

The role of genetic factors in determining variability in treatment response to drugs used in the management of respiratory disease has only recently attracted attention. This is perhaps surprising given that it has been clear for many years that there is marked inter-individual

variability in treatment response to given drugs. In this review I will discuss the evidence for genetic polymorphism in the target proteins of respiratory drugs used in the management of asthma and chronic obstructive pulmonary disease (COPD). It must however be remembered that genetic variability can be important in treatment response for drugs used in the treatment of other respiratory disease. Perhaps the best example is the contribution of hepatic metabolising enzyme polymorphisms to the risk of developing peripheral nerve toxicity in individuals treated with isoniazid: slow acetylators (10 % of the population) are at increased risk of neurotoxicity due to the reduced hepatic metabolism of isoniazid.

The major target proteins of drugs used in the treatment of airflow obstruction are G-protein coupled receptors, steroid receptors, the phosphodiesterase family of isoenzymes and metabolising enzymes involved in leukotriene metabolism. These will be systematically reviewed below.

β_2 ADRENOCEPTORS

By far the largest literature on the influence of genetic polymorphisms to disease susceptibility and treatment response concerns β_2 adrenoceptor polymorphism. The β_2 adrenoceptor is situated on chromosome 5q.31 and following initial work by Liggett and co-workers [1] which identified 4 single base substitutions which resulted in amino acid changes in the receptor as well as 5 degenerate single base substitutions (ie polymorphism which do not alter the amino acid code of the receptor) extensive studies have been performed to address the potential functionality of the nondegenerative polymorphisms.

The 4 polymorphisms which alter the amino acid code of the receptor are situated at codons 16, 27, 34 and 164. Of these the valine to methionine 34 substitution appears to be of no functional significance and is also rare and hence has not been studied in human populations. However the other 3 polymorphisms all occur in the Caucasian population two being common. All 3 appear to be functionally relevant.

Perhaps the most interesting from the pharmacogenetic point of view is the polymorphism at codon 164 (threonine to isoleucine). This polymorphism is relatively uncommon with an allelic frequency of 3 % in the Caucasian population. Because each individual has 2 genes for the β_2 adrenoceptor homozygous individuals would be expected to be rare: to date none have been studied formally. When the isoleucine 164 version of the receptor was expressed in recombinant cell lines there was a reduction in agonist induced activation of adenylyl cyclase and also altered receptor sequestration following long term agonist stimulation [2]. Interestingly the amino acid 164 sits deep in transmembrane domain IV and is very close to the proposed "exosite" where the hydrophobic tail of the long acting β_2 agonist salmeterol is believed to bind. Because the threonine - isoleucine 164 polymorphism is non conservative one would predict charge alteration in this region of the 4th transmembrane spanning domain and it seems likely therefore that individuals carrying this polymorphism may display an altered response to salmeterol. Because no individual homozygous for this polymorphism has yet been studied the response of such individuals to salmeterol (or other β agonists) remains unknown.

In contrast to the amino acid 164 polymorphism the polymorphisms at amino acid 16 (arginine-glycine) and 27 (glutamine-glutamate) are common in Caucasian populations. Neither amino acid substitution confers altered agonist binding efficiency: this is hardly surprising

given that both polymorphisms are in the N terminal extra cellular part of the receptor. However these polymorphisms have effects on receptor downregulation in both transformed cell systems and in primary cell cultures of known genotype. The glycine 16 variant of the receptor confers increased receptor downregulation following agonist exposure, whereas the glutamate 27 variant is protected in part from receptor downregulation [3,4]. Two published studies have suggested that individuals with asthma carrying the glycine 16 variant (which downregulates to a greater extent than the arginine 16 version of the receptor) have altered bronchodilator responses following treatment with β agonists. In a single blind placebo controlled cross over trial with formoterol bronchodilator subsensitivity was observed following 2 weeks treatment with formoterol 24 g b.d. in those individuals homozygous for the glycine 16 variant, whereas individuals with the arginine 16 variant of the receptor had maintained bronchodilator responses [5]. In the second study asthmatic children were more likely to have significant reversibility to inhaled β_2 agonist if they did not have the glycine 16 variant of the receptor [6]. However, a third study, examining the potential contribution of this polymorphism to adverse asthma control in asthmatics taking fenoterol failed to demonstrate any contribution from Glycine 16 [7].

