Genetic polymorphism of CYP2D6, GSTM1 and NAT2 and susceptibility to haematological neoplasias

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Xenobiotic-metabolizing enzymes constitute an important line of defence against a variety of carcinogens. Many are polymorphic, constituting the basis for the wide inter-individual variation in metabolic capacity and possibly a source of variation in the susceptibility to chemical-induced carcinogenesis. The aim of this study was to determine the existence of any association between the main genetic polymorphisms of cytochrome P450 2D6 (CYP2D6), gluta-thione S-transferase M1 (GSTM1) and N-acetyltransferase 2 (NAT2) and an altered risk for haematological neoplasias. A total of 160 patients and 128 controls were genotyped by means of PCR–RFLP-based assays. Mutated alleles comprising *CYP2D6*4*, *GSTM1*0*, *NAT2*5A*, **5B*, **5C*, **6* and **7* were analysed along with the wild-type alleles. The results showed a higher frequency of CYP2D6 extensive metabolizers carrying two functional alleles in the leuk-aemia group, when compared with controls (76.6 versus 57.0%, $P = 0.008$). No differences were found in the case of Hodgkin and non-Hodgkin lymphomas. Analysis of the GSTM1 and NAT2 polymorphisms failed to show an association with any of the neoplasias, although a near significant increase in fast acetylators was also found in the leukaemia group (50.0 versus 35.9%, $P = 0.06$). The results suggest an association of extensive metabolism with an increased risk for leukaemia, possibly by an increase in the metabolic activation of chemical carcinogens or linkage to another cancer-causing gene. Opposite findings presented in other studies may reflect geographical differences in the type of environmental carcinogens to which different populations are exposed.

Introduction

Xenobiotic-metabolizing enzymes constitute one of the first lines of defence against environmental chemicals and are present in all higher organisms. A number of enzymatic systems, covering a vast range of substrates, have evolved as an adaptive response to environmental aggression and include the cytochrome P450s, the glutathione S-transferases and the N-acetyltransferases (1). Many of these enzymes are genetically polymorphic and are responsible for wide inter-individual variation in drug metabolism (2). This variation may also result in different patterns of susceptibility to the effects of

carcinogenic substances and several studies have attempted to link genetic polymorphisms of xenobiotic-metabolizing enzymes [most commonly of cytochrome P450 2D6 (CYP2D6), glutathione S-transferase M1 (GSTM1) and N-acetyltransferase 2 (NAT2)] with an altered risk for some cancers, particularly those with an important environmental contribution, such as lung, bladder and colon cancer (for reviews see refs 3,4).

CYP2D6 (debrisoquine hydroxylase) is one of the most thoroughly studied enzymes since lack of its activity is the basis for adverse events occurring during therapy with some drugs (5). About 5–10% of Caucasians have inactivating mutations in both alleles of the *CYP2D6* gene (22q13.1) and have been designated poor metabolizers (PM) (6). The most frequent inactivating mutation among Caucasians is the splice site G1934A transition (*CYP2D6*4* allele) that causes a trun-cated protein (7). Other rarer alleles have been described and correlated with variable metabolic activity (8–10).

The glutathione S-transferases (GSTs) have been classified into four classes of cytosolic enzymes (GSTA, M, P and T) and a microsomal enzyme, with further subdivisions (11). The *GSTM1* locus (1p13) has at least three allelic variants, *GSTM1*A* and **B*, which differ by a single base in exon 7, and *GSTM1*0*, in which the entire gene is deleted. Individuals homozygous for *GSTM1*0* (GSTM1 null genotype) express no protein and represent ~50% of most Caucasian populations (12). GSTM1 metabolizes a wide range of toxic and mutagenic compounds, including some arising from phase I metabolism by cytochrome P450s.

