Hum Genet (2001) 109:95–98
DOI 10.1007/s004390100553

ORIGINAL INVESTIGATION

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A polymorphism in the promoter region of catalase is associated with blood pressure levels

Received: 15 February 2001 / Accepted: 22 May 2001 / Published online: 4 July 2001

Abstract Catalase is an important antioxidant enzyme that detoxifies H_2O_2 into oxygen and water and thus limits the deleterious effects of reactive oxygen species (ROS). Because chronic exposure to excess ROS may contribute to vascular damage, we investigated whether genetic variation in catalase was associated with susceptibility to essential hypertension (EHYT) in 324 individuals (at least 50 years old) who were randomly sampled from an isolated population living in Xiangchang, China. They were screened for genetic variation in the promoter of catalase by direct sequencing. In total, four single nucleotide polymorphisms (SNPs) were identified. The association between the SNPs and EHYT was investigated by a linear regression model under phenotypic selection; in our analyses, we used both SBP>150 mmHg and SBP>160 mmHg as thresholds. A SNP 844 bp upstream of the start codon (SNP-844) demonstrated strong evidence of association with EHYT (SBP>150 mmHg: F=5.09, P=0.008; SBP>160 mmHg: F=7.13, P=0.002). This is the first study to implicate genetic variation in catalase in susceptibility to EHYT and suggests that polymorphisms in promoter regions may be particularly relevant to the study of complex diseases.

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Introduction

Catalase is an important antioxidant enzyme whose physiological role is to detoxify H_2O_2 into oxygen and water and thus limit the deleterious effects of reactive oxygen species (ROS; Diesserdoth and Dounce 1970). It is widely recognized that an excess of ROS contributes to the aging process and degenerative diseases (Mates et al. 1999; Griendling and Alexander 1997; Rosenfeld 1998). Because of its physiological role, catalase is a very attractive blood pressure candidate gene. Recently, two observations have provided indirect evidence intimating at a possible role of catalase in essential hypertension (EHYT): (1) hypertensive individuals have statistically significant higher plasma hydrogen peroxide levels compared with normotensive subjects (Lacy et al. 1998), and (2) hypertensive type 2 diabetes mellitus patients have increased catalase activity compared with controls (Sözmen et al. 1999). The purpose of this study has been directly to investigate whether genetic variation in catalase contributes to susceptibility to EHYT in an isolated Chinese population.

Materials and methods

Study population

We randomly sampled 324 individuals who were at least 50 years old from an isolated population living in Xiangchang, Yuexi County, Anhui Province, China. Although it is unclear how long ago this population was founded, the number of founders is expected to be small, because 80% of sampled individuals carry only six different surnames. Furthermore, a study of four Y chromosome microsatellite markers has indicated that individuals with the same surname are generally descendants of the same individual who lived in Xiangchang at most 10–20 generations ago (data not shown). All subjects in this study are ethnically Han Chinese, and no individual of minority origin was sampled. Family histories of the subjects were pursued to ensure that the study subjects were not closely related. Informed consent from each subject was obtained regarding participation in this study.

Phenotyping

Each subject was visited at their home by a physician, and both systolic and diastolic blood pressure (SBP and DBP, respectively) was measured twice at least 20 min apart by a random zero sphygmomanometer. The average of the two readings was used for the analyses reported here. Individuals who were taking or had taken anti-hypertensive medication were not included in this study.

SNP identification and genotyping

DNA was extracted from isolated buffy coat by using a standard phenol/chloroform protocol. Individuals in the top 11% (37 individuals; SBP>178 mmHg) and the bottom 7% (22 individuals; SBP <104 mmHg) of the SBP distribution were selected for SNP identification. This group of 59 individuals will be referred to as the screening cohort.

The promoter region of the human catalase gene is not well defined. Studies of the rat catalase promoter suggest that sequences up to 3 kb upstream of the start codon may affect gene expression (Van Remmen et al. 1998*)*. Therefore, in the absence of similar experimental data in humans, we chose to screen 1677 bp of the putative promoter region and 5' UTR (herein denoted as –1677 bp upstream of the start codon, where +1 denotes the A in the translation start codon ATG) for genetic variation. The entire promoter sequence was amplified in one single polymerase chain reaction (PCR) in either a PE-9600 or PE-9700 (Perkin-Elmer, Fostor City, Calif.) and was used as a template for subsequent sequencing. In total, five primer pairs were used for sequencing (sequences available upon request). Following PCR, products were purified by the Wizard Purification kit (Promega, Madison, Wis.), and the sequence of each individual was obtained by three overlapping sequencing reactions with BigDye and Dye Terminator chemistry (Perkin-Elmer). Sequencing reactions were run on an ABI 373A or ABI 377 semi-automatic sequencer and were scored by using Sequence Analysis and Sequence Navigator software (Perkin-Elmer). Identified SNPs were genotyped by direct sequencing.