In contrast to the codon 16 polymorphism, polymorphism at codon 27 does not appear to dictate response to β_2 agonist drugs, although in one study both allelic association and linkage was observed between the aminoacid 27 polymorphism and IgE levels in asthmatic families [8]. These data may possibly be explained by linkage disequilibrium with other potential candidate genes on chromosome 5q including the Th_2 cytokine cluster.

Finally it is important to note that the amino acid 16 and 27 polymorphisms are in strong linkage disequilibrium in the Caucasian population and that there are racial differences in the distribution of these polymorphisms [9]. In particular the glutamate 27 polymorphism is rare in both the Japanese and Black Afro-Caribbean population (unpublished data).

HISTAMINE H_1 RECEPTORS

Although antagonists at histamine H_1 receptors are relatively poor bronchodilators there is marked variability in the response of individuals in their response to inhaled histamine. Because there is a good correlation between PD20 values obtained with both methacholine and histamine within individuals this would suggest that inter-individual variability of the bronchoconstrictor response to inhaled histamine is a feature of non specific bronchial hyperreactivity rather than due to genetic variability of the response to histamine alone. Nonetheless, we have recently screened the coding region for the histamine H_1 receptor for variants by single stranded confirmational polymorphism analysis (SSCP): this method is around 95 % sensitive for detecting single base substitutions or other polymorphisms. In marked contrast to the β_2 adrenoceptor we were unable to find any single base substitutions or other polymorphisms which altered the amino acid code of the histamine H_1 receptor, although we did find 1 degenerate polymorphism at codon 356 (A-G, allelic frequency 3.8 %) [10]. Hence it appears that genetic variation in the coding region of the H_1 receptor gene is unlikely to contribute to variability in individual responses to histamine.

MUSCARINIC RECEPTORS

Both muscarinic M_2 and M_3 receptors are expressed on airway smooth muscle. The muscarinic M_3 receptor is likely to be important in the contractile response of airway smooth muscle to muscarinic agonists such as acetylcholine released following vagal stimulation. The exact function of the muscarinic M_2 receptors present on these cells remains unclear: the M_2 receptor is negatively coupled to adenylyl cyclase and hence stimulation of these receptors can partially prevent the relaxant action of drugs such as β_2 agonists working through receptors positively coupled to adenylyl cyclase.

There are no described polymorphisms in either the M_2 or the M_3 receptor in the current literature. Whilst we have not screened the coding region (or regulator regions) of the M_3 receptor for polymorphism we have performed a limited screen of the coding region for the M_2 receptor and to date have not found evidence for polymorphism. There is a known polymorphic marker approximately 3 kilobases upstream of the M_2 gene however which could be used for linkage studies.

5-LIPOXYGENASE

The 5-lipoxygenase pathway is important in generation of the bronchoconstrictor leukotrienes, of which the most potent is leukotriene (LT) D4. Whilst the receptor for LTD4 has not been cloned the metabolising enzyme 5-lipoxygenase has been screened for polymorphic variants. Drazen and co-workers have described a number of variants in the promoter for this gene (called alleles 1-5) which interfere with potential Spl/Egr-1 transcriptional factor binding sites. Using reporter gene approaches this group has been able to show that these promoter polymorphisms alter the transcriptional activity of a surrogate reporter gene implying that individuals carrying the different alleles for this promoter polymorphism may have altered transcriptional activity of the 5-LO gene *in vivo* [11]. There are at present no published data on the potential for these polymorphisms to alter response to 5-lipoxygenase inhibitors in asthmatic populations. In addition to known polymorphisms in the 5-lipoxygenase promoter gene there is also a known polymorphism in the LT C4 synthase gene which in one study was shown to be associated with aspirin induced asthma.

GLUCOCORTICOID RECEPTORS

Whilst it is clear that there is a wide spectrum in steroid responsiveness in asthma with some individuals appearing to be essentially steroid resistant and other individuals having a good clinical response to steroids the biochemical basis for this variability remains unclear. In one study the glucocorticoid receptor from steroid sensitive and steroid resistant asthmatics was sequenced and no genetic basis for the variability in treatment response was found [12]. However there are many potential downstream targets for the glucocorticoid receptor which to date have not been examined for genetic polymorphism.