The N-acetyltransferases (NATs) are responsible for the acetylation of amino, hydroxyl and sulphydryl groups of many compounds, including a large number of arylamine carcinogens (13). The well-known bimodal distribution in the rate of elimination of isoniazid (an antituberculosis drug) has been the basis for the classic division of patients into fast and slow acetylators. The *NAT* locus (8p22) comprises two functional genes (*NAT1* and *NAT2*) and a pseudogene (*NATP*) (14). *NAT2* has long been known to be responsible for the inter-individual variation in acetylation rate and several alleles have been described (15,16). The most frequent *NAT2* alleles consist of the wild-type fast allele *NAT2*4* and five slow alleles *NAT2*5A*, **5B*, **5C*, **6* and **7* (6). The presence of at least one wild-type allele confers a fast acetylator phenotype, whereas two mutant alleles are required for the slow acetylator phenotype.

Many xenobiotic-metabolizing enzymes act sequentially in the disposal of toxic substrates. Drugs or carcinogens can be activated into more toxic forms by cytochrome P450s (phase I metabolism) and then conjugated by GSTs or NATs (phase II metabolism) to increase the solubility of the product in such a way as to facilitate its excretion (17,18).

Considering the role in detoxification played by these enzymes, the existence of common polymorphisms and previ-ous reports of their association with some cancers (3,4), and considering the possibility of an environmental contribution

to haematological malignancies, we aimed at determining whether any association exists between the main genetic polymorphisms of CYP2D6, GSTM1, NAT2 and an altered susceptibility to haematological neoplasias.

Materials and methods

Subjects

A group of 160 unrelated Portuguese patients with haematological neoplasias was studied (84 males, 76 females, mean age $\pm$ SD 49.9 $\pm$ 21.1). This group was divided according to the type of neoplasia into acute lymphocytic leukaemia (22 cases), chronic lymphocytic leukaemia (13 cases), acute myeloid leukaemia (18 cases), chronic myeloid leukaemia (11 cases), non-Hodgkin lymphoma (71 cases) and Hodgkin lymphoma (25 cases). All patients were undergoing chemotherapy, on an out-patient basis, at the University Hospital of Coimbra, Portugal. A control group of 128 unrelated Portuguese Caucasian volunteers (56 males, 72 females, mean age $\pm$ SD 30.8 $\pm$ 14.2) was also studied. Controls were recruited among members of hospital and faculty staff and students and were considered healthy with no history of cancer or other chronic diseases. All subjects were from the same ethnic group. Apart from a few minority groups, the Portuguese are virtually an ethnically homogeneous population, genetically related to other Iberian populations (19). Age differences between cases and controls were not considered to influence the study since previous data showed no correlation between genotype and age (20,21). All subjects gave their informed consent. Ten millilitre venous blood samples drawn into EDTA were obtained from each subject and genomic DNA was isolated by conventional methods (22).

CYP2D6 genotyping

A PCR–RFLP assay was used for the detection of the G1934A mutation responsible for the poor metabolizer allele *CYP2D6*4*, as described by Gough *et al.* (7). Amplification was carried out in a 50 µl reaction volume containing 150 ng of primer 5′-GCTTCGCCAACCACTCCG-3′, 150 ng of primer 5′-AAATCCTGCTCTTCCGAGGC-3′, 1 U of *Taq* DNA polymerase, 200 µM each dNTP, 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris–HCl (pH 9.0), 5% DMSO and 100–200 ng of DNA. After an initial denaturation at 94°C for 5 min, 30 cycles were performed consisting of annealing at 60°C for 1 min, extension at 72°C for 2 min and denaturation at 94°C for 1 min. This yielded a 334 bp fragment. Digestion of 20 µl of the PCR product was carried out with 5 U of the restriction enzyme *Bst*N1. Fragments were then separated on an 8% polyacrylamide gel and stained with ethidium bromide. The wild-type allele was identified by the presence of 230 and 104 bp size fragments. Alleles with the G1934A mutation do not have the restriction site for *Bst*N1 and produce only undigested 334 bp fragments.

