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CYP2D6 gene polymorphism in Caucasian smokers: lung cancer susceptibility and phenotype–genotype relationships

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Abstract

Individual susceptibility to smoking-related cancers is proposed to partly depend on a genetically determined ability to metabolise tobacco carcinogens. We previously reported on the association between the activity of the xenobiotic-metabolising enzyme CYP2D6 and lung cancer risk in a hospital-based case–control study among French Caucasian smokers. Here we extended the study to address the effect of four gene-inactivating mutations (*CYP2D6*3*, **4*, **5* and **16*) and the gene duplication of the *CYP2D6* gene (*CYP2D6*2×2* or *CYP2D6*1×2*) on lung cancer risk in the same population (150 patients with primary lung carcinoma of squamous cell or small cell histology and 172 controls). The risk of lung cancer associated with the *CYP2D6* poor metaboliser genotype (odds ratio 1.5, 95% confidence interval 0.5–4.3) did not differ from that in the reference category of extensive metaboliser and ultra-rapid metaboliser genotypes combined. Lung cancer risks for the *CYP2D6* PM genotype amongst light smokers (tobacco consumption $\leqslant$20 g/day) or heavy smokers (>20 g/day) were not significantly different. The present findings agree with the discrepancy between the phenotype-based and genotype-based studies indicated by the recent meta-analyses.

Keywords: *CYP2D6*; Lung cancer; Tobacco smoking; Polymorphism

1. Introduction

Individual susceptibility to smoking-related cancers is proposed to partly depend upon a genetically determined ability to metabolise tobacco carcinogens. The *CYP2D6* gene, which encodes the cytochrome P450 2D6 enzyme, has a well-defined phenotypic polymorphism. The polymorphism has been shown to have a genetic basis, with a multiplicity of variant alleles identified [1,2]. CYP2D6 has an important role in pharmacology, it metabolises approximately 25% of commonly used drugs. With regard to tobacco smoking, CYP2D6 has been implicated in the metabolism of nicotine [3], and it has been shown to catalyse activation of the tobacco-specific pulmonary carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), (reviewed in [4]), although more recent data point to a major role for CYP2A6 in both of these biotransformation processes [5]. In addition, carcinogen–DNA adducts have been shown to be reduced in individuals who do not express functional CYP2D6 protein [6]. The presence of *CYP2D6* mRNA has been demonstrated in lung tissue [7]. It is therefore conceivable to assume that individuals with a reduced capacity to metabolise CYP2D6 substrates, i.e. poor metabolisers (PMs), may have a decreased risk of lung cancer compared with extensive metabolisers (EMs).

The association between CYP2D6 polymorphism, determined either with a phenotyping or genotyping test, and lung cancer has been investigated in numerous epidemiological studies, with inconsistent results. Early studies employed phenotyping with a probe drug,

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but later work has often been based on genotypic assessment [8,9]. Deficiency of CYP2D6 enzyme activity has been shown to be due to the inheritance of two alleles coding for inactive or no enzyme. Such alleles include deletions of the whole *CYP2D6* gene and a large number of sequence alterations resulting in no or reduced activity [1,10]. It has been shown, however, that assessment of the gene deletion (*CYP2D6*5*), and the two most frequent inactivating mutations in Caucasians (*CYP2D6*3* and *CYP2D6*4*), detects more than 90% of the PMs in European Caucasian populations [10,11]. In addition to defective alleles, a duplication (or amplification) of the functional *CYP2D6* gene (*CYP2D6*2×2* or *CYP2D6*2×N*) has been discovered and shown to result in an ultra-rapid metaboliser (UM) phenotype [12]. This allele is present in a relatively small percentage of European Caucasians, but considerable variation has been observed between different ethnic groups [13].

We previously reported on the association between CYP2D6 enzyme activity, determined using dextromethorphan as the probe drug, and lung cancer in a hospital-based case–control study among Caucasian smokers [14]. In that study, no effect of CYP2D6 activity was found on the overall lung cancer risk, but a significant association was observed between CYP2D6 activity and daily tobacco consumption. Here we extended the study to investigate the effect of four main *CYP2D6* gene-inactivating mutations and gene duplication on lung cancer risk in the same population. In addition, we compared genotyping results with those obtained by phenotyping.

