Pharmacogenetics and ecogenetics

by D. P. Agarwal and H. W. Goedde

Institute of Human Genetics, University of Hamburg, Butenfeld 32, D-2000 Hamburg 54 (Federal Republic of Germany)

Key words. Pharmacogenetics; ecogenetics; enzyme polymorphism.

Introduction

The term *pharmacogenetics* refers to genetically controlled variations between individuals in the absorption, distribution, biotransformation and clearance of drugs and food, and the response to them. A relatively recent addition to this important discipline of human genetics is the area of *ecogenetics,* which deals with multiple and changing interactions between genotypes and a variety of environmental agents or xenobiotics such as industrial chemicals, pollutants, insecticides, pesticides and their degradation products. With increasing knowledge of the ecogenetic constitution of individuals belonging to different ethnic groups, inherited predispositions to disease caused by environmental factors become more evident. In the past few years, a number of pharmacogenetic conditions in man have been observed which lead to a better understanding of the underlying principles of interethnic variation in response to drugs and toxicants[30, 34, 54, 76]. The study of pharmacogenetics has also helped in elucidating basic mechanisms of drug action in the human body[28, 57, 72]. Genetic polymorphism in enzymes and other proteins is largely responsible for inter-individual, interethnic and racial differences in drug metabolism, which result in alterations of the pharmacokinetic and pharmacodynamic properties of therapeutic agents and environmental chemicals. Dietary factors and nutritional status considerably influence absorption, plasma protein binding, distribution, biotransformation and excretion of drugs, altering therapeutic response and toxicity. In table 1, major polymorphic enzyme systems associated with pharmacogenetic variability are presented. Although a majority of pharmacogenetic conditions are single gene phenomena resulting in inborn errors of metabolism, polygenic metabolic abnormalities in drug response have also been observed.

This chapter will review selected examples of pharmacogenetic and ecogenetic entities and their implications in human genetic research.

1. Serum cholinesterase polymorphism and suxamethonium sensitivity

Application of suxamethonium (succinyldicholine), a short-acting muscle relaxant, leads to prolonged apnea in certain individuals. The pharmacogenetic basis of this sensitivity has been found to be a genetically determined variation in serum cholinesterase (E C 3.1.1.8), commonly known as pseudocholinesterase[10, 27, 29]. Patients who suffer suxamethonium-related prolonged apnea either have very low levels of serum cholinesterase activity or possess an atypical form of the enzyme protein which cannot break down the drug efficiently. Individuals with cholinesterase deficiency are potentially at high risk to anti-cholinesterase insecticides. Application of a purified cholinesterase preparation from normal serum reduced the duration of suxamethonium-related apnea considerably[26, 28]. Therapeutic application of such preparations, which are available commercially, in cases of pesticide poisoning has been reported[40]. The usual diagnostic test is based on the resistance of the variant enzyme to inhibition with dibucaine[51]. While the normal enzyme is inhi-

Table 1. Some common pharmacogenetic conditions caused by enzyme variants

Enzyme defect	Drugs producing abnormal reponse	Symptoms	Inheritance	Incidence
Cholinesterase deficiency	Succinyldicholine (suxamethonium)		Autosomal recessive	Frequent in Caucasians, rare in Negroes, absent in Orientals
Paraoxonase polymorphism	Paraoxon (parathion)	Pesticide toxicity	Autosomal recessive	About 70% Caucasians deficient type
N-acetyl transferase polymorphism	Isoniazid Phenelzine Hydralazine Sulfonamides Arylamines	Polyneuritis; psychosis; arylamine-induced carcinogenesis	Autosomal recessive	About 70% Caucasians slow acetylators
Oxidase polymorphism	Phenytoin Dicoumarol Guanoxan Phenacetin	Drug toxicity and poor response in slow metabolizers	Autosomal recessive	Rare
	Debrisoquine Sparteine			6–9% in Caucasians 30% in Orientals
Glucose 6-phosphate dehydrogenase deficiency	Antimalarial drugs; Fava beans; Sulfonamides	Hemolytic anemia	X-Chromosomal recessive	Common in African and Mediterranean populations

Table 2. Frequency of the atypical cholinesterase allele E_1^a in different populations[73]

Low frequency (< 0.002)		Intermediate frequency (0.005–0.01)		High frequency (> 0.014)	
Thais	0	Japanese	0.0047	North Africans	0.0142
Koreans	0	Australians	0.0051	Czechoslovakians	0.0144
Japanese	0	Negroes (Seattle)	0.0053	Brazilians	0.0149
Eskimos	0	Lapps	0.0076	Greeks	0.0162
South American		Indian tribes		Germans	0.0162
Indians	0	(Mexico)	0.0093	White Americans	0.0163
Negroes (Congo)	0.0009	Maroccan Jews	0.0098	Portuguese	0.0168
Chinese (Taiwan)	0.0015			Berbers	0.0182
Filipinos	0.0024			Finns	0.0188
				British	0.0192
				Israelis	0.0312

bited more than 70% by dibucaine, the atypical heterozygotes and homozygotes show inhibition between 40–65% and less than 20% respectively.