Statistical analysis

Allele frequencies were estimated by gene counting, and significant departures from the Hardy-Weinberg equilibrium (HWE) were tested by either a chi-square test or a permutation method (Weir 1996) where appropriate. Initially, we tested for preliminary association of the identified SNPs with blood pressure levels by comparing the allele frequencies between individuals in the top 11% and bottom 7% of the blood pressure distribution (i.e., in the screening cohort) by simple χ^2 statistics. Subsequently, SNPs demonstrating nominal evidence for association were then genotyped in the remaining sample, and association of SNPs with SBP and DBP was investigated by a regression method under phenotypic selection. Specifically, under phenotypic selection, associations were tested in individuals whose SBP was above a defined threshold. In our analyses, we used both SBP>150 mmHg (n=100 individuals) and SBP>160 mmHg (n=73 individuals) as thresholds. Truncation selection increases both the frequency of the predisposing allele and its observed genetic effects and, hence, increases the power to detect the trait locus.

We briefly describe here the simple regression method for localizing a quantitative trait locus (Boerwinkle et al. 1986). Let y_i be the trait value of the i-th individual in the sample ($i = 1,...,n$), so that

$$y_i=\mu+w_i\gamma+x_i\alpha+z_i\delta+e_i$$

where, μ is the overall population mean, w_i is a k dimensional vector of covariate observations, γ is the regression coefficient associated with w_i, and e_i is a normally distributed random variable with $E[e_i]=0$ and $Var(e_i)=\sigma_e^2$. Dummy variables, x_i and z_i, are defined as,

$$x_i=\begin{cases} 2P_a & M_i = AA \\ P_a - P_A & M_i = Aa \\ -2P_A & M_i = aa \end{cases}$$

$$z_i=\begin{cases} P_a^2 & M_i = AA \\ P_A P_a & M_i = Aa \\ -P_A^2 & M_i = aa \end{cases}$$

where P_A and P_a are the frequencies of alleles A and a at the marker locus, respectively, and α and δ are the regression coefficients associated with x_i and z_i (or the estimated additive and dominance effects), respectively.

Let

$$SS_{ful}=\sum_{i=1}^{n}(y_i-\mu-w_i\gamma-x_i\alpha-z_i\delta)^2$$

$$SS_{red}=\sum_{i=1}^{n}(y_i-\mu-w_i\gamma)^2, \text{ and}$$

$$MSE=\frac{1}{n-k-3}\sum_{i=1}^{n}(y_i-\mu-w_i\gamma-x_i\alpha-z_i\delta)^2$$

The statistic for testing H_0: $\alpha=0$ and $\delta=0$ versus H_1: $\alpha \neq 0$ *or* $\delta \neq 0$, *or* $\alpha \neq 0$ *and* $\delta \neq 0$ for the presence of a trait locus is defined as

$$F_q=\frac{(SS_{red}-SS_{ful})/2}{MSE}$$

By standard statistical theory, F_q follows an F distribution with *2* and *n-k-3* degrees of freedom under the null hypothesis.

In silico promotor analysis

SNPs identified in the promoter region of catalase were interrogated for the presence of regulatory and transcription factor binding sites by Matinspector software v2.2 (Quandt et al. 1995).