2. Patients and methods

2.1. Subjects

The study population was described previously [14]. Briefly, Caucasian individuals were recruited from 1988 to 1992 in 10 French hospitals, of which 9 are located in Paris. Only incident cases with histologically confirmed primary squamous or small cell carcinomas of the lung were included. A control group, frequency matched for age, sex and hospital, consisted of patients without previous or current malignant diseases. Only regular smokers, defined as people having smoked five cigarettes or more (or cigars or pipes) per day for at least 5 years, were included. Both former smokers (defined as individuals having stopped smoking at least 1 year prior to the diagnosis) and current smokers were included. Patients were recruited by seven trained study interviewers. Each interviewer had to include both cases and controls. A questionnaire was filled out for each subject during a personal interview where information on lifetime tobacco use and alcohol consumption, personal medical history, current use of medications and occupational history were recorded. Blood samples from 150 lung cancer patients (98 squamous carcinomas and 52 small cell carcinomas) and 172 controls fulfilling the above-mentioned criteria were collected and stored at −20°C. The main diagnoses in the controls were rheumatological (33%), infectious and parasitic (10%), respiratory (9%), cardiovascular (8%), digestive (6%) and traumatological (6%) diseases. The main motive for admission to hospital was related to general symptoms (7%) for the other categories. Most of the study subjects were men (93% of cases and 95% of controls). The mean age was slightly higher for lung cancer patients (58.4 years) than for controls (55.0 years). Daily tobacco consumption was similar in cases and controls (26.3 g versus 25.1 g, non-significant (NS)). Cases had smoked longer than controls (38.0 years versus 32.2 years, $P < 0.001$) and the average number of pack-years was significantly higher among cases than among controls (42.4 versus 32.3, $P < 0.001$). Nineteen per cent of the cases and 7% of the controls reported a history of occupational asbestos exposure ($P < 0.001$).

2.2. CYP2D6 *genotyping*

Lymphocyte DNA was extracted from 10 ml of peripheral blood using standard protocols. In addition to the wild-type allele (*CYP2D6*1*), four deficient variant alleles (**3*, **4*, **5* and **16*) together with the gene amplification (*CYP2D6*2×2* or *CYP2D6*1×2*, no distinction was made between these two alleles) were determined. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to detect the base pair deletion in exon 4 (*CYP2D6*4* allele) as described by Hirvonen and colleagues [15], as well as the frame shift mutation in exon 5 (*CYP2D6*3* allele) given in Smith and colleagues [16]. The two different deletion polymorphisms (*CYP2D6*5* and **16*) and duplication (*CYP2D6*2×2*) of the *CYP2D6* gene were detected using Southern blotting and hybridisation [12]. Briefly, total genomic DNA (2.5 μg) was digested with *Eco*RI. Following gel electrophoresis DNA fragments were transferred onto Zetaprobe GT-membranes (Bio-Rad, 1982) using standard Southern blotting procedures and hybridised with a [^{32}P]-dCTP-labelled cDNA probe (kindly supplied by F. Gonzales, NIH, Bethesda, USA) at +65°C overnight. The presence of a 12.1 kb band revealed the *CYP2D6*2×2* allele, whereas the *CYP2D6*5* and *CYP2D6*16* alleles were detected by the presence of bands at 13.0 and 11 kb, respectively.

Individuals with two wild-type *CYP2D6* alleles were considered as extensive metabolisers (EMs). Those with two inactivating alleles of the gene, and thus devoid of CYP2D6 activity, were designated as poor metabolisers (PM). Carriers of one wild-type allele and one inactivating allele were predicted as intermediate metabolisers and marked as heterozygous intermediate metabolisers

(HEMs). Individuals with the *CYP2D6*2×2* allele and without any inactivating allele were predicted as UMs. Those with one inactivating allele and the gene duplication were assumed to be comparable with individuals with two functional alleles, and therefore considered as phenotypic EMs. One control subject among the cases was found to be homozygous for the *CYP2D6*4* allele and also carried the duplicated gene. Similarly, one carried both *CYP2D6*4* and **3* alleles in addition to the duplication. These 2 subjects were thus predicted as PMs.