As shown in table 2, the frequency of the atypical gene is quite different in various populations[73]. In Thai, Japanese, Eskimo and some South American populations, no atypical variant has been reported so far. Other common variants of cholinesterase include a fluoride-resistant one, and a silent gene variant with very low or no activity[6, 46, 58].

Although most of the individuals who have an atypical enzyme are expected to be genetically predisposed to suffer from prolonged apnea after suxamethonium application, the apnea cases encountered in the clinical practice do not always show a variant form on the basis of conventional tests[75]. When such unexplained cases were reexamined using succinyldicholine as substrate, they were found to possess altered substrate specificity and apparently represent a group of hitherto unknown variants[1–3, 31, 32].

Since radioactive peptides labeled with diisopropyl fluorophosphate prepared from the atypical enzyme moved more towards the cathode than those from the normal enzyme, a mutational change from an acidic amino acid to a basic amino acid in the esteratic site seems to be present in the dibucaine-resistant variant[31]. The amino acid sequence of the active site peptide obtained from the normal enzyme was found to be Gly-Glu-Ser-Ala-Gly-Ala-Ser-Ala-Val-Ser-Leu. From the difference in electrophoretic mobility of the active-site peptides from normal and atypical enzyme variants, the probable structure of the atypical human enzyme was deduced as Gly-His-Ser-Ala-Gly-Ala-Ser-Ala-Val-Ser-Leu[83].

2. Glucose-6-phosphate dehydrogenase deficiency and hemolytic anemia

Red cell G6PD (E.C.1.1.1.49) shows extensive polymorphism. A number of enzyme variants show poor or no activity. The G6PD deficiency is a sex-linked genetic condition as the gene for the enzyme is located on the X-chromosome. The gene's normal function is important for the maintenance of erythrocyte membrane integrity. Individuals with the variant enzyme are susceptible to a large number of industrial chemicals (napthalene, TNT, naladixic acid etc.), drugs (analgesics, sulphonamides, antimalarials, non-sulphonamide antibacterial agents etc.) and food (fava beans). The most common clinical consequence is hemolytic anemia, the severity of which depends upon the type of enzyme deficiency. A large number of variants have been characterized on the basis of electrophoretic and kinetic properties[50]. The frequency of G6PD deficiency among various populations is shown in table 3.

Table 3. Incidence of G6PD deficiency in various populations

Population	Variant	% Enzyme activity	Incidence
African	Gd A^-	10–20%	20% Population
Mediterranean	Gd Med.	0– 5%	35% Population
Chinese	Gd Canton	4–25%	20–30% Population
Thai	Gd Mahidol	5–16%	20% Population

3. Acetylator phenotype and susceptibility to arylamine-induced bladder cancer

The genetically determined polymorphism of human liver-N-acetyltransferase (E.C.2.3.1.5) is responsible for rapid or slow acetylation of a series of drugs, e.g. isoniazid, hydralazine, phenelzine, dapsone, sulphasalazine and other sulfa drugs. While slow acetylators exhibit toxic reactions to original drug compounds, rapid acetylators are subject to an increased risk from toxic acetylated metabolites[8, 14, 28]. Slow acetylators are homozygous for an autosomally recessive gene and rapid acetylators are either homozygous or heterozygous for a dominant gene[19, 78, 79]. While Caucasians, Negroes and South American Indians have a higher percentage of slow acetylators, Eskimos, Japanese and Chinese are predominantly rapid acetylators (table 4). A number of reports describe the association of acetylation phenotype with side effect of many drugs[8, 14, 78]. Serotonin has been found to be a physiological substrate of N-acetyltransferease. Thus, the acetylation pathway may compete with monoamine oxidase mediated serotonin degradation and polymorphic forms of N-acetyltransferase could interfere with neurotransmitter-related psychotropic drug response[28].

