DNA Analysis of Polymorphism in Drug or Xenobiotics Metabolism.

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Hereditary differences in activities of drug metabolizing enzymes among individuals have led to a phenotypic classification of humans as poor or extensive metabolizer. The metabolic genotype may be important in drug overdose with substances subjected to detoxication.

Here we present determination of the GSTM1 (glutathione S-transferase) gene deletion and the NAT2 (N-acetyltransferase) genotype, and also the influence of allelic difference at the ALDH2 (aldehyde dehydrogenase) gene locus on the ethanol loading in healthy Japanese individuals' DNAs.

Genotyping assays using DNA sample can be of great help at drug therapy and clinical or postmortem diagnosis.

GSTM1 POLYMORPHISM

GSTM1, one of the genetic loci encoding human GST isoenzymes, is associated to the GSTμ isoenzyme. In three alleles at the GSTM1 locus, GSTM1*0 (null) corresponds to a gene deletion. Almost 50% of people show the μ isoenzyme deficiency, which is caused by GSTM1*0/*0. Absence of the detoxifying role of GST against xenobiotics is considered to increase the risk of lung cancer (Comstock 1990; Groppi 1991).

We have examined the distribution of the GSTM1*0/*0 genotype in a Japanese population. According to Comstock (1990) and Groppi (1991), the regions from exon 4 to exon 5 and from intron 4 to exon 5, respectively, in the GSTM1 gene were amplified by PCR. Among 65 subjects, 32 lacked the PCR product and were determined as null homozygote.

NAT2 GENOTYPE

Polymorphic NAT2, which catalyzes the N-acetylation of various arylamines and hydrazines, divides into slow or rapid acetylator. The acetylation polymorphism is considered to be susceptible to bladder cancer and colorectal cancer. Four NAT2 alleles differ at single base substitutions which modify the restriction enzyme cleavage sites. About 40% of Caucasians are slow acetylators, who do not possess NAT2*1 allele (Hickman 1991).

We have amplified the sequence containing the polymorphic sites in the NAT2

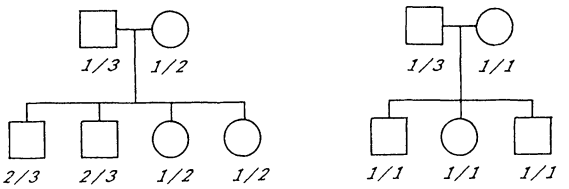

Figure 1. Determination of the NAT2 Genotype in Two Families.

Table 1. NAT2 Genotype Distribution in Healthy Japanese Examined.

Genotype	No. subjects	%
1/1	30	46
1/2	10	15
1/3	19	29
1/4	0	0
2/2	1	2
2/3	4	6
2/4	0	0
3/3	1	2
3/4	0	0
4/4	0	0
Total	65	100

ALDH2 Genotype	No. subjects	%
*ALDH2*1/ALDH2*1*	35	48
*ALDH2*1/ALDH2*2*	32	44
*ALDH2*2/ALDH2*2*	6	8
Total	73	100

Table 2. ALDH2 Genotype Distribution in Healthy Japanese Examined.

Table 3. Relationship between ALDH2 Genotype and the Ethanol Sensitivity.

	n	ALDH2 Phenotype	Mean Max. Acetaldehyde Level(μM)	Mean β_{60} Value (mg/mℓ/hr)
ALDH2*1/ALDH2*1	11	active	3.9± 1.9	0.16±0.03
ALDH2*1/ALDH2*2	15	inactive	20.6± 7.5	0.14±0.02
ALDH2*2/ALDH2*2	4	inactive	84.8±29.4	0.11±0.03

gene by PCR (Abe 1993) and the allele was determined as NAT2*2, *3 or *4 when the fragment was not cleaved by BamHI, TaqI or KpnI, respectively. The NAT2*1 allele was detected by cleavage of three enzymes (Fig. 1). The allele NAT2*4 was not detected and 6 Japanese among 65 were slow acetylators (Table 1).

ALDH2 GENOTYPE AND ALCOHOL LOADING TEST

In an individual ethanol sensitivity, allelic differences at the ADH2 and ALDH2 loci (Hsu 1985; Xu 1988) have an important role.

We distinguished the polymorphism at the ALDH2 locus in Japanese individuals' DNAs using the technique of PCR and allele-specific oligonucleotide probes. The activity of the ALDH2 isozyme in their hair roots was also detected. The subjects were orally administered 0.4 g/kg of ethanol to investigate the sensitivity (Yamada 1992; Yamamoto 1993). In people possessing the ALDH2*2 allele a consequent elevation of blood acetaldehyde after alcohol intake due to less or no enzyme activity causes strong sensitivity to ethanol (Tables 2 and 3).

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REFERENCES

Abe M, Suzuki T, Deguchi T (1993) An improved method for genotyping of N-acetyltransferase polymorphism by polymerase chain reaction. Jpn J Hum Genet 38:163-168

Comstock KE, Sanderson JS, Claflin G, et al (1990) GST1 gene deletion determined by polymerase chain reaction. Nucl Acids Res 18:3670

Groppi A, Coutelle C, Fleury B, et al (1991) Glutathione S-transferase class μ in French alcoholic cirrhotic patients. Hum Genet 87:628-630

Hickman D, Sim E (1991) N-Acetyltransferase polymorphism. Biochem Pharmacol 42:1007-1014

Hsu LC, Tani K, Fujiyoshi T, et al (1985) Cloning of cDNAs for humnan aldehyde dehydrogenases 1 and 2. Proc Natl Acad Sci USA 82:3771-3775

Xu Y, Carr LG, Bosron WF, et al (1988) Genotyping of human alcohol dehydrogenase at the ADH2 and ADH3 loci following DNA sequence amplification. Genomics 2:209-214

Yamada M, Yamamoto K, Ueno Y, et al (1992) Genotypes of alcohol-metabolizing enzymes and ethanol sensitivity. In: Kaempe B (ed) Forensic Toxicology. Mackeenzie Press, Copenhagen, pp.497-504

Yamamoto K, Ueno Y, Mizoi Y, et al (1993) Genetic polymorphism of alcohol and aldehyde dehydrogenase and the effects on alcohol metabolism. Jpn J Alcohol Drug Depend 28:13-25