INTERETHNIC VARIABILITY IN HUMAN DRUG RESPONSES

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ABSTRACT:
The scientific study of interethnic differences in responses to drugs has been extant for 80 years. Many of these differences have been described at the phenotypic level, and some have been explained by genetic factors. However, it is frequently difficult to disentangle accurately the hereditary and environmental influences in phenotypic comparisons. This is where the recent developments in knowledge of the genes responsible for drug receptors are starting to make a big impact. The beta 2 adrenoceptor is described; it has three genetic polymorphisms. The different genotypes influence responses to agonists such as albuterol (Salbutamol). New gene frequency data including those for Saudi Arabians, Indians, and Africans are shown. The expanding body of knowledge about genetic (and interethnic) variability in drug receptors is likely to be important in clinical medicine.

Phenotypic Interethnic Comparisons

Early studies on interethnic differences in responses to medications were made in the U.S.A. Paskind (1921) investigated the effect of 0.002 mg of atropine sulfate hypodermically on 20 white and 20 colored men in Cook County Hospital, Chicago. Initial slowing of the heart rate reaching a maximum in 10 to 15 min was observed frequently in whites but not in colored subjects (Fig. 1; Table 1).

Chen and Poth (1929) at Johns Hopkins (Baltimore, MD) measured the change in the transverse diameter of the pupil after the instillation of various mydriatics. Figure 2 shows a typical result 1 h after 10% l-ephedrine. The action was greatest in 10 Caucasians, intermediate in 10 Chinese, and least in 10 African Americans (Table 2).

Since those early observations, the whole science of pharmacogenetics has come into being. Many interethnic differences in drug responses have been found to be caused by differences in the metabolic biotransformations of drugs. However, there are clear pointers that this is not the whole story.

Two examples have been selected to illustrate this point.

1. In an investigation of the effect of morphine on Caucasians and Chinese, it was found that the former had significantly more respiratory depression than the latter at the same plasma morphine concentrations (Zhou et al., 1993, Fig. 6).

2. A study by Olatunde and Evans (1982) used the QT interval (Goldberger and Goldberger, 1981, Figs. 6–10) of the electrocardiogram to measure the response to quinidine in Nigerians and Caucasian British healthy subjects. The QT interval was corrected for a heart rate of 60 beats per minute (QTc). Intraerythrocytic quinidine concentrations were measured after a single 200 mg oral dose. The healthy Nigerians showed higher levels (see Olatunde and Evans, 1982 Fig. 1). The increase in QTc interval from baseline was significantly greater in the healthy white subject (Olatunde and Evans, 1982, Fig. 2). The snag in the interpretation of this study is that the Nigerians were studied in Lagos and the whites in Liverpool. Thus, it is not clear whether the responsiveness of the heart was caused by genetic or environmental factors.

The Elucidatory Power of Genetic Polymorphisms

In the field of drug metabolism many polymorphisms have been found wherein the same alleles occur in different frequencies in different ethnic groups. In other instances, different alleles have been found in different populations. Many examples show how these discoveries have shed light on interethnic variability in responses to drugs. Now that we can examine alleles directly, the obfuscations inherent in phenotypic studies of the kind referred to above can be circumvented. This is because there has been a fairly sudden advance in our knowledge of drug receptors, and it is becoming clear that they are subject to genetic variability in a manner similar to drug-metabolizing enzymes.

The Challenge of Receptors

As pointed out in Rang et al. (1999), the protein targets for drug action on mammalian cells can be broadly divided into receptors, ion channels, enzymes, and carrier molecules. According to Rang et al. (1999), “Receptors differ from the others. They form the sensing elements in the system of chemical communications that coordinate the function of all the different cells in the body. The chemical messengers being hormones, transmitter substances or other mediators such as cytokines and growth factors”.

The plethora of information accumulated in recent years can be seen in the 1999 Receptor & Ion Channel Nomenclature Supplement published with the March issue of TIPS (Alexander et al., 1999). It consists of 108 pages. Our knowledge of molecular genetics makes it likely that there are genetic variants for many of the receptors listed in this supplement; some will be polymorphic (see Alexander et al., 1999).

So the problem is one of selection. I have elected to concentrate on
the β2-adrenergic receptor, whose encoding gene ADRB2 is on chromosome 5 (at 5q31–32) and which is genetically different from the β1- and β3-adrenoceptors, whose genes are ADRB1 and ADRB2 at 10q24–26 and 8p11–12, respectively (see Alexander et al., 1999).

