Founder Effect for the Highly Prevalent R337H Mutation of Tumor Suppressor p53 in Brazilian Patients with Adrenocortical Tumors

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Emilia M. Pinto
Ana Elisa C. Billerbeck
Maria Candida B.F. Villares
Sorahia Domenice
Berenice B. Mendonça
Ana Claudia Latronico

Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular LIM/42, Disciplina de Endocrinologia e Metabologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil.

ABSTRACT

The incidence of adrenocortical tumors in children from the Southern region of Brazil is higher than in other parts of the world. This fact has been related to the identification of an inherited missense mutation of the p53 (R337H) at high frequency (78-97%) in Brazilian children with adrenocortical tumors. Given the high frequency of this germline mutation in the Brazilian population, it is very likely that the R337H mutation has arisen from a common origin. In this study, we analyzed two highly polymorphic intragenic markers (VNTRp53 and p53CA) in 22 patients (16 children and 6 adults) with adrenocortical tumors carrying the germline R337H mutation and 60 normal individuals using GeneScan Fragment Analysis software. We found six and sixteen different alleles for the VNTRp53 and p53CA polymorphic markers, respectively. Two distinct alleles, both with 122 bp, were found in 56.8% (VNTRp53) and 54.5% (p53CA) of the 44 alleles from patients with adrenocortical tumors associated with the R337H mutation. Differently, these same VNTRp53 and p53CA alleles were found in 18.3% and 14.2% of 120 alleles from normal individuals, respectively ($p<0.01$, Chi-square test). An identical haplotype for p53 *locus* was also identified in 95% of the apparently unrelated Brazilian patients with adrenocortical tumors carrying the R337H mutation. In conclusion, we demonstrated a strong evidence of co-segregation between two intragenic polymorphic p53 markers and the germline R337H mutation, indicating that this mutation has originated from a single common ancestral in the great majority of the Brazilian patients with adrenocortical tumors. **(Arq Bras Endocrinol Metab 2004;48/5:647-650)**

Keywords: Adrenal tumors; p53; Polymorphic markers; Founder effect.

RESUMO

Efeito Fundador da Altamente Prevalente Mutação R337H do Gene Supressor de Tumores p53 em Pacientes Brasileiros com Tumores Adrenocorticais.

A incidência dos tumores adrenocorticais em crianças das regiões sul e sudeste do Brasil é maior que em outras partes do mundo. Este fato tem sido atribuído a identificação com alta frequência (78-97%) da mutação R337H no p53 em crianças brasileiras com tumores adrenocorticais. Considerando a elevada freqüência desta mutação germinativa na população brasileira, é provável que a mutação R337H tenha uma origem comum. Neste estudo, analisamos 2 marcadores polimórficos intragênicos (VNTRp53 e p53CA) em 22 pacientes (16 crianças e 6 adultos) com tumores adrenocorticais portadores da mutação germinativa R337H e em 60 indivíduos normais, através do programa *GeneScan* de análise de fragmentos. Seis e 16 alelos dos marcadores polimórficos VNTRp53 e p53CA foram respectivamente identificados. Dois alelos, ambos com 122 bp, foram identificados em 56,8% (VNTRp53) e 54,5% (p53CA) dos 44 alelos dos pacientes com tumores adrenocorticais. Em contraste, estes mesmos marcadores foram encontrados respectivamente em 18,3% e 14,2% dos 120 alelos

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dos indivíduos normais (p< 0,01, teste do chi-quadrado). Identificamos também, um haplótipo idêntico para o *locus* p53 em 95% dos pacientes com tumores adrenocorticais com a mutação R337H. Em conclusão, demonstramos uma forte evidência de co-segregação entre dois marcadores polimórficos intragênicos do p53 e a mutação germinativa R337H, indicando que esta mutação teve origem num ancestral comum na maioria dos pacientes brasileiros com tumores adrenocorticais. **(Arq Bras Endocrinol Metab 2004;48/5:647-650)**

Descritores: Tumores adrenais; p53; Marcadores polimórficos; Efeito fundador.

ADRENOCORTICAL TUMORS IN CHILDREN and adolescents are rare events. It is estimated that approximately 25 cases of pediatric adrenocortical tumors occur in the United States per year (1, 2). However, the high incidence of adrenocortical tumors in children from Southern region of Brazil is particularly remarkable, since it has been estimated to be approximately 10-15 times greater than the worldwide incidence (3). The identification of a unique p53 germline mutation, R337H, at a very high frequency (97-78%) of Brazilian children with adrenocortical tumors suggests that genetic predisposition plays an important role in the etiology of these tumors in the Brazilian population (4, 5).