GSTM1 genotyping

A PCR method described by Zhong *et al.* (23) was used to determine the presence/deletion of *GSTM1*. Aliquots of 50–100 ng of DNA were amplified in a 25 µl volume reaction containing 200 ng of primer (P1) 5′-CGCCATCTT-GTGCTACATTGCCCG-3′, 100 ng of primer (P2) 5′-ATCTTCTCCTCTTCT-GTCTC-3′, 100 ng of primer (P3) 5′-TTCTGGATTGTAGCAGATCA-3′, 1 U of *Taq* DNA polymerase, 200 µM each dNTP, 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris–HCl (pH 9.0) and 5% DMSO. After an initial denaturation at 94°C for 5 min, 30 cycles were performed consisting of annealing at 52°C for 1 min, extension at 72°C for 1 min and denaturation at 94°C for 1 min. Products were run on a 2% agarose gel and stained with ethidium bromide. P1 and P2 can anneal either to the *GSTM1* or the *GSTM4* genes and give rise to a 157 bp fragment which serves as an internal control of the PCR reaction. P3 anneals only to the *GSTM1* gene and, together with P1, amplifies a 230 bp fragment which is specific for the *GSTM1* gene. This method does not distinguish heterozygotes from wild-type homozygotes.

NAT2 genotyping

Analysis of the six most common *NAT2* alleles was based on the assay described by Smith *et al.* (24), which is >95% predictive of phenotype. Aliquots of 200–400 ng of DNA were amplified in a 100 µl volume reaction containing 250 ng of primer 5′-GCTGGGTCTGGAAGCTCCTC-3′, 250 ng of primer 5′-TTGGGTGATACATACACAAGGG-3′, 2 U of *Taq* DNA polymerase, 200 µM each dNTP, 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris–HCl (pH 9.0) and 5% DMSO. After an initial denaturation at 94°C for 5 min, 34 cycles were performed consisting of annealing at 60°C for 30 s, extension at 72°C for 45 s and denaturation at 94°C for 30 s, completed with a final cycle of 60°C for 5 min and 72°C for 5 min. This results in the amplification of a 540 bp fragment. Subsequently, 20 µl of the PCR product were digested with 5 U of each of the restriction enzymes *Kpn*I, *Taq*I, *Dde*I and *Bam*HI for the detection of mutations C481T, C590A, A803G and G857A, respectively. Restriction fragments were separated on an 8% polyacrylamide gel and stained

with ethidium bromide. The wild-type allele was identified by complete digestion by *Kpn*I, *Taq*I and *Bam*HI, but not *Dde*I. Mutations either destroyed the recognition sites of the first three enzymes or created a new one for the fourth. Alleles *NAT2*5A*, *5C*, *6* and *7* were identified by the presence of mutations C481T, A803G, G590A and G857A, respectively. The *NAT2*5B* allele was identified by the presence of both C481T and A803G.

Statistical analysis

χ^2 tests were used to examine differences of allele and genotype frequencies between patients and controls. When group size was <40 or when expected values in contingency tables were less than five, Fisher's exact test was used. Possible interactions between genotypes shown to be significantly different in patients and controls were also studied and combined frequencies analysed by the same procedure.

Results

Allele and genotype frequencies of *CYP2D6*, *GSTM1* and *NAT2* are presented in Tables I, II and III, respectively.

The results showed a higher frequency of CYP2D6 homozygous extensive metabolizers in the leukaemia group when compared with the control group [76.6 versus 57.0%, $P = 0.008$, odds ratio 2.46 (95% CI 1.25–4.84)]. An increase in the wild-type allele frequency was also observed in this group [85.2 versus 75.4%, $P = 0.028$, odds ratio 1.87 (95% CI 1.06–3.29)]. No significant differences were observed in the lymphoma groups.

Frequencies of NAT2 fast and slow acetylators in the control group were 35.9 and 64.1%, respectively. No significant differences were observed in any neoplasia, although the frequency of fast acetylators in the leukaemia group showed an increase approaching statistical significance [50.0 versus 35.9%, $P = 0.06$, odds ratio 1.78 (95% CI 0.97–3.28)].