2.3. Phenotyping test

Data on CYP2D6 activity was available for 129 out of 172 (75%) control individuals. This enzymatic capacity was scored through the use of the probe drug dextromethorphan as previously described [14]. The main criteria for exclusion were: refusal to give informed consent, inability to take oral medications or to be interviewed, presence of severe renal or liver disease or chronic heart failure, and use of medications known or suspected to interfere with the phenotyping test (neuroleptics, antidepressants, anti-arrhythmics, β-blockers and drugs containing cimetidine, debrisoquine, dextromethorphan, dextropropoxyphene, diltiazem, guanoxan, phenacetine, phenformine or phenotiazine). All other medications administered during the last week before phenotyping test were abstracted from medical records. The CYP2D6 activity was expressed as the MR (metabolic ratio) of dextromethorphan to its metabolite dextrorphan. Individuals were classified as EMs ($MR \leqslant 0.3$) or PMs ($MR > 0.3$) by their phenotype. The reproducibility of phenotype assignment has been assessed for a sample of individuals in two different laboratories.

2.4. Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression using the generalised linear interactive modelling statistical package [17]. All ORs were adjusted for sex and age (< 50, 50–54, 55–59, 60–64, $\geqslant 65$ years). Because of the very strong association between smoking and lung cancer, ORs were also adjusted for smoking by including in the model smoking status (ex-smokers/current smokers), inhalation (no/yes), duration of smoking in years ($\leqslant 25$, 26–35, > 35) and daily consumption of tobacco in g/day ($\leqslant 20$, 21–30, > 30). Occupational exposures to asbestos (no/yes) and arsenic (no/yes) were also considered as confounding factors.

Interactions between *CYP2D6* genotypes and smoking-related variables were studied to test the equality of the effect of *CYP2D6* genotypes across levels of smoking exposure. These interactive effects were assessed by the likelihood ratio test to compare the goodness of fit of the model with and without the interaction term [18]. For that purpose, the average daily consumption of tobacco, the duration of smoking and the number of pack-years (number of packs smoked per day multiplied by number of years of smoking; 20 cigarettes per pack) were expressed as categorical variables defined with two levels of smoking exposure according to the approximate median in the control population.

The low overall prevalence of the *CYP2D6* UM genotype prevented us from investigating the effect of this genotype on lung cancer. *CYP2D6* UM and EM genotypes were therefore combined in all analyses. Due to a small number of individuals with the *CYP2D6* PM genotype, the combined *CYP2D6* UM and EM genotype category was considered as the reference in order to provide more stable risk estimates. One control individual and 2 lung cancer patients had one *CYP2D6*4* allele and the gene duplication. It was not possible to elucidate whether the wild-type or the *CYP2D6*4* allele was the duplicated one. Therefore, we could not explicitly predict the phenotype (EM or HEM) of these individuals, and decided to exclude them from the statistical analyses.

3. Results

Table 1 shows the frequency distribution of *CYP2D6* genotypes among lung cancer patients and controls. Among lung cancer patients, the frequencies of the genotypes representing UM, EM, HEM and PM were 2.7%, 52.0%, 39.9% and 5.4%, respectively. The corresponding frequencies among the controls were 4.7%, 62.6%, 26.3% and 6.4%.

*CYP2D6*4* was the most common inactivating allele detected; 37.8% of the cases and 26.9% of the controls carried this allele (allele frequencies 0.206 and 0.149, respectively). The frequencies detected are shown in Table 1. From the five lung cancer cases with the gene duplication, 4 (80%) were predicted to be UMs, and 1 (20%) as a PM (**3/*4×2*). In 10 controls, 8 (80%) were UMs, 1 (10%) an EM, and 1 (10%) a PM (**4/*4×2*) (Table 1). The two gene duplication carriers predicted to be PMs had metabolic ratios of dextromethorphan to dextrorphan above the antimode 0.3 (4.6 and 1.3, respectively).

The risk of lung cancer among the individuals with the PM genotype did not differ significantly from that among cases with the EM or UM genotype ($OR = 1.5$, 95% CI 0.5–4.3) (Table 2). The results remained the same when squamous cell carcinomas and small cell carcinomas were considered separately (data not shown). We also examined the interactive effects between the *CYP2D6* genotypes and daily tobacco consumption at two levels ($\leqslant 20$ g/day and > 20 g/day) on lung cancer risk. No significant interaction was

Table 1
Predicted phenotypes and the detected genotypes among lung cancer cases ($n = 148$) and hospital controls ($n = 171$)