Interestingly, the R337H mutation was also identified in the DNA of asymptomatic first degree relatives of patients with adrenocortical tumors and R337H, suggesting low penetrance of this p53 mutation for adrenocortical tumor development (4, 5). Adrenocortical tumors of patients with R337H mutation frequently display deletion of the entire chromosome 17 (3). In addition, *in vitro* analysis revealed that the p53 tetramerization domain containing the R337H mutation was less stable than the wild type domain (6). The stability of this mutant was highly sensitive to pH in the physiological range, suggesting that this missense mutation caused a pH-dependent p53 dysfunction (6).

Given the high frequency of the inherited R337H mutation in the Brazilians with adrenocortical tumors, it is most likely that this p53 mutation has originated from a single founder. To address this hypothesis, we compared the allelic distribution of two highly informative polymorphic markers into the *p53* gene between apparently unrelated Brazilian patients with adrenocortical tumors carrying the R337H mutation and normal individuals in an attempt to clarify the origin of the p53 mutation

Patients and Methods

The study was approved by the Ethics Committee of Hospital das Clínicas, São Paulo, Brazil, and informed written consent was obtained from all patients. We selected 22 patients (16 children and 6 adults) who had adrenocortical tumors associated with the germline R337H mutation. The median age was 1.2 years, ranging from 0.6 to 54 years. All patients were Caucasians. Sixty normal unrelated Brazilian individuals (30 children and 30 adults) were studied as controls.

DNA Analysis

Genomic DNA was extracted from peripheral blood leukocytes of all patients and controls according to standard protocols. Two highly informative polymorphic markers, VNTRp53 and p53CA, were amplified by polymerase chain reaction. The VNTRp53 is a pentanucleotide repeat $(AAAAT)_n$ within intron 1 (7), whereas P53(CA)n is a dinucleotide repeat polymorphism of the human *p53* gene (8). The oligonucleotide primers were labeled with fluorescent dye (TET™ or FAM™). Genomic DNA was amplified in a 50 µl reaction mixture of 1x PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 25-10 pmol of each primer and 0.5-0.8U *Taq Polymerase* (Amershan Pharmacia). The VNTRp53 PCR reaction mixture was denatured for 5 min at 93°C, and cycled 35 times (93°C for 1 min, 62°C for 1 min and 72°C for 1 min), followed by a 30 min extension at 72°C. The p53CA PCR reaction mixture was denatured for 3 min at 94°C, and cycled 35 times (94°C for 1 min, 60°C for 2 min and 72°C for 5 min), followed by a 30 min extension at 72°C. Internal size standard TAMRA 350 (Applied Biosystems, Foster City, CA) was added to 2 µL of PCR products and 24 µL of formamide and those were submitted to capillary electrophoresis in an Automatic Sequencer (ABI Prism 310 Genetic Analyzer) followed by GeneScan Fragment Analysis (Applied Biosystem, Foster City, CA).

Statistical Analysis

Differences in genotype frequencies between patients with adrenocortical tumors carrying the germline R337H mutation and normal controls for each polymorphic site were tested with Chi-square statistics.

Results

Six different alleles were identified for the VNTRp53 marker within the first intron of the *p53* gene, ranging from 112 to 137 bp (Table 1). The alleles from 117 to 137 bp were previously described (7), whereas the allele corresponding to 112 bp has not been previously identified in other populations. The 122 bp allele

Table 1. Allele frequencies of the VNTRp53 and p53CA polymorphic markers in the human p53 gene of 22 Brazilian patients with adrenocortical tumors carrying the R337H mutation and 60 normal controls.