The GSTM1 null genotype was present in 57.8% of controls and did not differ significantly from patients.

The frequency of individuals presenting with a combination of the homozygous extensive metabolizer and fast acetylator genotypes were also determined and shown to be significantly increased in the leukaemia group compared with controls [43.8 versus 20.3%, $P = 0.0007$, odds ratio 3.05 (95% CI 1.58–5.88)].

Discussion

The results showed a higher frequency of CYP2D6 homozygous extensive metabolizers (EM) in the leukaemia group when compared with the control group [$P = 0.008$, odds ratio 2.46 (95% CI 1.25–4.84)]. An increase in the wild-type allele frequency was also observed [$P = 0.028$, odds ratio 1.87 (95% CI 1.06–3.29)]. When interpreting these results, one must take into account the fact that in many cases, phase I metabolism forms toxic intermediate compounds which are more harmful than the original substrate, until further transformation is carried out (17,18). Many environmental carcinogens require metabolic activation by cytochrome P450 to exert deleterious effects (25). The increased activation of pro-carcinogens by extensive metabolizers could confer an increased risk of carcinogenesis and this could be expected to occur as a gene dosage effect, being most noticeable in the case of two functional alleles. Phenotype/genotype correlation studies have indeed demonstrated a higher metabolic activity for the wild-type homozygotes relative to PM individuals and heterozygous individuals (9,26). Other *CYP2D6* alleles have been described, including duplications conferring ultra-rapid metabolism, but have not been considered in this study due to their low frequency (9,10). Misclassification of *CYP2D6* genotypes is a possibility since the genotyping assay allows the identification of only those PM individuals carrying the G1934A mutation.

Table I. *CYP2D6* genotype and allele frequencies in patients and controls (%)

	Controls (n = 128)	All neoplasias (n = 160)	All leukaemias (n = 64)	ALL (n = 22)	CLL (n = 13)	AML (n = 18)	CML (n = 11)	NHL (n = 71)	HL (n = 25)
Genotype									
EM	57.0	71.3[a]	76.5[b]	77.3	76.9	83.3	63.6	64.8	76.0
HEM	36.7	23.1	17.2	18.2	7.7	16.7	27.3	29.6	20.0
PM	6.3	5.6	6.3	4.5	15.4	0	9.1	5.6	4.0
Allele									
wt	75.4	82.8[c]	85.2[d]	86.4	80.8	91.7	77.3	79.6	86.0
m	24.6	17.2	14.8	13.6	19.2	8.3	27.7	20.4	14.0

ALL, acute lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma.
EM, homozygous extensive metabolizer (two functional alleles); HEM, heterozygous extensive metabolizer (one functional allele); PM, poor metabolizer (no functional alleles); wt, wild-type; m, mutant (*CYP2D6*4*).
[a]Statistically significant relative to controls, $P = 0.012$.
[b]Statistically significant relative to controls, $P = 0.008$.
[c]Statistically significant relative to controls, $P = 0.028$.
[d]Statistically significant relative to controls, $P = 0.028$.

Table II. *GSTM1* genotype frequencies observed in patients and controls (%)

	Controls (n = 128)	All neoplasias (n = 160)	All leukaemias (n = 64)	ALL (n = 22)	CLL (n = 13)	AML (n = 18)	CML (n = 11)	NHL (n = 71)	HL (n = 25)
GSTM1[a]	42.2	42.5	40.6	36.4	61.5	44.4	18.2	43.7	44.0
*GSTM1*0/*0*	57.8	57.5	59.4	63.6	38.5	55.6	81.8	56.3	56.0

Abbreviations as in Table I.
[a]Gene present (includes both homozygotes and heterozygotes).