Phenotype predicted	Genotype detected		Cases	Controls
			n (%)	*n* (%)
UM	*CYP2D6*1/CYP2D6*2×2*[a]		4 (2.7)	8 (4.7)
		Total	4 (2.7)	8 (4.7)
EM	*CYP2D6*1/CYP2D6*1*		77 (52.0)	106 (62.0)
	*CYP2D6*5/CYP2D6*2×2*[a]		0	1 (0.6)
		Total	77 (52.0)	107 (62.6)
HEM	*CYP2D6*1/CYP2D6*3*		7 (4.7)	4 (2.3)
	*CYP2D6*1/CYP2D6*4*		48 (32.4)	35 (20.5)
	*CYP2D6*1/CYP2D6*5*		4 (2.7)	5 (2.9)
	*CYP2D6*1/CYP2D6*16*		0 (0.0)	1 (0.6)
		Total	59 (39.9)	45 (26.3)
PM	*CYP2D6*3/CYP2D6*4*		0	3 (1.8)
	*CYP2D6*3/CYP2D6*4×2*[b]		1 (0.7)	0
	*CYP2D6*4/CYP2D6*4*		6 (4.1)	5 (2.9)
	*CYP2D6*4 CYP2D6*5*		0	2 (1.2)
	*CYP2D6*4/CYP2D6*16*		1 (0.7)	0
	*CYP2D6*4/CYP2D6*4×2*		0	1 (0.6)
		Total	8 (5.4)	11 (6.4)

UM, ultra-rapid metaboliser; EM, extensive metabolisers; HEM, heterozygous intermediate metaboliser; PM, poor metaboliser.
[a] Not determined, whether it was the *CYP2D6*2* or *CYP2D6*1* allele that was duplicated.
[b] Not determined, whether it was the *CYP2D6*4* or *CYP2D6*3* allele that was duplicated.

found (χ^2 for homogeneity = 0.60, 2 degrees of freedom, NS). The ORs of lung cancer did not differ among light smokers and heavy smokers in the different classes of *CYP2D6* genotype (Table 2). The results were not modified when duration of smoking ($\leqslant 35$ years and > 35 years) or pack-years of smoking ($\leqslant 35$ pack-years and > 35 pack-years) were considered (data not shown). However, the statistical power in these analyses to detect differences in lung cancer risks was low due to the relatively small sample sizes.

Both phenotype and genotype determinations were available for 129 control subjects. Fig. 1 shows the distribution of the metabolic ratio by *CYP2D6* genotypes. Overall, the two determinations gave concordant results for 121/129 control individuals (93.8%, 95% CI 88.1–97.3%). As shown in Fig. 1, there were two genotypic PMs (**3/*4,* and **4/*4*), for whom the MR was below the MR antimode of 0.3. Among the genetic heterozygotes, there were four individuals with a PM phenotype. Similarly, two genotypic EMs were phenotypically determined as PMs, possibly due to unrecorded medication with drug(s) that can interfere with the *CYP2D6* phenotyping.

4. Discussion

This case–control study did not detect a difference in the risk of lung cancer between individuals with the PM genotype compared with those with an EM or UM genotype. Our results are in accordance with those reported in two recent genotyping studies [19,20] and

Table 2
CYP2D6 genotype and tobacco smoke exposure: number of cases/controls and odds ratios (OR) and 95% confidence intervals (CI) of lung cancer

Phenotype predicted	Total		Tobacco consumption			
			⩽20 g/day		>20 g/day	
	Cases/controls[a]	OR[b] (95% CI)	Cases/controls	OR[c] (95% CI)	Cases/controls	OR[c] (95% CI)
UM or EM	79/114	1 [Ref.]	34/57	1 [Ref.]	45/57	1.2 (0.6–2.3)
HEM	57/44	1.9 (1.1–3.2)	29/21	2.1 (0.9–4.2)	28/23	2.1 (1.0–4.6)
PM	8/11	1.5 (0.5–4.3)	5/7	2.1 (0.5–8.2)	3/4	1.1 (0.2–5.8)

UM, ultra-rapid metaboliser; EM, extensive metaboliser; HEM, heterozygote intermediate metaboliser; PM, poor metaboliser; OR, odds ratio; CI, confidence interval.
[a] For 4 cases and 2 controls, smoking exposure data were missing.
[b] Adjusted for sex, age, daily tobacco consumption, duration of smoking, smoking status, inhalation, asbestos exposure and arsenic exposure.
[c] Adjusted for sex, age, duration of smoking, smoking status, inhalation, asbestos exposure and arsenic exposure.

