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Evaluation of Single Nucleotide Polymorphism Responsible for the Determination of Earwax Type in Koreans

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Abstract : The *ABCC11* gene is a member of the ATP-binding cassette gene family. A single nucleotide polymorphism (SNP) in the *ABCC11* gene is associated with the determination of human earwax type. The purpose of this research is to evaluate the allelic frequencies of this gene in Korean population samples. A total of 619 individuals were analyzed by *Dde I* restriction fragment length polymorphisms (RFLPs). The genotype frequency of dry earwax in Koreans was very high. The proportions of dry/dry genotype found were 99% (99/100) in normal controls; 98.1% (207/211) in patients with schizophrenia; 99% (99/100) in patients with breast cancer; 96% (48/50) patients with uterine myoma; 98.3% (59/60) in obesity subjects; 100% (43/43) in patients with stomach cancer, and 98.2% (54/55) in attention deficit hyperactivity disorder (ADHD) patients. The wet/wet genotype was not found. There were no significant differences in the distribution of earwax genotypes between controls and experimental groups. These results suggest that the frequency of dry earwax are very high, and therefore the SNP determining earwax type will not be useful in genetic studies of the process involved in various diseases or pathologic conditions in Korean populations.

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Key words : Earwax type, Polymorphism, *ABCC11* gene

Introduction

Earwax (cerumen) is a secretory product of ceruminous apocrine glands in the auditory canal of humans and many other mammals. There are two genetic types of earwax (1). The wet type is dominant and the dry type is recessive. Yoshiura et al. (2) reported that the determinant of earwax type is a single base change (single nucleotide polymorphism, SNP) in the *ABCC11* gene. In that report, 99 Koreans examined had only the AA genotype corresponding to dry type earwax.

In this context, the present study aims to reevaluate the genotype distribution of the *ABCC11* gene responsible for the determination of ear wax type in Korean population by using restriction fragment length polymorphisms (RFLPs) and to compare the distribution between some pathologic patients and controls.

Materials and Methods

A total of 619 individuals were investigated. Peripheral blood samples were obtained from 100 healthy controls, 211 schizophrenia patients, 60 obesity subjects, and 55 attention deficit hyperactivity disorder

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(ADHD) patients. Normal tissues of tumor samples were collected from 100 breast cancer, 50 uterine myoma, and 43 stomach cancer patients.

Polymerase chain reaction (PCR) amplification for single nucleotide polymorphism (SNP) in exon 4 of the *ABCC11* gene was performed using the following primers (5′-3′): forward, TGCAAAGAGATTCCACC-AGTT; reverse, TAAGTGCCAGGGACGTGGTT. PCR was performed in 20 μL volume including 100 ng genomic DNA, 0.5 unit AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA, U.S.A.), 2 μL of 10× PCR buffer, 2 mM $MgCl_2$, and 0.2 mM dNTP. Cycling conditions were 95°C for 10 min, then 40 cycles of 94°C for 45 sec, 56°C for 45 sec, and 72°C for 45 sec, with a final extension step of 10 min at 72°C.

PCR products of 421 bp were cut with 1 unit of restriction enzyme, *Dde I* for 16h at 37°C. Enzyme digested DNA was analyzed by electrophoresis using 2% agarose gel and stained with ethidium bromide to be viewable.

The distribution of polymorphisms was compared between each of the experimental groups and controls using the chi-square test. Statistical analysis using the chi-square test was also performed a comparison between total experimental patients and controls.

Results

Dde I restriction fragment length polymorphism (RFLP) in the *ABCC11* gene responsible for determination of earwax type was studied in a total of 619 Koreans.

The amplified 421 bp fragment contained the *Dde I* polymorphic site in the exon 4 of the *ABCC11* gene. *Dde I* restriction of the PCR products resulted in two different patterns of fragments. If the 421 bp PCR products have an *Dde I* site at codon 180, *Dde I* cuts them into three fragments (241 bp, 111 bp, 69 bp : allele A).

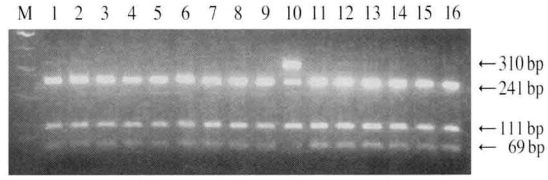

Fig. 1. RFLP analysis of PCR products digested by *Dde I* in patients with uterine myoma. The A and G alleles are determined as 241+111+69 bp and 310+69 bp bands, respectively. Lane 10 is an example of AG heterozygote and the remainings are AA homozygote. M is a size marker.

