Distribution of Catechol O-Methyltransferase Genotypes in a healthy Korean population

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= 국문초록 =

정상 한국인에서 Catechol O-methyltransferase 유전형의 분포

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배경 및 연구방법 : Catechol O-methyltransferase(COMT)는 교감신경계 작용에 있어 주요 효소중의 하나이며 근래에는 각종 정신질환 또는 유방암 등과의 연관성 여부로 주목 받고 있다. 정상 한국인에서 COMT 유전형의 분포를 알기 위해 저자들은 PCR 법을 이용하여 254명의 유전자형을 진단하였고 그 결과를 타인종에서의 수치와 비교하였다.

연구결과 : 254명의 한국인에서 53%는 homozygotic Val158 type(COMT^V COMT^V), 37%는 mixed type(COMT^V·COMT^L), 10%는 homozygotic Met158 type(COMT^L COMT^L)으로 판단되었다. 이러한 구성비는 일본인의 것과 매우 유사하였으며 백인종을 대상으로 한 보고들과 비교해 볼 때 활성도가 낮은 대립유전자(COMT^L)의 분율이 의미있게 낮은 것으로 나타났다($p < 0.001$).

결론 : 본 연구 결과는 향후 한국인을 대상으로 한 각종 질환과 COMT 유전형과의 연관성을 추구하는 연구를 위한 기본적인 정보가 될 것이다.

Introduction

Catechol O-methyltransferase (COMT), the ubiquitous enzyme with its genetic locus on the

short arm of chromosome 22, metabolizes some endogenous catecholamines like epinephrine, norepinephrine etc. As COMT is one of the major enzymes involved in the catecholaminergic system

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its possible influence on human behavior or psychiatric illness have been investigated¹⁻⁴⁾. The report of allelic deletion of COMT in patients with the congenital anomaly named Velo-Cardio-Facial syndrome (VCFS) which commonly accompanies schizophrenia, attention deficiency hyperactivity disorder (ADHD) or bipolar disorder led to the researches to find out the relationship of these psychiatric diseases with COMT⁵⁻⁶⁾.

COMT has also been focused on because of its role in the metabolic elimination of levodopa, the antiparkinsonian drug. Currently marketed COMT inhibitor like tolcapone is used to reduce symptomatic fluctuation and on-off phenomenon in levodopa therapy. Since COMT has long been known to exist as a polymorphic enzyme from population studies on its phenotype⁷⁻⁹⁾, the effect of its inhibitor would show corresponding interindividual variation.

Another topic on COMT under current investigation is its role in breast cancer via catechol-estrogen elimination. Oxidative metabolites of estrogen like 16- α -hydroxyestrone, estrogen catechol, and estrogen quinone may give genetic damage¹⁰⁻¹¹⁾ and the subjects COMT activity to eliminate these metabolites might be related to cumulative genetic damage¹²⁾. Therefore, the knowledge about the distribution of COMT activity and its genotype in an ethnic group will provide the basic information needed for COMT inhibitor therapy or allelic association study of some neurologic or psychiatric diseases.

Recently, the genetic locus determining the enzymatic activity of COMT was discovered¹³⁾. It is reported that valine found at the 158th amino acid of COMT with high enzyme activity is replaced by methionine in COMT with low enzyme activity. Nowadays, genotype screening in a large popula-

tion has become possible by polymerase chain reaction. Hence, researches on the relationship of COMT genotype with various diseases like breast cancer, parkinsonism and some psychoses are done with this method¹⁴⁻¹⁷⁾. To evaluate the genotypic characteristics of COMT in healthy Korean people, that will present a basic information for further disease-related COMT research in Koreans and other Asian population, the authors performed population study for genotype distribution of COMT in a healthy Korean people.

Materials & Methods

Subjects

Two hundred and fifty four unrelated Korean adults undergoing health screening in Seoul National University Hospital gave written informed consent to the genotyping study under the same IRB's approval. They were 191 men and 63 women aged 21 to 58 years (mean 34, S.D. 6.5 years) and were devoid of any specific health problems with normal AST and ALT values. Genomic DNA was extracted from peripheral blood leukocytes using kits of Takara Co (Dr Gentle, Tokyo, Japan).

COMT Genotyping

The Val158Met polymorphism of HSCOMT 2 gene (Guanine Adenine exchange in position 1947, Gene bank accession number z26491) was tested by using the oligonucleotide primer TCGTG-GACGCCGTGATTCAGG and AGGTCTGACA-ACGGGTCAGGC (Bioneer : Seoul, Korea) described by Li *et al.* 18). The PCR product of 217bp was digested by the restriction enzyme *Nla* III

Fig. 1. RFLP analysis of COMT codon 158 polymorphism.

- The lanes numbered 1, 3, 4, 5, 6, and 7 show COMT^HCOMT^H genotype.
- The lanes numbered 2 and 9 show COMT^HCOMT^L genotype.
- The lane numbered 8 show COMT^LCOMT^L genotype.

(New England Biolabs : Beverly, MA, USA). Digestion for 3 hours at 37°C gave the fragments of 114, 83, and 20 bp in high activity genome (coding Valine) and 96, 83, 20, and 18 bp in low activity genome (coding Methionine). We performed electrophoresis at 100 V for 35 minutes with 3 % Metaphor agarose gel(FMC, Maine, USA) containing

0.5 g/ml ethidium bromide. The smaller fragments like 18 bp and 20 bp were not seen by electrophoresis. Genotype determination relied on the existence of 114 or 96 bp fragments(Fig. 1).