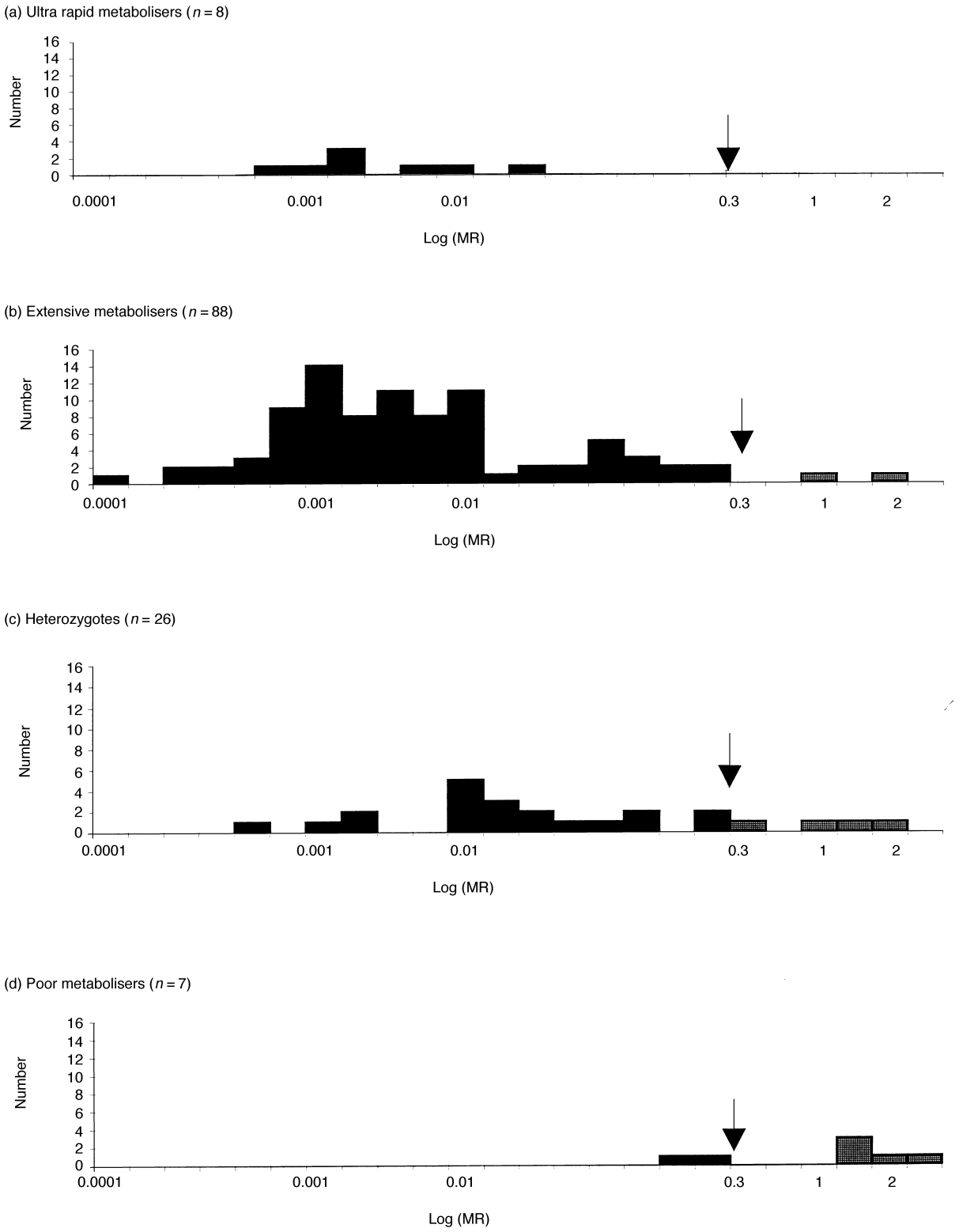

Fig. 1. (a–d) Distribution of the metabolic ratio (MR) (log MR; antimode indicated by an arrow) by the ultra-rapid metaboliser (UM), extensive metaboliser (EM), heterozygote intermediate metaboliser (HEM) and poor metaboliser (PM) genotype in the 129 control individuals with both phenotype and genotype determinations available.

meta-analyses [8,9]. The meta-analyses found a consistent small decrease in the risk of lung cancer associated with the PM phenotype (OR 0.69, 95% CI 0.52–0.90, and 0.70, 95% CI 0.47–1.03; Refs. [8] and [9], respectively), while lung cancer studies based on genotyping assays did not indicate such an effect [8,9].

