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CYP2D6 GENETIC POLYMORPHISMS ARE ASSOCIATED WITH SUSCEPTIBILITY TO PITUITARY TUMORS

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SUMMARY

Several polymorphisms of drug-metabolizing enzymes have been implicated in the susceptibility to tumor development. The role of the *CYP2D6*, *GSTM1* and *GSTT1* genes has been extensively studied, with alleles conferring different metabolic efficiencies and tumor risk. We studied the relationship between the main polymorphisms of these genes and the susceptibility to develop pituitary tumors, by performing a case-control study comprising 235 patients and 256 controls which were genotyped by means of PCR-RFLP based assays. Frequencies of the *CYP2D6*1* and of the poor metabolizer allele *CYP2D6*4*, were determined along with the frequencies of the *GSTM1* and *GSTT1* null genotypes. *CYP2D6* genotype frequencies were similar in patients and controls (p=0.087). *CYP2D6*1* and *CYP2D6*4* allele frequencies were 83.8%, 16.2% in cases and 78.3%, 21.7% in controls, showing a significant difference between the two groups (p=0.012). There were no significant differences between the frequencies of the *GSTM1* and *GSTT1* null genotypes in both groups. No association was found between histological type and any of the studied polymorphisms. Our data suggest an association of the *CYP2D6*1* allele and the susceptibility to pituitary adenomas, which could be due to an increased metabolism of unidentified procarcinogens or to linkage disequilibrium with another gene involved in pituitary tumorigenesis.

Keywords: pituitary tumors and CYP2D6, GSTM1 *and* GSTT1 *genetic polymorphisms*

RESUMO

POLIMORFISMOS DO GENE *CYP2D6* ESTÃO ASSOCIADOS A UMA SUSCEPTIBILIDADE PARA OS TUMORES DA HIPÓFISE

Enzimas envolvidas no metabolismo de genotóxicos têm sido associadas a uma susceptibilidade para alguns tumores. Existe um marcado polimorfismo de genes codificadores destas enzimas a que corresponde diferentes capacidades metabolizadoras que podem conferir diferentes graus de susceptibilidade para esses tumores. Neste estudo pretendeu-se avaliar o papel dos polimorfismos dos genes *CYP2D6*, *GSTM1* e *GSTT1* na susceptibilidade individual para o desenvolvimento de tumores da hipófise tendo sido estudados 235 doentes com diferentes macroadenomas hipofisários e 256 controlos. Foram determinadas as frequências *CYP2D6*1*, *CYP2D6*4*, bem como a dos genótipos nulos *GSTM1* e *GSTT1*. As frequências genotípicas do gene *CYP2D6* foram idênticas nos doentes e controlos (p=0,087). As frequências alélicas *CYP2D6*1*

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e *CYP2D6*4* foram 83,8%, 16,2% nos doentes e 78,3%, 21,7% nos controlos, evidenciando uma diferença significativa entre os dois grupos (p=0,012). As frequências dos genótipos nulos *GSTM1* e *GSTT1* foram idênticas nos doentes e controlos. Não se observou qualquer associação entre os polimorfismos estudados e o tipo histológico do tumor. Estes resultados sugerem uma associação entre o genótipo *CYP2D6*1* e susceptibilidade para os tumores da hipófise cujo mecanismo se poderá dever quer a um aumento da metabolização de um procarcinogénio desconhecido ou a um desequilíbrio de ligação com outro gene envolvido no processo de génese tumoral.

Palavras-chave: tumores da hipófise e polimorfismos dos genes CYP2D6, GSTM1 *e* GSTT1

INTRODUCTION

Individual susceptibility for the development of tumors depends on a complex interaction between genetic and environmental factors[1,2]. Susceptibility to chemical carcinogens plays an important role in the development of many neoplasias. Several polymorphisms of human drug-metabolizing enzymes influence this individual susceptibility. Among the most studied polymorphic genes that have been associated with several tumors are the *CYP2D6, GSTM1* and *GSTT1* genes[3,4]. The cytochrome P450 family (including the CYP2D6) and the glutathione-S-transferases μ1 and θ1 (GSTM1 and GSTT1) are important enzymes of phase I and phase II, respectively, of detoxification of many potential carcinogenic and mutagenic products[5,6]. The genes that encode these enzymes are highly polymorphic with alleles conferring different efficiencies and possibly tumor risk. The most frequent inactivating mutation of the *CYP2D6* gene is the splice site c.506,-1g>a transition (*CYP2D6*4*). Poor metabolizer (*CYP2D6*4/ CYP2D6*4*), in which both alleles have inactivating mutations, and extensive metabolizer (*CYP2D6*1/CYP2D6*1 or CYP2D6*1/ CYP2D6*4*) genotypes have been associated with different risks of certain pathologies including lung cancer and others[7,8]. The *GSTM1* and *GSTT1* genes are also polymorphic and they have a null allele variant in which the entire gene is absent. The null genotype for both enzymes (*GSTM1*0/GSTM1*0* and *GSTT1*0/GSTT1*0*) has been associated with many different types of tumors (7-9). The role of these genes in the development of pituitary tumors is unknown with only two small studies described in the literature (10,11). In order to achieve a better insight into the relationship between the main genetic polymorphisms of *CYP2D6, GSTM1* and *GSTT1* genes and the individual susceptibility to develop pituitary tumors, we performed a case-control study and established the frequencies of the *CYP2D6, GSTM1* and *GSTT1* most common genotypes in patients with different pituitary adenomas.