More recently, attention has been drawn to a possible association between acetylation phenotype and bladder cancer[18, 78, 79]. Aromatic amines, a class of potent carcinogens, are acetylated in the human body. A significantly higher percentage of patients with bladder cancer were found to possess the slow acetylator phenotype as compared to a control group. It is likely that rapid acetylators exposed to tobacco smoke or synthetic dye-intermediates render arylamines non-carcinogenic by faster degradation via acetylation. However, it is not ruled out that slow acetylators have greater survival with bladder cancer than rapid-acetylator phenotypes[18].

4. Paraoxonase polymorphism and parathion toxicity

Serum paraoxonase (E.C. 3.1.1.2) which hydrolyzes paraoxon, a metabolite of the widely-used insecticide parathion, to the non-toxic p-nitrophenol has been shown to be polymorphic[23,24]. Two alleles at one autosomal locus with low activity (gene frequency = 0.70) and high activity (gene frequency = 0.30) have been observed in Caucasian populations[11,64]. The low activity and high activity enzyme forms differ in their NaCl activation, inhibition with EDTA, apparent Km values, calcium requirement and pH optimum[15]. While carriers of the high activity variants are possibly better protected against organo-phosphorous insecticides, individuals with the low activity phenotype have an increased risk of toxic reactions with parathion and structurally related metabolites. Interethnic differences in the distribution of gene frequency of low activity allele were found in studies comparing Indians, Malays, Kenyans, Ethiopians, Nigerians, Chinese, Indonesians, Japanese, Vietnamese, and Atacameño Indians[25,37]. A relatively low frequency of the low activity gene was noted in Africans and Asians, and a total absence of this allele was found in Australian aborigines.

Recently, it was shown that a single gene locus determines the paraoxonase and arylesterase activities, and the ratio of serum paraoxonase to arylesterase activity was found to be distributed trimodally within a Caucasian population from the United States[15,63]. The characteristic is inherited as a simple Mendelian trait.

Table 4. Distribution of acetylators of isoniazid in different populations

Population	Number	Slow acetylators (%)	Fast acetylators (%) Hetero-zygotes	Homo-zygotes
South Indians (Madras)	1477	59	35.6	5.4
Caucasians	1958	58.6 (52–68)	35.9	5.5
Negroes	531	54.6 (49–65)	38.6	6.8
Eskimos	485	10.5 (5–21)	43.8	45.7
Japanese	2141	12.0 (10–15)	45.3	42.7
Chinese	682	22	49.8	28.2

5. Alpha$_1$-antitrypsin deficiency and pulmonary emphysema

A number of genetic variants of alpha$_1$-antitrypsin or protease inhibitor (Pi) have been detected in various populations[12,20,37]. In tables 5 and 6 the distribution of Pi phenotypes in different European populations is shown. The variant forms of Pi differ in inhibitor activity and electrophoretic properties. Severe genetic deficiency of Pi is associated with the development of lung emphysema[20]. While the homozygous ZZ phenotype has a frequency of about 0.05% in Europeans, the Mz heterozygote has a frequency of about 4.5%[55]. The risk of a ZZ phenotype developing lung emphysema is about 30 times higher than that of the general population[56]. There is an even greater risk for heterozygotes of developing chronic obstructive pulmonary disease in combination with smoking and environmental pollution[59]. However, little is yet known about the specific genetic and/or environmental factors that lead to pulmonary disorders in Pi type ZZ individuals.

Table 5. Gene frequencies and phenotype distribution of α_1-antitrypsin (Pi) in Caucasians[55]

Allele	Gene frequency*	Phenotype	Frequency*	Relative concentration in serum	Predisposition for pulmonary insufficiency and lung emphysema
Pi^M	0.969	PiMM	93.01	100	–
Pi^S	0.019	PiSS	0.25	60	(++)
		PiMS	5.30	80	(+)
Pi^Z	0.008	PiZZ	0.05	10	++++
		PiMZ	1.07	50–60	++

*The values do not sum up to 100% since other very rare alleles occur.

6. Polymorphism of mixed-function oxidases

Mixed-function oxidases are responsible for the oxidative metabolism of a number of drugs, like diphenyhydramine, phenytoin, dicoumarol, antipyrine, amylobarbitone and debrisoquine-sparteine. The genetic control of the drug oxidation system in man is very complex, as it is subject to change and adaptation by means of enzyme induction[62]. Increased demethylation of diphenyhydramine was observed in Orientals as compared to Caucasians, but the number of subjects was too small to allow any definitive conclusions[54].