The lung cancer risks associated with *CYP2D6* genotypes were similar among light smokers ($\leqslant$20 g tobacco/day) and heavy smokers (>20 g/day). The ORs for the HEM genotype were somewhat increased in both smoking categories, similar to that in the total population. Since the effect was not seen consistently in all groups, we interpret the finding to be due to chance rather than to indicate a true association. It has been suggested that the difference in the risk of lung cancer associated with the *CYP2D6* phenotype is restricted to non-adenocarcinoma histology, and that this could at least partially explain inconsistencies in the literature data. Our results do not support this view, as our study population consisted of cases with squamous cell carcinoma and small cell carcinoma with no significant difference observed in risk between PM and EM or UM genotypes. This finding is in keeping with another study on Caucasian lung cancer cases not finding a difference in the risk between the various cell types [20].

To study the phenotype–genotype correlation, we compared the metabolic ratio of dextromethorphan within the control population to the genotypes assessed. The phenotyping procedure revealed a bimodal distribution of the log(MR), where individuals with the PM phenotype accounted for 8.5% ($n=11/129$). The genotype assessment detected 5.4% ($n=7/129$) of the control individuals as PMs. These values are in agreement with those reported for Caucasian populations (5–10%) [10,13,21]. The frequency of gene duplication similarly agreed with that observed earlier among Caucasians [10,22]. To compare our results with those of the literature, we tabulated the phenotype–genotype concordance in twelve different studies, which had predicted the metabolic phenotype by genetic analyses (Table 3). The concordance found between the phenotype and genotypes in our study (93.8%) agrees with literature data (93.4–100%).

Discrepant phenotype–genotype determinations were initially observed for 8 out of our 129 control individuals (6.2%), and the samples were re-analysed for both genotype and phenotype assessments. All the genotypes were reconfirmed, whereas 1 individual classified as EM in the first phenotypic determination, with a metabolic ratio close to the antimode value, was found to be a PM in re-analysis of the MR. A number of drugs have well-known properties to interfere with CYP2D6 enzyme activity and are thus likely to produce misclassification into phenotype assignment [23]. To circumvent potential false phenotyping results from this source, all individuals who according to hospital records had taken such medication were excluded from the study. However, for the remaining subjects, we could not formally exclude possible patient self-medication or administration of such drugs without formal medical prescription.

The genotype assessments included the most prevalent *CYP2D6* inactivating alleles covering more than 90% of the PMs [2,10]. It is, however, possible that a few individuals carried rare inactivating alleles not searched for in the present study as reported for some Caucasian populations [10,21]. The reasons for the MR values of <0.3 detected for two genotypic PMs are more difficult to explain but may have been caused, e.g. by metabolism of dextromethorphan by other CYP enzymes, such as CYP3A4 [24]. Genotyping indicated for 1 case and 1 control that a defective allele (probably **4*) was duplicated. For both of these, the phenotyping result confirmed the PM status. This is in accordance with data showing that with a low frequency some of the

Table 3
Studies on the *CYP2D6* phenotype–genotype association

Author [Ref.]	Study population	Phenotyping		Genotyping		Concordance (%)
		Probe drug	Number of PMs (%)	Variant alleles determined	Number of PMs	
Heim [25]	22 EMs, 9 PMs	Multiple	–	**3,*4*	9	100
Evans [26]	116 healthy volunteers	DMP	20 (17.2)	**3,*4,*5*	13	93.9
Broly [27]	249 EMs, 86 PMs	SPT, DBQ	–	**3,*4,*5,*9*	78	96.4
Dahl [28]	167 EMs	DBQ	–	**3,*4*	31	99.5
Daly [29]	73 healthy volunteers, 32 PMs	DBQ	2 (2.7)	**3,*4,*5*	2	100
Hirvonen [15]	20 healthy volunteers	DBQ	3 (15)	**3,*4,*5*	3	100
Douglas [30]	11 EMs, 14 PMs	DMP		**3,*4*	13	96.0
Bock [31]	194 healthy volunteers	SPT	15 (7.7)	**3,*4,*5*	9	96.9
Brosen [32]	77 EMs, 91 PMs	SPT	–	**3,*4,*5*	80	93.4
Zimmermann [33]	83 healthy volunteers	DMP	5 (6.0)	**3,*4*	5	100
Sachse [11]	589 volunteers (healthy or with diseases)	DBQ, DMP	49 (8.3)	>10	46	99.5
Marez [1]	42 EMs, 21 PMs	DBQ, DMP, SPT	–	>10	21	95.2
Laforest (this study)	129 hospital controls without cancer	DMP	11 (8.5)	**3,*4,*5,*16, 2XN*	7	93.8