Table III. *NAT2* genotype and allele frequencies observed in patients and controls (%)

	Controls (n = 128)	All neoplasias (n = 160)	All leukaemias (n = 64)	ALL (n = 22)	CLL (n = 13)	AML (n = 18)	CML (n = 11)	NHL (n = 71)	HL (n = 25)
Genotype									
Fast/Fast	6.2	5.6	9.4	0	23.1	11.1	9.1	1.4	8.0
Fast/Slow	29.7	36.9	40.6	40.9	30.8	50.0	36.4	38.0	24.0
Slow/Slow	64.1	57.5	50.0[a]	59.1	46.1	38.9	54.5	60.6	68.0
Allele									
*4	21.1	24.1	29.7	20.5	38.5	36.1	27.3	20.4	20.0
*5A	3.1	1.9	1.6	4.5	0	0	0	2.8	0
*5B	37.9	42.2	38.3	40.9	34.6	38.9	36.4	45.1	44.0
*5C	2.3	1.3	0.8	0	3.8	0	0	1.4	2.0
*6	32.8	29.1	29.7	34.1	23.1	25.0	36.5	28.2	30.0
*7	2.7	1.6	0	0	0	0	0	2.1	4.0

Abbreviations as in Table I.
Fast alleles, *NAT2*4*; slow alleles, *NAT2*5A*, *5B*, *5C*, *6*, *7*.
[a]$P = 0.06$.

Other inactivating mutations may exist, however, studies indicate that this mutation occurs in ~80% of Caucasian PMs (7). In our case, this would mean that the true proportion of PMs in the leukaemia group would be 7.9% (6.3% ÷ 80%), meaning misclassification of one (1.6%) individual. This would not have modified our results substantially, even if we assumed that no change occurred in the control group.

Interestingly, a previous study reported an increase in PMs among leukaemia patients (27). It was hypothesized that PM individuals were at increased risk for this neoplasia because of the impaired detoxification of an unidentified chemical. This apparent discrepancy in results, which has also been reported for lung cancer (28), could reflect geographical differences in environmental exposure to carcinogens.

At this point we cannot rule out the possibility that the apparent increase in homozygotes is due to loss of heterozygosity of peripheral leukocytes used for DNA extraction. Regarding this last point, it is interesting to note that in our study there was also a decrease in the frequency of *CYP2D6* heterozygotes among leukaemia patients (the same occurred in the previously mentioned study; 27). Allele loss by cells used for genetic analysis could be a source of bias, since the genotyping assay does not distinguish between the homozygous and any hemizygous state. Further studies are required to clarify this point, for example by using other cells as the source of genomic DNA.

Another explanation for the findings would be the existence of linkage disequilibrium of the *CYP2D6* locus with other neighbouring genes involved in carcinogenesis.

We also investigated whether other fully active genotoxin-

metabolizing enzymes were over-represented in our patients by analysing the polymorphisms of the *GSTM1* and *NAT2* genes, which have frequently been associated with predisposition to other neoplasias (3,4). *GSTM1* analysis failed to reveal any association with the neoplasias considered in this study. As far as *NAT2* analysis is concerned, no significant difference in fast and slow acetylator frequencies was detected between patients and controls, however, there was a near significant increase in fast acetylators in the leukaemia group [$P = 0.06$, odds ratio 1.78 (95% CI 0.97–3.28)], thus confirming the overall tendency for higher metabolic activity in this group. There have also been reports of associations between fast acetylation and lung (29) and colon cancer, including a recent study with Portuguese patients (30), although it is unclear whether they share common causative factors with the cancers in the present study.

In order to assess the existence of any interaction between the overexpressed genotypes, we calculated the frequency of the simultaneous presence of homozygous EM and fast acetylator genotypes and found a clear increase in the leukaemia group [$P = 0.0007$; odds ratio 3.05 (95% CI 1.58–5.88)], which suggests a synergistic effect of both genotypes.