Alleles (bp)	**R337H mutation (44 alleles)**		**Normal Controls (120 alleles)**	
VNTRp53	Number	Percent (%)	Number	Percent (%)
112	0	0	1	0.8
117	0	0	7	5.8
122	**25**	**56.8***	**22**	**18.3***
127	11	25	60	50
132	7	15.9	28	23.3
137	1	2.3	2	1.7
P53CA				
102	0	0	5	4.2
104	0	0	3	2.5
106	0	0	0	0
108	0	0	3	2.5
110	0	0	2	1.7
112	1	2.3	5	4.2
114	1	2.3	8	6.7
116	2	4.5	11	9.2
118	2	4.5	14	11.7
120	5	11.4	16	13.3
122	**24**	**54.5***	**17**	**14.2***
124	3	6.8	15	12.5
126	1	2.3	4	3.3
128	4	9	9	7.5
130	1	2.3	8	6.7
132	0	0	2	1.7
134	0	0	2	1.7

* p< 0.01

containing 7 unit repeats was identified in 25 of the 44 alleles (56.8%) from patients with adrenocortical tumors associated with the germline R337H mutation. Differently, this same allele was found in 22 of 120 alleles (18.3%) from the normal controls.

Sixteen different alleles were identified for the p53CA polymorphic marker, ranging from 102 to 134 bp (Table 1). The 122 bp allele was found in 24 of the 44 alleles (54.5%) from patients with adrenocortical tumors associated with the R337H mutation. Differently, this same allele was found in 17 of 120 alleles (14.2%) from the normal controls. The allele distribution was significantly different ($p<0.01$, Chi-square test) between patients with adrenocortical tumors and R337H mutation and normal controls. Segregation studies showed that an identical haplotype for the p53 *locus* was identified in 95% of the apparently unrelated Brazilian patients with adrenocortical tumors carrying the R337H mutation. In addition, this same haplotype was always present in tumor DNA, usually affected by LOH.

Discussion

We demonstrated a strong evidence of co-segregation between two distinct alleles within the p53 gene and the germline R337H mutation in Brazilian patients with adrenocortical tumors. The allelic frequencies were significantly different between these patients who harbored the R337H mutation and normal individuals, suggesting that the R337H mutation originated from a common ancestral in the Brazilian population.

The clear evidence of a founder effect for the R337H mutation contrasts with a previous study performed by Ribeiro *et al.* (4), who analyzed four different polymorphic markers along the short arm of chromosome 17, including the same two intragenic *p53* markers studied here and two other flanking markers (D17S1832 and D17S786) in 17 patients carrying the R337H mutation. They demonstrated that at least some mutant alleles arose independently, thus eliminating the presence of a founder effect (4). However, the small number of unrelated patients studied, the physical distance of the two flanking markers in relation to *p53 locus* (8-12 centirays) and the absence of a control group, did not rule out the presence of a founder effect in this study. Additionally, an identical allele (represented by number 3) for the intragenic marker VNTRp53 was identified in 16 of 17 patients studied (4). However, these data were not accounted for in this study.

The majority of the population in the southern region of Brazil consists mainly of European extraction, such as Portuguese, Italian, Spanish and German, with native Brazilian Indian and African influence (9). The high incidence of adrenocortical tumors in patients from this region is therefore explained by the presence of the germline R337H mutation, which was inherited from a common founder several decades ago. This fact is suggested by identification of the R337H mutation in asymptomatic relatives of patients with adrenocortical tumors as old as 60 years old. Nevertheless, R337H has never been described as a common mutation of the p53 in other populations.

We have previously demonstrated that the second event, crucial for adrenocortical tumor development, is characterized by the loss of chromosome 17 harboring the normal p53 allele in most of affected patients (3). However, the etiologic factor for the occurrence of this second event, especially during childhood, remains unknown. Interestingly, the peak age of occurrence of adrenocortical tumors in Brazilian children is under of 4 years of age, suggesting that the second event also occurs very early in life or even during fetal life. Alterations of the pH in adrenocortical tissue, such as those observed during adrenal fetal apoptosis, have been speculated as participating in this

still unknown process (6).

In conclusion, an identical haplotype for *p53 locus* was identified in 95% of the apparently unrelated Brazilian patients with adrenocortical tumors carrying the R337H mutation. The allelic frequencies were significantly different between affected patients carrying the R337H mutation and the normal population, indicating that this mutation originated from a single common ancestral in most of the Brazilian patients with adrenocortical tumors.

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Address for correspondence:

Ana Claudia Latronico
Laboratório de Hormônios e Genética Molecular
LIM/42 - Hospital das Clínicas, Faculdade de Medicina - USP
Av. Dr. Enéas Carvalho de Aguiar, 155, 2 andar, Bloco 6, 05403-900 São Paulo, SP - Brasil
FAX: (5511) 3083-0626
e-mail: anacl@usp.br