The β2-Adrenergic Receptor

This β2-AR1 is a typical metabotropic or 7-transmembrane-spanning receptor whose structure has been well studied (see Reihau et al., 1993 as reproduced in Hall, 1996, Fig. 1; Rang et al., 1999, Figs. 2.3B and 2.7). This receptor has been found to exhibit genetic polymorphisms (Reihau et al., 1993), the two most important being Gly/Arg at codon 16, and Glu/Gln at codon 27. Another polymorphism is at codon 164, but here the frequency of variant alleles is low.

There has been much interest in the relationships between these polymorphisms and various disorders, especially asthma (See Büscher et al., 1999), but this is not the subject of this discussion. Here the initial focus will mainly be on how these polymorphisms are related to the responsiveness of bronchial smooth muscle to β-agonist drugs.

An in vitro study was carried out by Green et al. (1995) using human airway smooth muscle cells derived at autopsy from persons without pulmonary disorders. The response to a test stimulus following a 24-h exposure to isoproterenol showed that the homozygous Glu27 genotype conferred pronounced desensitization and down-regulation. (Green et al., 1995, Table 2).

In vivo studies have shown

1. A significantly greater degree of bronchodilator desensitization of responses to formoterol with homozygous Gly16 than with homozygous Arg16 asthmatics (see Tan et al., 1997, Fig. 1).
2. In a survey of FEV1 in asthmatic children, Martinez et al. (1997) found that Arg16 homozygotes were 5.3 times more responsive to albuterol than Gly16 homozygotes, and heterozygotes were 2.3 times more responsive (Martinez et al., 1997, Table 3).
3. Albuterol concentrations in the blood were measured by Lima et al. (1999) in eight black and eight white asthmatics after oral ingestion of 8 mg. The percentage change in FEV1 was also measured sequentially. While there was no significant difference between the two groups in the blood albuterol concentrations, the airway responsiveness was markedly greater over 8 h in Arg16/Arg16 homozygotes than in the other two genotypes. No effect on albuterol-evoked FEV1 was observed at codon 27 (Lima et al., 1999, Fig. 1).

Other studies (discussed in detail by Lima et al., 1999) failed to show such a clear-cut difference between genotypes in their bronchodilation following albuterol administration. These studies probably differed because the drug was given by inhalation and the concentration acting on the bronchial β2 AR was variable. In the study by Lima et al. (1999), there was only a very small intersubject variability of plasma albuterol concentrations. These authors do not mention any interethnic differences in responsiveness within a single genotype (e.g., homozygous Arg16).

The β2-Adrenoceptor Polymorphisms in Different Ethnic Groups

The data (published and unpublished) now available have been tabulated by my collaborator Dr. Howard A. McLeod and are shown in Table 3. There is a highly significant regression of Arg16 allele frequency on Gln27 allele frequency, which is further evidence in favor of linkage disequilibrium as previously noted, for example, by Martinez et al. (1997) and Dewar et al. (1998).

The 13 data points obtained from Table 3 where there is information on both polymorphisms show wide variation and suggest the presence of two clusters, viz. Caucasians (1) and Africans and Chinese (2).

More data are available for the codon 27 than for the codon 16 polymorphism. There is a suggestion of the presence of a west-to-eastcline (possibly stepped). The main conclusion, therefore, is that the Chinese and Africans have high Arg16 and Gln27 frequencies compared with Caucasians.

Therapeutic Expectations

It might be expected that a Chinese population would require on average a much smaller dose of β2-AR agonist to achieve the same bronchodilation as a Caucasian population. Furthermore, the Chinese might desensitize more readily. However, this may be an unwarranted assumption. The reason for caution in the extrapolation is that the β2-AR is only the first link in a catenary intracellular process (Fig. 3). The proteins involved in this sequential process may be subject to genetic variation.

Genetic Factors Influencing β2-Adrenoceptor Function in Human Airways

Genetic heterogeneity has been found in G proteins. Vichow et al. (1999) described a C825T polymorphism in GNB3, the gene encoding the G beta-3 subunit of heterotrimeric G proteins (OMIM 139130). The 825 T allele, associated with the expression of a shorter splice variant (G beta-3 subunit) gave enhanced signal transduction via pertussis toxin-sensitive G proteins. Neutrophils containing the variant had an enhanced chemotaxis and faster migration in response to stimulation with interleukin 8.