Table 1. Distribution of earwax genotypes in controls and experimental groups

	AA (%)	AG (%)	GG (%)	*p* value
Schizophrenia (N=211)	207 (98.1)	4 (1.9)	0 (0)	0.557
Breast cancer (N=100)	99 (99)	1 (1)	0 (0)	1.000
Uterine myoma (N=50)	48 (96)	2 (4)	0 (0)	0.216
Obesity (N=60)	59 (98.3)	1 (1.7)	0 (0)	0.713
Stomach cancer (N=43)	43 (100)	0 (0)	0 (0)	0.511
ADHD (N=55)	54 (98.2)	1 (1.8)	0 (0)	0.606
Total (N=519)	510 (98.2)	9 (1.8)	0 (0)	0.594
Controls (N=100)	99 (99)	1 (1)	0 (0)	

ADHD, attention deficit hyperactivity disorder.

If, on the other hand, the products contain no such site, it digests into two fragments (310 bp, 111 bp : allele G) (Fig. 1).

The genotype distribution of the *ABCC11* gene at codon 180 was summarized in Table 1. 609 of 619 Koreans (98.4%) had the dry type earwax of genotype AA and only 10 (1.6%) had the wet type of AG genotype. The wet type of GG genotype was not detected in this experiment. There were no significant differences in the proportions of the earwax genotypes found in normal healthy controls and in the experimental groups.

Discussion

Earwax is a secretion product of the ceruminous apocrine glands. It plays an important role in the human

auditory tube, assisting in clearing, lubrication, and preventing dryness. It also provides some protection from bacteria, fungi, insects, and foreign bodies.

There are two types of earwax. The dry type of earwax is milky-white or yellowish-gray and brittle, whereas the wet type is brown, sticky, and wet. The wet earwax phenotype is completely dominant over to the dry type (3). Little attention has been given to this variation elsewhere, probably because Caucasians and Africans have only the wet type of earwax. The dry earwax type is seen frequently among East Asians. In Matsunaga's study (3), high frequencies of the dry type were seen in populations of Northern Chinese, Koreans, Tunguse, and Monglols. Petrakis et al. (4) and Ibraimov (5) reported a high frequency of the dry type in Native American Indians and Mongoloid populations, respectively. Interestingly, earwax type has been used by anthropologists to trace human migratory patterns, such as those of the Inuits (6).

There have been very few reports showing the incidence of earwax type in Koreans. In one study, on a total of 4,000 male Koreans, the incidence of dry earwax was 97.2%, while the wet type was only 2.8% (7). In this report, Cho and Kim argued that since Koreans have a very low incidence of wet cerumen compared to other Mongolian ethnic groups, this could be indirect evidence that Koreans may have a relatively pure blood line in anthropological terms.

Yoshiura et al. (2) showed that a nonsynonymous SNP (538G-A, at codon 180) in *ABCC11* exon 4 was responsible for determination of earwax type. The AA homozygotes correspond to dry earwax, and either GA heterozygotes or GG homozygotes to the wet type. In this report, a functional assay showed that cells with allele A showed a lower excretory activity for cGMP than those with allele G. In the worldwide frequency distribution of allele A among different ethnic populations, the allele A frequency showed a north-south and east-west downward geographic gradient; worldwide, it was highest in Koreans and Chinese, and a common dry-type haplotype was retained among various ethnic populations. These results suggest that the allele A arose in northeast Asia and thereafter spread through the world. In the 99 Koreans examined in that study, only the AA genotype corresponding to dry type earwax was found.

The amplified PCR product containing allele A has a *Dde I* restriction site, so it is possible to predict the genotype of earwax type by analysis of RFLPs. To my knowledge, this present study is the first RFLPs analysis for determining earwax type.

A total of 619 individuals were studied, including 100 normal controls, 211 schizophrenia, 100 breast cancer, 50 uterine myoma, 43 stomach cancer, and 55 attention deficit hyperactivity disorder (ADHD) patients, and 60 obese persons. There were no significant differences in the proportions of the earwax genotypes between normal healthy controls and experimental groups. These results suggest that the polymorphism for determining earwax type may be not associated with factors to develop some pathologic conditions, including stomach cancer, breast cancer, and others and probably there are no differences in effect of the polymorphism amongst the Korean population. To my knowledge, there is no report in the distribution of earwax genotypes between normal controls and experimental groups with various pathologic conditions in other countries.