PHOSPHODIESTERASE ISOENZYMES

It is now clear that there are many different phosphodiesterase isoenzymes [13]. On the basis of sequence homology these have been divided into a number of families: however the genes for phosphodiesterase isoenzymes are somewhat complex often with several exons leading to potential splice variants. In the airways it appears that members of the Type 3 and Type 4 isoenzyme families are likely to be the most important in both airway smooth muscle and in inflammatory cells. At present the only respiratory drugs targeted at this system and in widespread use are theophylline and its derivatives: these act as non-selective phosphodiesterase isoenzymes. Whether or not polymorphisms alter activity of individual phosphodiesterase isoenzymes has not been explored, although this would be a difficult topic for research give the multiplicity of phosphodiesterase isoforms.

CONCLUSIONS

It is estimated that approximately 1 in 1000 bases in coding region DNA and 1 in 500 bases in non-coding region DNA are polymorphic within the human genome. This however is clearly an oversimplification in that some genes show marked variability (eg the β_2 adrenoceptor, with a frequency of polymorphisms of >1 in 200) at one extreme and genes such as the histamine H_1 receptor of similar length but with a rate of polymorphism of 1 in 1500 at the other extreme. Given this high rate of genetic variability many polymorphisms will be identified which can potentially alter the function of a gene. In addition to coding region polymorphisms many regulatory sequences can potentially also be polymorphic and may alter transcriptional activity of that gene. Given this high rate of genetic variability it is critical to assess the functional relevance of identified polymorphisms before performing pharmacogenetic studies. Many genetic variants are likely to be clinically silent, producing no functional effects. However it is likely that genetic polymorphism within specific genes plays a major role in determining the inter-individual variability in treatment response to drugs used in the treatment of respiratory (and other) disease.

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Discussion: Pharmacogenetics of respiratory system drugs

M.T. Kinirons:
Has anybody looked at the relationship of some of these polymorphisms with sudden death?

I.P. Hall:
The β_2 adrenoceptor has been studied. There have been a number of epidemiological studies suggesting that giving asthmatic individuals high doses of β-agonists may predispose to sudden death. This was initially noted with isoprenaline inhalers, back in the late 1960s and resulted in them being removed from the market in many countries. Subsequently an epidemic of asthma deaths in New Zealand in the 1970s suggested fenoterol to be the clue. A couple of retrospective studies, (Hancox RJ *et al.*, Eur Resp J, 1998), (Weir TD *et al.* Am J Respir Crit Cared Med, 1998), have been done on this issue but they suggested that there is no association between genotype and sudden death.

N.L. Benowitz:
It is interesting to speculate about what the lack of tolerance related to agonism might mean for the cardiovascular system. The development of tolerance could be an important homeostatic mechanism. Do people who have the mutation for this β_2 gene have any cardiovascular physiological abnormalities?

I.P. Hall:
We have been working with John Cockcroft, who is a cardiovascular pharmacologist, and we did studies where we infused isoprenaline into the forearm vasculature of individuals. Then we looked at vasodilator responses to isoprenaline by plethysmography. The data, published in abstract form, show an association, which is actually more striking for venodilation than for arteriodilation, between a preserved response and a non-down-regulating genotype. There is also quite a marked racial difference in these polymorphisms. For example, the black South African population, which is known to have blunted vasodilator responses to isoprenaline, has got a markedly reduced frequency of the non-down-regulating, glutamate 27 genotype.

P. Rolan:
Treating patients some years ago, I noticed some who were very poorly controlled with oral doses of steroids of 30 mg or more. These patients did not seem to get better, but they became cushinoid. It was not clear what was happening to them as the drug was clearly entering the circulation. Do you have any experience of similar patients and do you have an explanation for these observations?

I.P. Hall:
There is a group of patients who are considered to have "glucocorticoid-resistant" asthma. I have never been convinced, because the distribution in terms of peak flow responses is not a bimodal distribution but unimodal. I think patients are labelled as glucocorticoid-resistant asthma if their response is less than a certain percentage. The difficulty obviously exists that is there may be some heterogeneity in the patients you are looking at, for example, some of these patients may not have asthma, they may have fixed air-flow obstruction. A group in

London sequenced the glucocorticoid receptor itself in half a dozen individuals who were said to be glucocorticoid resistant, and they did not find any polymorphic variance within it. However, there are a large number of downstream signalling molecules, which could potentially have some effect.

A. Breckenridge:
Most of your data was retrospective. Have you performed any prospective clinical trials with bronchodilators?