DMP, dextromethorphan; DBQ, debrisoquine; SPT, sparteine; EM, extensive metaboliser; PM, poor metaboliser.

duplicated alleles carry the *4 mutation [10,11]. In all, our present work is in keeping with two recent studies on phenotype–genotype relationships indicating that neither the phenotype nor genotype was completely accurate in the identification of poor metabolisers [2,21].

In conclusion, the present study on Caucasian smokers did not indicate a decreased risk of lung cancer associated with the *CYP2D6* poor metaboliser genotype. Stratification by tobacco smoke exposure or tumour histology did not alter the results. Our present finding is in accordance with those reported in recent meta-analyses. The discrepant results from the phenotype-based and genotype-based studies reported so far remain unexplained as yet. Obviously, one explanation may be that some functionally important variant *CYP2D6* alleles are still to be identified. Alternatively, as was recently suggested for Parkinson's disease [34], it may be possible that *CYP2D6* is in linkage disequilibrium with another locus at chromosome 22q13.

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References

1. Marez D, Legrand M, Sabbagh N, *et al*. Polymorphism of the cytochrome P450*CYP2D6* gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. *Pharmacogenetics* 1997, **7**, 193–202.
2. Griese E-U, Zanger UM, Brudermanns U, *et al*. Assessment of the predictive power of genotypes for the in-vivo catalytic function of *CYP2D6* in a German population. *Pharmacogenetics* 1998, **8**, 15–26.
3. Cholerton S, Boustead C, Taber H, Arpanahi A, Idle JR. *CYP2D6* genotypes in cigarettte smokers and non-tobacco users. *Pharmacogenetics* 1996, **6**, 261–263.
4. Hecht SS. Recent studies on mechanisms of bioactivation and detoxification of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific lung carcinogen. *Crit Rev Toxicol* 1996, **26**, 163–181.
5. Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. *J Pharmacol Exp Ther* 1997, **282**, 1608–1614.
6. Kato S, Bowman ED, Harrington AM, Blomeke B, Shields PG. Human lung carcinogen–DNA adduct levels mediated by genetic polymorphisms *in vivo*. *J Natl Cancer Inst* 1995, **87**, 902–907.
7. Guidice JM, Marez D, Sabbagh N, *et al*. Evidence for CYP2D6 expression in human lung. *Biochem Biophys Res Commun* 1997, **241**, 79–85.
8. Rostami-Hodjegan A, Lennard MS, Woods HF, Tucker GT. Meta-analysis of studies of the CYP2D6 polymorphism in relation to lung cancer and Parkinson's disease. *Pharmacogenetics* 1998, **8**, 227–238.
9. Benhamou S, Bouchardy C, Jacqz-Aigrain E. Inherent difficulties in epidemiological studies involving phenotyping. In Vineis P, Malats N, Lang M, *et al*, eds. *Metabolic Polymorphisms and Susceptibility to Cancer*. Lyon, International Agency for Research on Cancer, 1999.
10. Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Human Genet* 1997, **60**, 284–295.
11. Sachse C, Brockmöller J, Hildebrand M, Müller K, Roots I. Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics* 1998, **8**, 181–185.
12. Johansson I, Lundqvist E, Bertilsson L, Dahl M-L, Sjöqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 *CYP2D6* locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci USA* 1993, **90**, 11825–11829.
13. Ingelman-Sundberg M. Duplication, multiduplication and amplification of genes encoding drug metabolizing genes. Evolutionary, toxicological and clinical pharmaceutical aspects. *Drug Met Rev* 1999, **31**, 449–459.
14. Bouchardy C, Benhamou S, Dayer P. The effect of tobacco on lung cancer risk depends on CYP2D6 activity. *Cancer Res* 1996, **56**, 251–253.
15. Hirvonen A, Husgafvel-Pursiainen K, Anttila S, Karjalainen A, Pelkonen O, Vainio H. PCR-based *CYP2D6* genotyping for Finnish lung cancer patients. *Pharmacogenetics* 1993, **3**, 19–27.
16. Smith CAD, Gough AC, Leigh PN, *et al*. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. *Lancet* 1992, **339**, 1375–1377.
17. *GLIM. The Generalised Linear Interactive Modelling System*, 2nd edn. Oxford, The Nuffield Press, 1987.
18. Breslow NE, Day NE. The analysis of case–control studies. In *Statistical Methods in Cancer Research*, IARC Scientific Publications, Vol. 1, No. 32. Lyon, International Agency for Research on Cancer, 1980, 192–242.
19. Legrand-Andréoletti M, Stücker I, Marez D, *et al*. Cytochrome P450 *CYP2D6* gene polymorphism and lung cancer susceptibility in Caucasians. *Pharmacogenetics* 1998, **8**, 7–14.
20. Shaw GL, Falk RT, Frame JN, *et al*. Genetic polymorphism of *CYP2D6* and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 1998, **7**, 215–219.