POPULATION AND METHODS

Studied populations and tumor classification

A retrospective case-control study was performed involving a total of 491 individuals. We identified 235 cases, (84 males, 151 females, mean age±SD 50.1±15.3 years) comprising adenomas of various histologies (65 null cell adenomas, 64 somatotrophinomas, 58 macroprolactinomas, 27 corticotrophinomas, 12 gonadotrophinomas, 2 thyrotrophinomas and 2 pluri-hormonal adenomas. In 5 it was not possible to perform the immunohistochemical study, so they were classified as clinically nonfunctioning adenomas. The diagnosis of these tumors was initially based on clinical, radiological and standard biochemical and hormonal criteria and subsequently confirmed by histological and immunocytochemical examination except for most of the macroprolactinomas that were not surgically treated. The control group was composed of 256 healthy volunteers (150 males and 106 females, mean age±SD 36.8±13.7 years) recruited from Hospital and Faculty staff and blood donors. Age differences between controls and patients were not considered relevant as previous studies have shown no correlation between age and genotype[12,13]. All individuals were Caucasians. All gave informed consent and the study was approved by the Hospital ethics committee.

Genotype analysis

DNA was isolated from peripheral blood samples by standard methods. Detection of the c.506,-1g>a transition, corresponding to the recessive poor metabolizer allele *CYP2D6*4*, was carried out by PCR amplification of genomic DNA, followed by restriction enzyme digestion with *Bst*N1 using previously published procedures[14,15]. This allowed classification of individuals into extensive (*CYP2D6*1/CYP2D6*1*), heterozygous extensive (*CYP2D6*1/CYP2D6*4*) and poor (*CYP2D6*4/ CYP2D6*4*) metabolizers. *GSTM1* and *GSTT1* null geno-

types were detected by PCR amplification that also included fragments of the *GSTM4* and beta-globin genes, respectively, as internal controls, according to previous published methods[14,16,17]. This method does not distinguish heterozygous null genotypes from wild-type homozygous.

Statistical analysis

Chi-squared tests were used to examine differences of allele and genotype frequencies between patients and controls. Fisher's exact test was used when appropriate. Statistical significance was set at a p value <0.05 and adjusted for the number of comparisons by use of the Bonferroni correction. Analysis was also performed according to tumor histological type.

RESULTS

Allele and genotype observed frequencies of the *CYP2D6, GSTM1 and GSTT1* genetic polymorphisms are presented in tables I and II. The frequencies of the *CYP2D6*1/*1*, *CYP2D6*1/*4*, *CYP2D6*4/*4* genotypes were similar in patients and in controls ($\chi^2_{2d.f.}$=4.88, p=0.087). *CYP2D6*1* and *CYP2D6*4* allele frequencies were 83.8%, 16.2% in cases and 78.3%, 21.7% in controls, showing a significant difference between the two groups ($\chi^2_{1d.f.}$=6.28, p=0.012). No differences were detected when analyzed according to type of adenoma (table I).

Table I - CYP2D6 *genotype and allele frequencies*

		***CYP2D6* genotypes (%)**			**Alleles (%)**	
	n	****1/*1***	****1/*4***	****4/*4***	****1***	****4***
Control group	256	159 (62.1)	83 (32.4)	14 (5.5)	401 (78.3)	111 (21.7)
All adenomas	235	165 (71.4)	64 (27.2)	6 (2.6)[a]	394 (83.8)	76 (16.2)[b]
Null cell adenomas	65	47 (72.3)	17 (26.2)	1 (1.5)	111 (85.4)	19 (14.6)
Somatotrophinomas	64	45 (70.3)	19 (29.7)	0	109 (85.2)	19 (14.8)
Macroprolactinomas	58	37 (63.8)	18 (31. 0)	3 (5.2)	92 (79.3)	24 (20.7)
Corticotrophinomas	27	19 (70.4)	6 (22.2)	2 (7.4)	44 (81.5)	10 (18.5)
Gonadotrophinomas	12	10 (83.3)	2 (16.7)	0	22 (91.7)	2 (8.3)
Thyrotrophinomas	2	2	0	0	4	0
Pluri-hormonal adenomas	2	2	0	0	4	0
Clinically non functioning adenomas	5	3	2	0	8	2