Results

Study population

Among the 324 unrelated older individuals sampled, 141 (55%) were males, and 115 (45%) were females. The average age of the subjects was 62.2 years old. The average (±SD) SBP for individuals in this sample was 144± 30 mmHg, and the average (±SD) DBP was 85± 15 mmHg. No significant difference in blood pressure levels (for both SBP and DBP) were observed between genders. The correlation between SBP and DBP was 0.85; 53.5% of the individuals were hypertensive, which was significantly higher compared with that in neighboring areas (28%; Xu et al. 1996) or the national average of rural

Table 1 Characteristics of the identified SNPs

SNP name[a]	SNP type	Frequency	Location
SNP-844	C/T	0.684/0.316	Promoter
SNP-330	G/A	0.931/0.069	Promoter
SNP-89	T/A	0.694/0.306	Promoter
SNP-20	A/G	0.690/0.310	5' UTR

[a]Numbering corresponds to number of basepairs upstream of the translation start site

Table 2 Results of association analyses with SNP-844 (*numbers in parentheses* number of individuals contributing to each genotypic mean)

Threshold[a]	Regression[b]		Genotypic mean[c] (mmHg)		
	F	*P*-value	CC	CT	TT
SBP>150 mmHg	5.09	0.008	164.0/89.6 (47)	170.3/87.5 (43)	176.6/88.0 (10)
SBP>160 mmHg	7.13	0.002	169.7/89.6 (32)	174.9/87.5 (35)	193.3/88.0 (6)

[a]Phenotypic threshold used to select individuals for inclusion in the regression analysis
[b]Regression model as described in Methods section
[c]Genotypic mean reported as SBP/DBP

populations in China (28.1%; Tao et al. 1995) for the same age group.

Identified SNPs

In total, four SNPs were identified in the catalase promoter and 5' UTR. Table 1 presents the name, type, allele frequencies, and location for these SNPs. All SNPs were in HWE.

Association of SNPs with EHYT

All identified SNPs were initially tested for association in the screening cohort as described above. Of the four SNPs, only SNP-844 showed evidence of association (P=0.02) and therefore was genotyped in the remaining 265 individuals. Table 2 shows that SNP-844 is strongly associated with EHYT for both of the phenotypic thresholds considered (i.e., SBP>150 mmHg, P=0.008 and SBP>160 mmHg, P=0.002). The genotypic means for SNP-844 are also shown in Table 2, and individuals homozygous for the SNP-844 T allele have considerably higher SBP. Interestingly, SNP-844 is only associated with SBP and not DBP (P=0.78).

Predicted regulatory elements

In silico analysis predicts SNP-844 to lie within several interesting transcription factor recognition sequences (Fig. 1). The SNP-844 C allele contains putative MZF1 and AP2 binding sites. MZF1 is thought to regulate the expression of genes that play a critical role in hematopoiesis (Perrotti et al. 1995), and AP2 regulates epidermal and neural-specific gene expression (Mitchell et al. 1991). The SNP-844 T allele contains putative Ikaros-2 and LYF-1 binding sites. Ikaros-2 is a master regulator during lymphocyte development (Molnar and Georgopoulos 1994), and LYF-1 may be a general activator of genes whose expression is restricted to the B- and/or T-lymphocyte lineages (Lo et al. 1991).

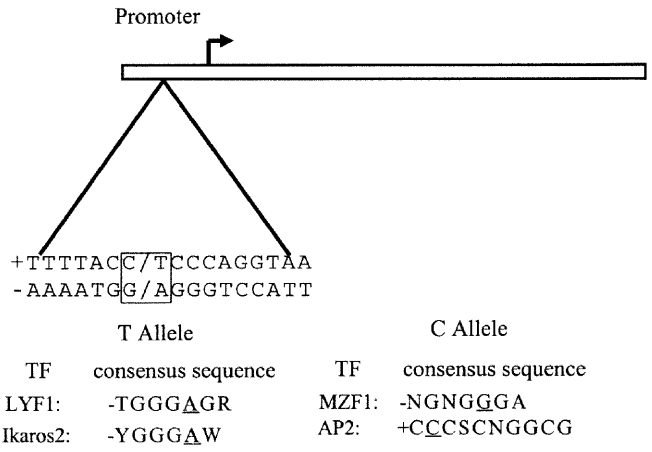

Fig. 1 Putative transcription factor (*TF*) binding sites in which SNP-844 lies. The *arrow* corresponds to the translation start site, and the C/T SNP is *boxed*. DNA strands are denoted as + and –. The C allele is predicted to bind the transcription factors MZF1 (– strand) and AP2 (+ strand), whereas the T allele is predicted to bind Ikaros2 (– strand) and LYF1 (– strand). The IUPAC string consensus binding sequences of each of these TFs are listed (see Quandt et al. 1995), and the position of the SNP allele is *underlined*

Discussion

In this report, we have shown that variation in the promoter region of the catalase gene is significantly associated with EHYT in an isolated Chinese population. Catalase is an important antioxidant enzyme whose physiological role is to limit the deleterious effects of ROS. Previous studies have suggested that ROS may confer susceptibility to EHYT (Griendling and Alexander 1997; Lacy et al. 1998; Sözmen et al. 1999), although there are studies to the contrary (Orie et al. 1999).