In the light of these results, one cannot ignore the fact that all the patients were recruited from a chemotherapy centre. Patients with higher metabolic activity might dispose of cytostatic drugs at a higher rate than that appropriate for adequate remission therapy, thus requiring longer periods of treatment (31). If this is so, then at any given time one would expect to find a higher proportion of active metabolizers among patients undergoing chemotherapy, constituting a source of selection bias. However, this is unlikely to have influenced the results since patients were enrolled in the study before initiating the chemotherapy cycles. Further studies are also underway to assess the influence of these phenotypes on treatment efficacy.

In conclusion, our findings seem to suggest an influence of genetic polymorphisms of xenobiotic-metabolizing enzymes, particularly CYP2D6 polymorphisms, on the susceptibility to some haematological neoplasias, possibly by an increase in the metabolic activation of chemical carcinogens or linkage to another cancer-causing gene. Contradictory data presented in other similar studies may imply a different pattern of carcinogen exposure in the Portuguese population, although more extensive research is needed to clarify this issue. Further research in this field may allow the identification of risk factors and contribute towards understanding the molecular mechanisms underlying the process of carcinogenesis.

Acknowledgements

References

1. Nebert,D.W. (1997) Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? *Am. J. Hum. Genet.*, **60**, 265–271.
2. Weber,W.W. (1997) *Pharmacogenetics*. Oxford University Press, New York, NY.
3. Smith,G., Stanley,L.A., Sim,E., Strange,R.C. and Wolf,C.R. (1995) Metabolic polymorphisms and cancer susceptibility. *Cancer Surv.*, **25**, 27–65.
4. d'Errico,A., Taioli,E., Chen,X. and Vineis,P. (1996) Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers*, **1**, 149–173.
5. Eichelbaum,M. and Gross,A.S. (1990) The genetic polymorphism of debrisoquine/sparteine metabolism—clinical aspects. *Pharmacol. Ther.*, **46**, 377–394.
6. Meyer,U.A. and Zanger,U.M. (1997) Molecular mechanisms of genetic polymorphisms of drug metabolism. *Annu. Rev. Pharmacol. Toxicol.*, **37**, 269–296.
7. Gough,A.C., Miles,J.S., Spurr,N.K., Moss,J.E., Gaedigk,A., Eichelbaum,M. and Wolf,C.R. (1990) Identification of the primary gene defect at the cytochrome P450 CYP2D6 locus. *Nature*, **347**, 773–776.
8. Daly,A.K., Brockmöller,J., Broly,F. *et al.* (1996) Nomenclature for human CYP2D6 alleles. *Pharmacogenetics*, **6**, 193–201.
9. Sachse,C., Brockmöller,J., Bauer,S. and Roots,I. (1997) Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet.*, **60**, 284–295.
10. Marez,D., Legrand,M., Sabbagh,N., Lo Guidice,J.M., Spire,C., Lafitte,J.J., Meyer,U.A. and Broly,F. (1997) Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterisation of 48 mutations and 53 alleles, their frequencies and evolution. *Pharmacogenetics*, **7**, 193–202.
11. Mannervik,B., Awasthi,Y.C., Board,P.G. *et al.* (1992). Nomenclature for human glutathione transferases. *Biochem. J.*, **282**, 305–308.
12. Seidegard,J., Vorachek,W.R., Pero,R.W. and Pearson,W.R. (1988) Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. *Proc. Natl Acad. Sci. USA*, **85**, 7293–7297.
13. Grant,D.M. (1993) Molecular genetics of the *N*-acetyltransferases. *Pharmacogenetics*, **3**, 45–50.