The total incidence of dry earwax of genotype AA was 98.4%, while the wet type of AG genotype was 1.6%. These results demonstrate that the frequencies of earwax phenotype in this report is similar to those observed in Cho and Kim's study (7), and support the finding that the dry earwax allele is most prevalent in Koreans.

If the Korean population is 50 millions, according to this result, the number of GG wet type is about 3,200 (q^2=0.000064) and there was no GG genotype in Koreans in this study; this means that most wet earwax type may stem from the heterozygote AG allele and

Koreans with GG wet type earwax are very rare or not exist.

In conclusion, Koreans have the highest frequency of the dry earwax type in the world. The wet type of earwax with allele AG heterozygote is rare, and wet type with allele GG homozygote may be very rare or not exist. And the SNP determining earwax type will not be useful in genetic studies of the process involved in various diseases or pathologic conditions in Korean populations.

References

Adachi B : Das Orhenschmalz als Rassenmerkmal und Rassengeruch ('Achselgeruch') nebst dem Rassenunterschied der Schweissdrusen. Z Rassenk 6: 273-307, 1937.

Yoshiura K, Kinoshita A, Ishida T, Ninokata A, Ishikawa T, Kaname T, Bannai M, Tokunaga K, Sonoda S, Komaki R, Ihara M, Saenko VA, Alipov GK, Sekine I, Komatsu K, Takahashi H, Nakashima M, Sosonkina N, Mapendano CK, Ghadami M, Nomura M, Liang DS, Miwa N, Kim DK, Garidkhuu A, Natsume N, Ohta T, Tomita H, Kaneko A, Kikuchi M, Russomando G, Hirayama K, Ishibashi M, Takahashi A, Saitou N, Murray JC, Saito S, Nakamura Y, Niikawa N : A SNP in the *ABCC11* gene is the determinant of human earwax type. Nat Genet 38: 324-330, 2006.

Matsunaga, E : The dimorphism in human normal cerumen. Ann Hum Genet 25: 273-286, 1962.

Petrakis NL, Molohan KT, Tepper DJ : Cerumen in American Indians: genetic implications of sticky and dry types. Science 158: 1192-1193, 1967.

Ibraimov AI : Cerumen phenotypes in certain populations of Eurasia and Africa. Am J Phys Anthropol 84: 209-211, 1991.

Bass EJ, Jackson JF : Cerumen types in Eskimos. Am J Phys Anthropol 47: 209-210, 1977.

Cho SH, Kim BY : Cerumen and axillary odour in Koreans. J Korean Otol 25: 76-80, 1982.

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한국인에서 귀에지형을 결정하는 단일염기서열다형성의 평가

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간추림 : *ABCC11* 유전자는 ATP결합 카세트 유전자군의 일종이다. *ABCC11* 유전자에는 귀에지형을 결정하는 단일염기서열다형성(SNP)이 존재한다. 이 연구의 목적은 귀에지 관련 유전자의 대립유전자의 빈도를 한국인에서 조사하고자 한다. 총 619명의 대상자에서 *Dde I* 제한효소를 이용하여 제한단편길이다형성(RFLPs)을 분석하였다. 정상 대조군에서 건성/건성 유전자형이 99% (99/100); 정신분열증의 경우 98.1% (207/211); 유방암의 경우 99% (99/100); 자궁근종의 경우 96% (48/50); 비만인의 경우 98.3% (59/60); 위암의 경우 100% (43/43) 그리고 주위력결핍과다활동장애(ADHD)의 경우 98.2% (54/55)로 나타났다. 습성/습성 유전자형은 나타나지 않았으며, 대조군과 실험군과의 유전자형의 분포에는 유의성이 없었다. 이상의 결과에서 한국인에서 대부분의 경우 건성 귀에지를 가지며, 귀에지를 결정하는 SNP는 한국인에서 나타나는 여러 질병과 병적인 신체 조건의 경우에서 유전적 연관성을 연구하는데 적합하지 않는 것으로 생각된다.

찾아보기 낱말 : 귀에지형, 다형성, *ABCC11* 유전자

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