21. Leathart JBS, London SJ, Steward A, Adams JD, Idle JR, Daly AK. CYP2D6 phenotype–genotype relationships in African–Americans and Caucasians in Los Angeles. *Pharmacogenetics* 1998, **8**, 529–541.
22. Agúndez JA, Martinez C, Ladero JM, *et al*. Debrisoquin oxidation genotype and susceptibility to lung cancer. *Clin Pharmacol Ther* 1994, **55**, 10–14.
23. Bertz RJ, Granneman GR. Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinetics* 1997, **32**, 210–258.
24. von Moltke LL, Greenblatt DJ, Grassi JM, *et al*. Multiple human cytochromes contribute to biotransformation of dextromethorphan *in vitro*: role of CYP2C9, CYP2C19, CYP2D6 and CYP3A. *J Pharm Pharmacol* 1998, **50**, 997–1004.
25. Heim M, Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* 1990, **1**, 529–532.
26. Evans WE, Relling M. Concordance of P450 2D6 (debrisoquine hydroxylase) phenotype and genotype: inability of dextromethorphan metabolic ratio to discriminate reliably heterozygous and homozygous extensive metabolizers. *Pharmacogenetics* 1991, **1**, 143–148.
27. Broly F, Gaedigk A, Heim M, Eichelbaum M, Morike K, Meyer UA. Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of *CYP2D6* in a European population. *DNA Cell Biol* 1991, **10**, 545–558.
28. Dahl ML, Johansson I, Palmertz MP, Ingelman-Sundberg M, Sjoqvist F. Analysis of the *CYP2D6* gene in relation to debrisoquin and desipramine hydroxylation in a Swedish population. *Clin Pharmacol Ther* 1992, **51**, 12–17.
29. Daly AK, Armstrong M, Monkman SC, Idle ME, Idle JR. Genetic and metabolic criteria for the assignment of debrisoquine 4-hydroxylation (cytochrome P4502D6) phenotypes. *Pharmacogenetics* 1991, **1**, 33–41.
30. Douglas AM, Atchison BA, Somogyi AA, Drummer OH. Interpretation of a simple PCR analysis of the *CYP2D6(A)* and *CYP2D6(B)* null alleles associated with the debrisoquine/sparteine genetic polymorphism. *Pharmacogenetics* 1994, **4**, 154–158.
31. Bock KW, Schrenk D, Forster A, *et al*. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. *Pharmacogenetics* 1994, **4**, 209–218.
32. Brosen K, Nielsen PN, Brusgaard K, Gram LF, Skjodt K. *CYP2D6* genotype determination in the Danish population. *Eur J Clin Pharmacol* 1994, **47**, 221–225.
33. Zimmermann T, Schlenk R, Pfaff G, Lach P, Wildfeuer A. Prediction of phenotype for dextromethorphan O-demethylation by using polymerase chain reaction in healthy volunteers. *Arzneimittelforschung* 1995, **45**, 41–43.
34. Wilhelmsen K, Mirel D, Marder K, *et al*. Is there a genetic susceptibility locus for Parkinson's disease on chromosome 22q13? *Ann Neurol* 1997, **41**, 813–817.