Statistical Analysis

The influence of sex and race (in comparison with other reports) on genotypic distribution was assessed by the χ -square test.

Results

The COMT genotype in 254 Koreans consisted of 53% of homozygotic Val158 type (COMT^H COMT^H), 37% of mixed type (COMT^H COMT^L) and 10% of homozygotic Met158 type (COMT^L COMT^L). The allele frequencies calculated from those ratios were 71% for COMT^H and 29% for COMT^L. Sex was not related to the allele frequency of the subjects (Table 1). The allele frequencies in other ethnic groups are shown in Table 2. The data in the ethnic groups other than Koreans were from the normal control groups for each of the genotyping researches to find the relationship between COMT genotype and psychiatric diseases. The distribution of COMT genotype in Koreans was significantly different from

Table 1. COMT genotype in healthy, unrelated Korean population

		Genotype Count : No. (Frequency)			Allele Frequency	
	No.	COMT ^H COMT ^H	COMT ^H COMT ^L	COMT ^L COMT ^L	COMT ^H	COMT ^L
Male	191	101(53%)	70(37%)	20(10%)	71%	29%
Female	63	34(54%)	23(36.5%)	6(9.5%)	72%	28%
Total	254	135(53%)	93(37%)	26(10%)	71%	29%

Table 2. Frequency profile of high and low activity alleles of COMT in Korean population and those in other ethnic groups. Allele frequency of Koreans is significantly different from those of caucasians

Ethnic group (Reference No.)	No.	Genotype Count: No (Frequency)			Allele Frequency	
		COMT ^H COMT ^H	COMT ^H COMT ^L	COMT ^L COMT ^L	COMT ^H	COMT ^L
Korean	254	135(53%)	93(37%)	26(10%)	71%	29%
Han Chinese (18)	98	66(67%)	29(30%)	3(3%)	82%	18% *
Japanese (15)	153	74(48%)	70(46%)	9(6%)	71%	29%
Caucasian(USA) (14)	78	20(26%)	33(42%)	25(32%)	47%	53% *
Spanish (19)	113	35(31%)	57(50%)	21(19%)	57%	43% *
Caucasian(Eur) (16)	121	29(24%)	62(51%)	30(25%)	49.5%	50.4% *

* : Significant difference of allele frequency compared with Koreans. All of the data except for the data in Koreans are from normal control groups of research articles in references.

those of Caucasians^{14,16,19}. Caucasians had greater allele frequency for COMT^L genotype than those in Asian peoples.

Discussion

Ethnic difference of some enzymes involved in the drug metabolism has been extensively searched so far. Screening and counting of the frequency of outliers with remarkably high or low metabolic activity has been a traditional way in population studies on the phenotype. But the enzymes like thiopurine methyltransferase (TPMT) or COMT do not show well discernible phenotype distribution, but rather widely overlapping enzyme activities in any ethnic groups studied^{7,9}. Genotyping of such enzymes instead of phenotyping is a simple way of evaluation of the frequency distribution. Though there are some silent mutations reported¹⁹, only a change in the 158th amino acid (Valine or Methionine) of COMT is believed to explain the

difference in enzyme activity. Such simplicity of mutation and the advanced molecular laboratory methods made the genotyping for large population and its interpretation performed by many groups of researchers.

Though the data referred for comparison were not from the studies originally designed to evaluate the interethnic differences in normal population, easily discernible interethnic differences were observed. The allelic frequency of Met158 (low activity) type in Koreans (29 %) was identical to that of Japanese (29 %) and greater than that of Chinese (18 %, $p < 0.001$, Table 2). Considering the fact that all subjects of the Chinese data were indigenous to southwestern China (Sichuan province), further study seems necessary to discuss interethnic differences between these three Asian populations. The χ -square test showed that Asian populations including Koreans have significantly lower Met158 genotype frequency than Caucasian groups ($p < 0.001$). As COMT genotype is known

to be inherited regardless of sex and the genotype does not change by aging, the reliability of the present comparison can be regarded as considerable. The clinical implication of this racial difference in genotype needs to be determined, but given the fact that COMT is a major enzyme in the catecholamine system, one may assess possible racial difference in the effect of some CNS drugs. In the epidemiologic research to find the implication of COMT genotype on breast cancer, its frequency in normal population would also be one of fundamental references.

Background & Method : Catechol O-methyltransferase (COMT) is one of the key enzymes in the sympathetic nervous system and have been studied for its role in psychiatric, neurologic diseases and estrogen-related cancer. For the evaluation of COMT genotype profile in normal healthy Koreans, authors performed population study by the PCR-based RFLP method.

Result : COMT genotypes in 254 Korean population consisted of 53 % of homozygotic Val158 type (COMT^H COMT^H), 37 % of mixed type (COMT^H COMT^L) and 10 % of homozygotic Met158 type (COMT^L COMT^L). These results were very similar to those of Japanese, while a smaller proportion of low activity allele ($p < 0.001$) was noted in Koreans when compared with Caucasians.

Conclusion : In the researches to find the implication of COMT genotype on many diseases in Korean people, the current result will be used as a fundamental data.

Key words : COMT · Genotype · Korean · Population.

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