*CYP2D6*1*, active metabolizer; *CYP2D6*4*, poor metabolizer
(a) *vs.* control group, $\chi^2$1d.f.=2.98, p=0.084, (*CYP2D6*4/*4* vs all others), $\chi^2$2d.f.=4.88, p=0.087, (all genotypes);
(b) *vs.* control group, $\chi^2$1d.f.=6.28, p=0.012

Frequencies of the *GSTM1*0/*0* and of the *GSTT1*0/*0* null genotypes were similar in cases and in controls. In the different types of adenomas these frequencies were also similar to the control group (table II).

Table II - GSTM1 *and* GSTT1 *genotype frequencies*

		***GSTM1* genotypes (%)**		***GSTT1* genotypes (%)[a]**	
	n	**(+)**	**(0)**	**(+)**	**(0)**
Control group	256	110 (43.0)	146 (57.0)	209 (81.6)	47 (18.4)
All adenomas	235[a]	108 (46.0)	127 (54.0)[b]	193 (82.5)	41 (17.5)[c]
Null cell adenomas	65	33 (50.8)	32 (49.2)	53 (81.5)	12 (18.5)
Somatotrophinomas	64	28 (43.8)	36 (56.2)	53 (82.8)	11 (17.2)
Macroprolactinomas	58	27 (46.6)	31 (53.4)	47 (82.5)	10 (17.5)
Corticotrophinomas	27	10 (37.0)	17 (63.0)	23 (85.2)	4 (14.8)
Gonadotrophinomas	12	9 (75.0)	3 (25.0)	10 (83.3)	2 (16.7)
Thyrotrophinomas	2	0	2	2	0
Pluri-hormonal adenomas	2	1	1	2	0
Clinically nonfunctioning adenomas	5	0	5	3	2

(+) Gene present (homozygous or heterozygous); (0) Homozygous deletion (*GSTM1*0/*0* or *GSTT1*0/*0*)
(a) In one case (macroprolactinoma) *GSTT1* genotype was not possible to study
(b) *vs.* control group, $\chi^2$1d.f.=0.33, p=0.57; (c) *vs.* control group, $\chi^2$1d.f.=0.058, p=0.81

DISCUSSION

In epidemiological studies, genetic polymorphisms of genotoxic metabolizing enzymes have been correlated with individual risk for the development of some tumors[1,2]. This may result from different activities of the polymorphic enzymes regarding activation or inactivation of different carcinogens. Many tumors have been associated with these polymorphisms, such as those from the lung, head and neck, digestive system, breast, bladder, prostate, skin, thyroid and hematological neoplasias[8,14,18-26]. However, studies often present conflicting results which also seem to vary according to ethnical/geographical context. In this study, genotype frequencies of the *CYP2D6, GSTM1* and *GSTT1* genes were described in 235 patients with different pituitary adenomas to determine if any particular genotype was associated with a greater susceptibility for these tumors or for specific histological types. The active metabolizer allele (*CYP2D6*1*) was found at a higher frequency in the group of patients. In a previous study, with fewer patients, this frequency was also higher although not significant[11]. While the functional significance of this finding is unclear, this has also been reported for tumors at other sites. Proposed mechanisms include increased susceptibility due to metabolic activation of unidentified

chemical carcinogens or linkage to another putative tumour-causing gene[25,26]. No significant association was found between *GSTM1* and *GSTT1* polymorphisms and tumour risk. When the adenomas were divided according to histological type there was no significant difference between the different sub-groups and the control group for the three genes studied. An increased frequency of the *GSTM1* null genotype has been previously reported in prolactinomas but the sample studied was small (10). In summary, these data suggest that *CYP2D6* gene polymorphisms are associated with susceptibility for the development of pituitary tumors and that the polymorphisms of the *GSTM1* and *GSTT1* genes do not seem to be associated with a greater risk for these tumors. However, further studies are needed to analyze the effect according to histological type and to determine if the association also occurs in other populations with different ethnic/geographical backgrounds. This may allow the identification of risk factors and contribute towards understanding the molecular mechanisms involved in the process of pituitary tumorigenesis.

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