Phenytoin metabolism is also under genetic control and racial differences in hydroxylation capacity have been reported[7,13]. *Antipyrine* undergoes at least 3 primary biotransformations, namely 3-hydroxylation, 4-hydroxylation and demethylation. A bimodal distribution in demethylation capacity in the Oriental but not in the Caucasian group was observed[49].

A small but statistically significant difference in *amobarbital* hydroxylation between Orientals and Caucasians was also reported; the Caucasians showed greater metabolic excretion[52]. The observed interethnic differences may not be genetic since amobarbital hydroxylation is enzymatically inducible.

Table 6. Distribution of α_1-antitrypsin phenotypes in 14 European populations[12]

Population	Sample size (n)	Relative genotype* frequencies (%) MM	MZ	ZZ
Northern Sweden	1869	98.34	1.66	0.00
Northwestern Finland	300	97.33	2.67	0.00
Finland	136	97.70	2.30	0.00
Norway	2830	96.93	3.00	0.07
Finland	548	97.26	2.74	0.00
Finland	223	99.10	0.90	0.00
Southern England	926	95.68	4.21	0.11
Ireland	1000	96.10	3.90	0.00
France (Bretagne)	280	95.35	4.65	0.00
France (Pyrenean groups)		1386	97.40	2.600.00
Germany	1474	97.69	2.24	0.07
Germany	408	98.78	1.22	0.00
Northern Italy	202	98.02	1.98	0.00
Central and Southern Italy	500	97.20	2.60	0.20
Pooled	12.082	97.26	2.70	0.04

*The M allele represents a collection of all alleles other than the Pi^Z allele. Thus, MM and MZ types in fact comprise collections of several genotypes.

Table 7. Debrisoquine and 4-hydroxybrisoquine excretion in five populations[53]

Population	n*	D	4HD	Total	r	P
Canadian Caucasian	80	1.17 ± 0.33 (14.6)	1.08 ± 0.43 (12.1)	1.52 ± 0.16 (32.9)	−0.42	0.001
Canadian Oriental	28	1.34 ± 0.31 (21.9)	0.85 ± 0.55 (7.1)	1.56 ± 0.16 (36.2)	−0.61	0.005
Saudi Arabian	102	0.66 ± 0.43 (4.6)	0.79 ± 0.44 (6.2)	1.08 ± 0.36 (11.9)	+0.48	0.0001
Ghanaian	80	1.07 ± 0.33 (11.6)	0.76 ± 0.41 (5.7)	1.29 ± 0.27 (19.7)	+0.02	0.87
Nigerian	123	1.29 ± 0.31 (19.3)	1.08 ± 0.40 (12.1)	1.56 ± 0.22 (36.5)	−0.18	0.05

*The n gives the number of subjects studied. The data D (debrisoquine), 4HD (4-hydroxydebrisoquine), and total indicate log percent of dose (mean ± SD) excreted in urine during 8 h after ingestion of a test dose of debrisoquine; the antilog of each mean is shown in parentheses. r and P indicate the Spearman rank correlation coefficients for D and 4HD and their P values, respectively.

The oxidative metabolism of *debrisoquine* shows genetic polymorphism, and individual differences in plasma drug concentration and antihypertensive effect have been observed[72]. Poor metabolizers have a higher drug to metabolite ratio (> 20) whereas extensive hydroxylators have ratios that are less than 12.5[53]. The incidence of deficiency of the capacity for debrisoquine hydroxylation is about 8–9% in the United Kingdom, and a deficiency in the capacity to oxidize spartein, an anti-arrhythmic drug, is found in about 5% of German individuals[16,47]. A close relationship between an individual's ability to hydroxylate debrisoquine and to N-oxidize sparteine has been established[9,17]. The deficiency varies widely among various ethnic groups, as shown in table 7. The poor or deficient metabolizers are homozygous for a recessive allele[72]. Poor metabolizers of debrisoquine also show an oxidation defect for other drugs like gaunoxan, phenacetin, nortryptaline, phenformin, perhexiline, phenytoin and metoprolol[47,53]. It has been suggested that poor metabolizers of debrisoquine may be less prone to intestinal and bronchial cancers than are the fast metabolizers[54].

7. *Polymorphism of alcohol metabolizing enzymes*

Racial and individual differences in the alcohol metabolism rate have been reported by several investigators[21,22,41,68–70]. As shown in table 8, Oriental and American Indian subjects were found to have significantly faster rates of alcohol metabolism than Caucasians.