A codon 19 Arg/Cys genetic polymorphism was described by McGraw et al. (1998) in the 19-amino acid peptide that regulates the mRNA translation of the β3-AR molecule. This peptide is encoded by a short open reading frame termed the 5′-leader cistron (5′LC) situated 102 base pairs upstream from the β3-AR coding block. The expression of β3-AR was 72% higher in COS-7 cells bearing 5′LC Cys as compared with those possessing 5′LC Arg (McGraw et al., 1998, Fig. 4). In human airway smooth muscle cells β2-AR expression

TABLE 1

<table>
<thead>
<tr>
<th>Change of Pulse Rate</th>
<th>Coloreds</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero and increase</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Decrease</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

Change of pulse rate 10 min after 1/60 g of atropine hypodermically in colored and white subjects

From Fisher’s exact test, p = 0.0007, Paskind (1921).

Abbreviations used are: AR, adrenoceptor; 5′LC, 5′-leader cistron; FEV1, forced expiratory volume in 1 s.
was twice as great in cells bearing 5′LC Cys (as compared with 5′LC Arg-bearing cells). A linkage disequilibrium was demonstrated between the 5′LC polymorphism and the β2-AR 16 and 27 polymorphisms described above. It seems possible that this new polymorphism may influence the response of bronchial smooth muscle to agonists, and it may exhibit interethnic variability.

It was pointed out by McGraw and Liggett (1997) that the principal mechanism of desensitization of β2-AR polymorphisms is phosphorylation by beta-AR kinase or other closely related G protein-coupled receptor kinases.

Various cell types in the lung were found to have different activities of these kinases. For example, mast cells had much more than smooth muscle. These kinases could play a role in desensitization in vivo in response to β2-agonists. Genetic polymorphisms have not been described in these enzymes yet. It is quite possible that they exist, and if so their interethnic distribution may be variable.

**Influence of β2 Adrenergic Receptor Polymorphisms in Nonpulmonary Conditions**

The β2-AR is a major lipolytic receptor in human fat cells. With the notion that the β2-AR polymorphisms might play a role in obesity, Large et al. (1997) genotyped 140 women with a large variation in body fat mass. The Glu27 homozygotes had an average fat mass excess of 20 kg (Large et al., 1997, Table 1). Neither allele at the Arg16Gly polymorphism was linked to obesity. An investigation of obese men did not give a similar result (Hellstrom et al., 1999). These findings in women will probably stimulate further work and may be applied to the known interethnic variability in obesity.

The codon 164 threonine/isoleucine polymorphism of β2-AR has not received much attention in humans because of the comparative rarity of the less frequent (isoleucine) allele. To investigate the functional significance of this polymorphism in the myocardium, Turki et al. (1996) prepared transgenic mice expressing 45 times the normal endogenous β2-AR. Mice with the mutant 164 Ile and mice with the wild-type threonine allele were studied. It was found in the mutant mice (as compared with the wild-type mice) that in vitro 1) the basal adenyl cyclase activity was lower and 2) maximal isoproterenol stimulated activity was lower. It was found that in vivo 1) resting heart rate and dP/dt (max) were less and 2) responses to infused isoproterenol were similarly less.

Liggett et al. (1998) determined the codon 164 polymorphism in 259 patients with advanced heart failure due to ischemic heart disease or dilated cardiomyopathy and 212 healthy controls. The allele frequencies did not differ between the two populations, but the 1-year survival (Liggett et al., 1998, Fig. 2) in the 10 heterozygous Ile 164-carrying patients was only 42% compared with 76% for patients endowed with a wild-type β2-AR (relative risk, 3.69; p = 0.002). No interaction was noted with race (among other factors), but there were only 3 non-Caucasians in the 10 individuals in the Ile 164 group.

The studies briefly described above suggest that innovative therapies in many fields will have to take into account interethnic variations in receptors.

**Other Genetic Polymorphisms in Drug Receptors that may be of Clinical Interest**

Alexander et al. (1999) reveals many receptors that could be relevant to clinical problems. Some are more attractive for investigation than others. I will call attention to one, namely, histamine 1 receptor HRH1 (OMIM 600167). The full structure of this gene, which is at chromosomal localization 3p 25, has been published (DeBacker et al., 1998).

Regarding histamine biotransformation, a C314T genetic polymorphism of histamine N-methyl transferase has been described (Yan et al., 1999). The T314 variant allele was found to be more common in asthmatics than in control subjects (allele frequencies, 0.19 versus 0.08; χ² = 12.40; p = 0.001; odds ratio = 2.62).

It is a common observation that some people become much more sleepy than others after the usual doses of both oral and inhaled antihistaminics. It would be of interest to know whether this phenomenon is influenced by the C314T polymorphism in histamine N-methyltransferase and by allelic variants of the HRH 1 when they come to light.