Whereas catalase is ubiquitously expressed, its abundance and activity varies across different tissues (Diesserdoth and Dounce 1970; Van Remmen et al. 1998). Therefore, it is particularly relevant that SNP-844 occurs in the promoter (770 bp upstream of the transcriptional start site) and is predicted to both create and destroy transcription factor binding sites (MZF1, Ikaros2, LYF-1, and AP2). However, these putative binding sites need to be experimentally verified, and it is not entirely clear how they would affect blood pressure levels.

Furthermore, our data do not allow us to determine whether SNP-844 is itself responsible for affecting SBP or whether it reflects linkage disequilibrium with variation in the remainder of the gene or perhaps in a nearby flanking gene. Moreover, it is possible that the observed association of SNP-844 with SBP is a statistical artifact resulting from a population substructure, a problem to which case-control studies are prone (Pritchard and Rosenberg 1999). However, given the homogeneity of this isolated Chinese population, it is unlikely the observed association of SNP-844 and SBP is spurious.

In summary, this is the first study to implicate genetic variation in the catalase gene with susceptibility to EHYT. Future studies will be required to replicate our findings across populations and to elucidate the molecular basis of this observation.

References

Boerwinkle E, Chakraborty R, Sing CF (1986) The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. Ann Hum Genet 50:181–194

Diesserdoth A, Dounce AL (1970) Catalase: physical and chemical properties, mechanisms of catalysis, and physiological role. Physiol Rev 50:319–375

Griendling KK, Alexander RW (1997) Oxidative stress and cardiovascular disease. Circulation. 96:3264–3265

Lacy F, O'Connor DT, Schmid-Schonbein GW (1998) Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. J Hypertens 16:291–303

Lo K, Landau NR, Smale ST (1991) LyF-1, a transcriptional regulator that interacts with a novel class of promoters for lymphocyte-specific genes. Mol Cell Biol 11:5229–5243

Mates JM, Perez-Gomez C, Nunez de Castro I (1999) Antioxidant enzymes and human diseases. Clin Biochem 32:595–603

Mitchell PJ, Timmons PM, Hebert JM, Rigby PW, Tjian R (1991) Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. Genes Dev 5:105–119

Molnar A, Georgopoulos K (1994) The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. Mol Cell Biol 14:8292–8303

Orie NN, Zidek W, Tepel M (1999) Reactive oxygen species in essential hypertension and non-insulin-dependent diabetes mellitus. Am J Hypertens 12:1169–1174

Perrotti D, Melotti P, Skorski T, Casella I, Peschle C, Calabretta B (1995) Overexpression of the zinc finger protein MZF1 inhibits hematopoietic development from embryonic stem cells: correlation with negative regulation of CD34 and c-myb promoter activity. Mol Cell Biol 15:6075–6087

Pritchard JK, Rosenberg NA (1999) Use of unlinked genetic markers to detect population stratification in association studies. Am J Hum Genet 65:220–228

Quandt K, Frech K, Karas H, Wingender E, Werner T (1995) MatInd and MatInspector – new fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Res 23:4878–4884

Rosenfeld ME (1998) Inflammation, lipids, and free radicals: lessons learned from the atherogenic process. Semin Reprod Endocrinol 16:249–261

Sözmen B, Delen Y, Girgin FK, Sözmen YS (1999) Catalase and paraoxonase in hypertensive type 2 diabetes mellitus: correlation with glycemic control. Clin Biochem 32:423–427

Tao S, Wu X, Duan X (1995) Hypertension prevalence and status of awareness, treatment and control in China. Chin Med J 108:483–489

Van Remmen H, Williams MD, Yang H, Walter CA, Richardson A (1998) Analysis of the transcriptional activity of the 5'-flanking region of the rat catalase gene in transiently transfected cells and in transgenic mice. J Cell Physiol 174:18–26

Weir BS (1996) Genetic data analysis II: methods for discrete population genetic data. Sinauer, Sunderland, Mass., USA

Xu X, Niu T, Christiani DC (1996) Environmental and occupational determinants of blood pressure in rural communities in China. Ann Epidemiol 7:95–106