Table 8. Alcohol metabolic rate in different populations[35]

Population	Number	Alcohol metabolic rate (elimination rate (mg/kg/h))
American Indians		
North American Indians	26	101.3
North American Indians	30	92.0
North American Indians	12	182.7
North American Indians	17	122.98
Canadian eskimos	21	109.8
Orientals		
Chinese	15	136.6
Chinese, Japanese, Korean, Taiwanese	24	146,0
Chinese	39	127.0
Japanese	47	133.0
Japanese	335	110–140
Hindu Reddis	35	122.9
Caucasians		
Americans	30	93.0
Canadians	58	103.6
Europeans	68	108.0

It is widely known that the majority of Asiatic Orientals of Mongoloid origin and North American Indians respond with severe alcohol sensitivity symptoms after drinking an acute dose of alcohol which has no significant effect in Caucasians (table 9). Alcohol sensitivity symptoms include facial flush, muscle weakness, increase in heart rate, vasodilation, low blood pressure, a hot feeling in the stomach, and elevation in skin temperature and ventilation[61,81,82]. Higher steady state blood acetaldehyde levels have been recorded in flushing subjects than in nonsensitive individuals[60,85]. Various reasons for the elevated blood acetaldehyde levels in persons who show sensitivity to ethanol have been discussed: a faster absorption rate of ethanol; differential base rates of alcohol metabolism, differences in the clearance rate of acetaldehyde in different ethnic groups; genetic polymorphism of alcohol dehydrogenase (ADH) and/or aldehyde dehydrogenase (ALDH).

Table 9. Alcohol sensitivity (facial flushing) in different populations and ethnic groups

Subject Group	Frequency of sensitivity (%)	Reference
Caucasians		
Europeans	4	Wolff (1972)
Europeans	10	Zeiner et al.(1979)
North Americans	12	Ewing et al. (1974)
American Indians		
North American Indians	50	Wolff (1973)
North American mongoloids	80	Wolff (1973)
American Indians with European ancestry	90	Wolff (1973)
Orientals		
Japanese	58	Mizoi et al. (1979)
Japanese	85	Morikawa et al. (1979)
Japanese, Korean, Chinese, Taiwanese	83	Wolff (1972)
Chinese	57	Zeiner et al. (1979)
Hapa Haole (Hawaii)	60	Wilson et al. (1978)
Vietnamese	60	Goedde et al. (1980)

Several variant forms of ADH have been detected in different populations which are controlled by separate gene loci (table 10). Among Japanese and Chinese, a very high incidence of an 'atypical' ADH has been observed.

Table 10. Distribution of alcohol dehydrogenase variants at ADH_2 locus

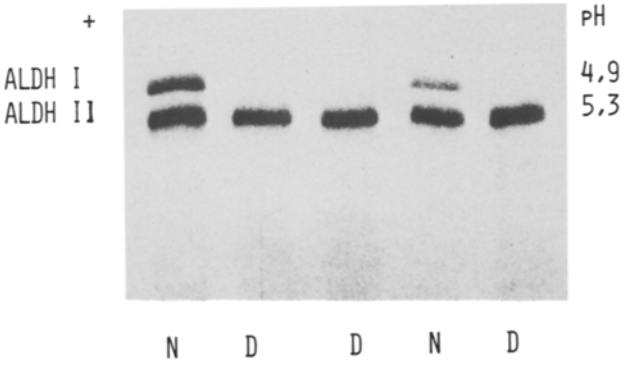
Country of origin	Number of liver specimens	'Usual' 1–1 (%)	'Atypical' 1–2 (%)	2–2 (%)
Switzerland	59	81.4	17.6	0.0
Germany	35	94.3	5.7	0.0
England	46	91.3	8.7	0.0
Japan	111	10.8	44.1	45.1
Japan	40	15	45	40
China	83	10.8	44.1	45.1
India	43	100	0	0

This variant form, controlled by the ADH_2 gene, has been implicated as being responsible for the production of higher than normal levels of acetaldehyde after drinking alcohol in subjects sensitive to alcohol[74]. The NAD-dependent ALDH is widely distributed in human organs and tissues. At least 4 isozymes of ALDH have been found in human liver which differ in their electrophoretic

Table 11. Blood acetaldehyde and ethanol levels in normal and deficient Japanese after drinking an acute dose of ethanol[35,44]