14. Blum,M., Grant,D.M., McBride,W., Heim,M. and Meyer,U.A. (1990) Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localisation and functional expression. *DNA Cell Biol.*, **9**, 193–203.
15. Vatsis,K.P., Weber,W.W., Bell,D.A. *et al.* (1995) Nomenclature for *N*-acetyltransferases. *Pharmacogenetics*, **5**, 1–17.
16. Cascorbi,I., Drakoulis,N., Brockmöller,J., Maurer,A., Sperling,K. and Roots,I. (1995) Arylamine *N*-acetylation (NAT2) mutations and their allelic linkage in unrelated caucasian individuals: correlation with phenotypic activity. *Am. J. Hum. Genet.*, **57**, 581–592.
17. Smith,C.A.D., Smith,G. and Wolf,C.R. (1994) Genetic polymorphisms in xenobiotic metabolism. *Eur. J. Cancer*, **30A**, 1935–1941.
18. Nebert,D.W., McKinnon,R.A. and Puga,A. (1996) Human drug-metabolizing enzyme polymorphisms: effects on risk of toxicity and cancer. *DNA Cell Biol.*, **15**, 273–280.
19. Arnaiz-Villena,A., Martínez-Laso,J., Gómez-Casado,E., Díaz-Campos,N., Santos,P., Martinho,A. and Breda-Coimbra,H. (1997) Relatedness among Basques, Portuguese, Spaniards, and Algerians studied by HLA allelic frequencies and haplotypes. *Immunogenetics*, **47**, 37–43.
20. Agundez,J.A., Rodriguez,I., Olivera,M., Ladero,J.M., Garcia,M.A., Ribera,J.M. and Benitez,J. (1997) CYP2D6, NAT2 and CYP2E1 genetic polymorphisms in nonagenarians. *Age Ageing*, **26**, 147–151.
21. Muiras,M.L., Verasdonck,P., Cottet,F. and Schächter,F. (1998) Lack of association between human longevity and genetic polymorphisms in drug-metabolizing enzymes at the NAT2, GSTM1 and CYP2D6 loci. *Hum. Genet.*, **102**, 526–532.
22. Miller,S.A., Dykes,D.D. and Polesky,H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res.*, **16**, 1215–1218.
23. Zhong,S., Wyllie,A.H., Barnes,D., Wolf,C.R. and Spurr,N.K. (1993) Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis*, **14**, 1821–1824.
24. Smith,C.A.D., Wadelius,M., Gough,A.C., Harrison,D.J., Wolf,C.R. and Rane,A. (1997) A simplified assay for the arylamine *N*-acetyltransferase 2 polymorphism validated by phenotyping with isoniazid. *J. Med. Genet.*, **34**, 758–760.
25. Guengerich,F.P. (1993) Cytochrome P450 enzymes. *Am. Sci.*, **81**, 440–447.
26. Sachse,C., Brockmöller,J., Hildebrand,M., Mülller,K. and Roots,I. (1998) Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolisers of debrisoquine. *Pharmacogenetics*, **8**, 181–185.
27. Wolf,C.R., Smith,C.A.D., Gough,A.C. *et al.* (1992) Relationship between the debrisoquine hydroxylase polymorphism and cancer susceptibility. *Carcinogenesis*, **13**, 1035–1038.
28. Rostami-Hodjegan,A., Lennard,M.S., Woods,H.F. and Tucker,G.T. (1998) Meta-analysis of studies of the CYP2D6 polymorphism in relation to lung cancer and Parkinson's disease. *Pharmacogenetics*, **8**, 227–238.
29. Cascorbi,I., Brockmöller,J., Mrozikiewicz,P.M., Bauer,S., Loddenkemper,R. and Roots,I. (1996) Homozygous rapid arylamine *N*-

acetyltransferase (NAT2) genotype as a susceptibility factor for lung cancer. *Cancer Res.*, **56**, 3961–3966.

30. Gil,J.P. and Lechner,M.C. (1998) Increased frequency of wild-type arylamine-*N*-acetyltransferase allele NAT2*4 homozygous in Portuguese patients with colorectal cancer. *Carcinogenesis*, **19**, 37–41.

31. Ratain,M.J. (1997) Pharmacology of cancer chemotherapy. In DeVita,V.T., Hellman,S. and Rosenberg,S.A. (eds) *Cancer: Principles and Practice of Oncology*. Lippincott-Raven, Philadelphia, PA, pp. 375–385.