I.P. Hall:
We are performing a prospective study in standard clinical trial conditions. What we have not done is to examine whether knowledge of a patients genotype is going to affect a general practitioner's decision to treat the patients with salmeterol. Although these differences that I have described are probably real, they may not be clinically relevant, because they may not alter the way asthmatic patients are treated. Tasking a nihilistic view, this type of patient could be treated with salmeterol or fenoterol for a while and if there is no response, the dose of inhaled steroids could be increased. If genotype did determine response to long-acting β agonists, I would argue it is probably more cost-effective to genotype the patient first, but the size of the study required to reach this conclusion is a minimum of 100 individuals and we have not done such a study.

A. Breckenridge:
The other very interesting implication is the drug-regulatory one. If you designed your study with a smaller tighter group to show efficacy in this group for whom you might predict treatment would work, it would be a very interesting way ahead for drug regulation.

I.P. Hall:
One could argue that the pharmaceutical industry would not want to know about a group of individuals who are going to be non-responders. I think that that is probably wrong, because what you really want to know is how effective your drug is, and if you do small Phase-II or Phase-III studies and by chance include in them a number of non-responders, you will inevitably underestimate the efficacy of your drug. On the other hand, I am not aware that any of the licensing agencies to date have formally asked for genetic information, although I have heard rumours that the FDA has started to consider this. I have been asked by pharmaceutical companies to genotype individuals in β agonist studies. And I do not know what that information is being used for, but I suspect that the companies are preparing for possible requests from the regulatory authorities.

A.J.J. Wood:
Didn't Liggett's group find an association with nocturnal asthma, and also with the desensitisation phenomena *in vivo*?

I.P. Hall:
There is a retrospective study looking at individuals with nocturnal asthma (Turki J *et al.*, J Clin Invest, 1995) showing that they are more likely to have the down-regulating Gly 16 genotype. There is also a good biopsy study published (Turki J *et al.*, Am J Phys-Lung

Cellular & Molecular Physiology, 1995) showing reduced β adrenoceptor number in individuals with Gly 16 genotype after treatment.

P.B. Watkins:

I had an interesting experience recently as a consultant to industry. Clinical trials revealed that genetic polymorphism appeared to identify 50% of the individuals who responded to the drug, and this made sense on a physiological basis. In this situation, the head of clinical development suggested that with the follow-up compounds with same mechanism genotyping could be used to reduce the sample size of the clinical trials, improving speed and cutting costs substantially. A person responsible for regulatory affairs commented that if only studies on people who are genotyped are done, it is likely that the drug would only be approved in individuals who had genotyping. He pointed out that this would be reducing the market for the drug by 50%. He also pointed out the genotyping raised ethical issues that society has not completely dealt with. Interestingly, a decision was made in that discussion not to pursue the genotyping any further, but to save genomic DNA samples on every individual enroled in clinical trials in case this information became desirable or was required by the FDA at a later date.

A.J.J. Wood:

Would you approve a drug to be used in a group that had not been tested? If you had carried out your studies in a single genotype and showed that there was a beneficial safety-toxicity ratio in that group, would you approve it for use in a different genotype?

A. Breckenridge:

No, of course you would not. But the drug company needs to indicate whether it would only be submitting for approval in those patients who had been genotyped. It depends in part on the nature of the disease, but if we are going to take therapeutics any further forward, a group like this should be encouraging this kind of approach.

I.P. Hall:

The drug companies can develop the test and sell it. However, there may be a conceptual problem. We do not have any problems measuring theophylline levels in patients on theophylline, but we seem to have a major problem in taking a blood sample to obtain someone's genotype.

M.M. Reidenberg:

You said that, in your view, it would be cost-effective for the general practitioner to determine the β adrenergic genotype. Could you expand on the basis for that opinion, please?

I.P. Hall:

At the moment we do not know it specifically for β-receptor genotypes. I said that if you could say whether or not a patient was going to respond to salmeterol (for example) depended on genotype, then it would be more cost-effective to genotype an individual if you set up a service to do it, than to do a sort of informal trial in that patient. The reason for that is that the cost of genotyping, if it were widely available, could easily come down to a few pounds per sample.

E.M. Sellers:

One of the main reasons why most pharmaceutical companies do not analyse their data with respect to differences in gender response to drugs, is that they feel they will be restricted in their labelling, if they find any differences. Since studies are powered to detect a main group effect a subgroup analysis may give a type II error of non-efficacy. If that happens with gender, it is not surprising to see the same reasoning occurring for genotyping.