ALDH Isozyme I	Peak levels (mean ± SD)	
	Acetaldehyde, μmol/l	Ethanol, mmol/l
Normal (n = 25)	2.1 ± 1.7	10.30 ± 1.85
Deficient (n = 19)	35.4 ± 12.8	10.93 ± 2.31

and kinetic properties[39,43]. The most rapidly migrating isozyme (ALDH I or E_2) is predominantly localized in mitochondria and has a low Km for acetaldehyde. While all Caucasians have this isozyme in their liver, in about 50% of Orientals this isozyme is missing[4,33,42]. A typical



Isoelectric focusing pattern of human liver ALDH isozymes. N = normal phenotype; D = deficient phenotype.

isozyme pattern in liver extracts separated by isoelectric focusing is shown in the figure.

The discovery of genetic variation in ALDH isozyme activity led to the suggestion that subjects deficient in the low Km ALDH I isozyme might be confronted with slow and impaired oxidation of acetaldehyde leading to vasomotor symptoms after drinking an acute dose of alcohol[5,33,35,44]. A positive correlation between facial flushing and elevated blood acetaldehyde level in conjunction with ALDH I deficiency was indeed observed (table 11). Thus, defective acetaldehyde metabolism due to a genetic enzyme defect and not its higher production through the superactive atypical ADH is responsible for the higher steady-state blood levels of acetaldehyde in alcohol-sensitive persons.

When various populations and racial groups were screened for ALDH isozyme I deficiency using hair root follicles as the source of enzyme protein, only populations with a mongoloid heritage showed the abnormality[36]. In table 12 the incidence of ALDH deficiency in various populations is shown. Substantial familial resemblances in the flushing response after alcohol intake have

Table 12. Incidence of ALDH I isozyme deficiency in different populations

Population	Number	% of individuals deficient
Japanese	184	44
Chinese	196	35
Vietnamese	82	57
Indonesians	30	39
Thais (Northern Thailand)	110	8
Atacamenos (Indians in Chile)	133	43
Caucasians (Europe)	224	0
Egyptians and Sudanese	160	0
Liberians	169	0
Kenyans	15	0

been observed and the trait appears to be inherited as an autosomal dominant characteristic[38,71].

As the genetically determined ALDH deficiency causes impairment of acetaldehyde metabolism in the mongoloid race, its prevalence may have exerted an influence on the drinking habits of deficient individuals in the course of time. The incidence of alcoholism has remained traditionally very low in Japan and China as compared to other advanced countries in the West. Due to the altered social structure, and occupational pressure, more and more Japanese, Chinese and Korean individuals drink alcohol, but owing to their inherent enzyme defect, they experience severe alcohol intolerance symptoms. In a recent survey in Japan, a significantly low frequency of the enzyme deficiency was encountered in alcoholics compared with non-alcoholic psychiatric patients, drug-dependent patients and healthy controls[45]. The incidence of ALDH deficiency in patients and control groups is shown in table 13. Individuals sensitive to alcohol by virtue of their inborn enzyme deficiency may be discouraged from abusing alcohol owing to initial aversive reactions. Thus, ALDH enzyme polymorphism may offer a biological protection against alcoholism.

The synchronizing effect of ethanol on the brain wave pattern (EEG) also shows inter-individual variation[66]. Twin studies have shown that ethanol-related EEG changes in monozygotic twins are similar to each other, as are repeated recordings from one person. However, dizygotic twins have different EEGs, in the resting state as well as the alpha and beta waves[65]. Alcoholics have a

Table 13. ALDH I deficiency in alcoholics and control groups in Japan[35,45]

Subject	Number	% with ALDH deficiency
Healthy controls	105	41
Alcoholics	175	2.3
Schizophrenics	86	41.9
Drug dependents	47	48.9

poorly synchronized EEG pattern compared to healthy subjects and might be predisposed to alcoholism by virtue of their psychopharmacological differences in response to ethanol[67].

Concluding comments

The study of pharmacogenetics and ecogenetics among different individuals and populations offers an unique opportunity to understand multiple, simultaneously occurring interactions between genes and the environment, and the subsequent phenotypic expression of heritable characters. Incorporation of appropiate ecogenetic diagnostic services into the general genetic services may be useful for public health monitoring in the prevention of occupational disease.

The modern methods of molecular biochemistry will allow the characterization of hereditary traits affecting drug metabolism at the DNA level. Gene identification, isolation and cloning will help to determine whether gene duplication and other such events have taken place leading to species differences in the metabolism of drugs and xenobiotics.

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