**A Voyage of Exploration**

We are embarked on a voyage of exploration of the unknown with regard to the β2-AR polymorphisms. The key requirements in the area of clinical practice will be to conduct comparative experiments similar to those briefly described above.
to those of Lima et al. (1999) in genotyped selected ethnic groups, e.g., Caucasians and Chinese, preferably in the same environment. Then the influence and importance of the polymorphisms in the two ethnic groups may be clarified.

The $b_2$-AR is only one example wherein we happen to know more than about most drug receptor polymorphisms. Obviously, this is an exciting area of pharmacology and therapeutics and as yet its interethnic aspect has not received much attention.

On 23 July 1999 a survey was carried out of the ISI database for the combined terms "drug receptor human" for 1998 and 1999. A total of 766 article abstracts were found and examined. None dealt with any aspect of interethnic variability in receptors. So it would seem that the whole subject is ripe for development.

"The Great Ocean of Truth"

The concept of “diathesis” has been in the minds of the medical profession for many years (diathesis: a permanent, hereditary or acquired condition of the body which renders it liable to certain special diseases or affections; a constitutional predisposition or tendency; Willis, 1681). The idea was that persons with a particular constitution (often presumed to be hereditary) would be more liable than others to become ill when subjected to certain environmental influences. We are now at an interesting point in history because we have recently acquired the technology and we are beginning to see exactly how this interplay of genetic endowment and environmental factors works. This is true in fields other than pharmacogenetics and ecogenetics; for example, in understanding resistance and susceptibility to infective disorders (see Levin and Newport, 1999, who describe mutations in the interferon gamma receptor 1 as causing susceptibility to mycobacterial infections, and Allen et al., 1999, who describe resistance to cerebral malaria conferred by the presence of southeast Asian ovalocytosis band 3).

In the elucidation of the scientific basis of practical therapeutics, it seems that now is the time to intensify the study of genetic and interethnic variability of receptors. They could be regarded as important undiscovered constituents in Newton’s “great ocean of truth” (“I do not know what I may appear to the world but to myself I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell

**TABLE 3**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Ethnic Group</th>
<th>Codon 27 Number of Persons Studied</th>
<th>Gln</th>
<th>Codon 16 Number of Persons Studied</th>
<th>Arg</th>
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<td>Liggett et al., 1998</td>
<td>Caucasian USA$^a$</td>
<td>212</td>
<td>0.58</td>
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<td>0.70</td>
<td>58</td>
<td>0.35</td>
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<tr>
<td>Large et al., 1997</td>
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<td>82</td>
<td>0.36</td>
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<tr>
<td>Hellstrom et al., 1999</td>
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<td>Caucasian Sweden$^e$</td>
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<td>Hellstrom et al., 1999</td>
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<td>43</td>
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<td>66</td>
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<tr>
<td>Meirhaeghe et al., 1999</td>
<td>Caucasian France</td>
<td>1152</td>
<td>0.59</td>
<td></td>
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<tr>
<td>Martinez et al., 1997</td>
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<td>188</td>
<td>0.61</td>
<td>188</td>
<td>0.37</td>
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<tr>
<td>Xie et al., 1999</td>
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<td>188</td>
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<td>188</td>
<td>0.46</td>
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<tr>
<td>McLeod et al., unpublished$^j$</td>
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<td>100</td>
<td>0.53</td>
<td>100</td>
<td>0.37</td>
</tr>
<tr>
<td>Martinez et al., 1997</td>
<td>Caucasian-Hispanic USA$^k$</td>
<td>40</td>
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<tr>
<td>Martinez et al., 1997</td>
<td>Hispanic USA$^l$</td>
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<td>Xie et al., 1999</td>
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<td>McLeod et al., unpublished$^m$</td>
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<tr>
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<td>Indian</td>
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<td>McLeod et al., unpublished$^p$</td>
<td>Chinese</td>
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<td>Chinese</td>
<td>104</td>
<td>0.92</td>
<td>104</td>
<td>0.59</td>
</tr>
</tbody>
</table>

$^a$ 91% Caucasian.
$^b$ Non-obese females.
$^c$ Obese females.
$^d$ Non-obese males.
$^e$ Obese males.
$^f$ Asthmatics.

![Sequence of intracellular events that occur following binding of an agonist to the $b_2$-adrenoceptor.](image)
than ordinary, while the great ocean of truth lay all undiscovered before me”).

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References