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Correlation of axillary osmidrosis to a SNP in the *ABCC11* gene determined by the Smart Amplification Process (SmartAmp) method☆

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Summary Axillary osmidrosis (AO) is caused by apocrine glands secretions that are converted to odouriferous compounds by bacteria. A potential link between AO and wet earwax type has been implicated by phenotype-based analysis. Recently, a non-synonymous single nucleotide polymorphism (SNP) 538G> A (Gly180Arg) in the human adenosine triphosphate (ATP)-binding cassette (ABC) transporter *ABCC11* gene was found to determine the type of earwax. In this context, we examined a relationship between the degree of AO and the *ABCC11* genotype. We have genotyped the SNP 538G> A in a total of 82 Japanese individuals (68 volunteers and 14 AO patients) by both DNA sequencing and the recently developed Smart Amplification Process (SmartAmp). The degree of AO in Japanese subjects was associated with the genotype of the *ABCC11* gene as well as wet earwax type. In most AO patients investigated in this study, the G/G and G/A genotypes well correlated with the degree of AO, whereas A/A did not. The specific SmartAmp assays developed for this study provided genotypes within 30 min directly from blood samples. In East Asian countries, AO is rather infrequent. Although the judgement of the degree of AO prevalence is subjective, the SNP 538G> A in *ABCC11* is a good genetic biomarker for screening for AO. The SmartAmp method-based genotyping of the *ABCC11* gene would provide an accurate and practical tool for guidance of appropriate treatment and psychological management for patients.

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Axillary osmidrosis (AO) or bromhidrosis is a physical condition that is often perceived, especially by younger women, as a distressing and troublesome problem. In fact, the condition of AO has been treated and recognised as a disease that is covered by the national health insurance system in Japan. Sweat produced by axillary glands, including apocrine, eccrine, apoeccrine and sebaceous glands, is odourless.[1] Secretions from apocrine glands can be converted to compounds by bacteria (*Corynebacteria*), which have an unpleasant smell associated with body odour.[1,2] The mechanism of producing the major odour-causing compounds in the human axilla is still a subject of controversy. Because ancillary odour first appears at puberty when apocrine glands become fully developed, early studies were first focussed on volatile odouriferous steroids.[3,4] More recently branched and unsaturated fatty acids as for example (*E*)-3-methyl-2-hexenoic acid (3M2H) and 3-methyl-3-hydroxylhexenoic acid (HMHA), have been identified as the key components of odor rather than steroids.[5] Released from apocrine secretions, 3M2H is carried to the skin surface bound to apolipoprotein D (also known as apocrine secretion odour-binding protein 2),[5] and is also produced from 3M2H-Gln by bacterial N-acyl-aminoacylase in the axilla.[6] In addition, volatile sulphur compounds, such as 3-methyl-3-sulphanylhexan-1-ol, have been identified as another source of typical sweat/onion-like smells.[7,8]

In East Asian countries, AO is rather infrequent; hence, the social norm is generally a natural expectation for axillary odourlessness. Histological findings reveal significantly numerous and larger-sized apocrine glands in osmidrosis patients, compared with the controls, suggesting the existence of two different phenotypes.[9,10] Furthermore, wet earwax has been shown to be always inherited as a Mendelian dominant trait, and accompanied with osmidrosis.[9,11,12] Human earwax is a secretory product of ceruminous apocrine glands and shown to be either wet or dry in consistency. Dry earwax is common in East Asians, whereas wet earwax is common in most all other populations. In 2006, a single nucleotide polymorphism (SNP) in the *ABCC11* gene, at nucleotide 538 (538G> A; 180Gly> Arg) in exon 4, was identified as a determinant for either wet or dry types of earwax.[13] The AA genotype was linked to dry earwax, whereas GA and GG were connected to the wet type. Moreover, a deleted mutation of 27 base pairs (bps) in exon 29 (Δ27) in the G allele shows an equivalent effect to the A allele on *ABCC11*. Both the SNP (538G> A) and the deletion (Δ27) are believed to cause an inactive form of the *ABCC11* product.[14]

ABCC11 is a novel ABC transporter identified by database search, and cloned from a cDNA library of human adult liver.[15–17] Known as multidrug-resistance-associated protein 8 (MRP8), the *ABCC11* gene product contains two ATP-binding cassettes (ABC) and 12 transmembrane helices. Human *ABCC11* and *ABCC12* gene are located on human chromosome 16q12.1 in a tail-to-head orientation with a separation distance of about 20 kb.[17] The predicted amino acid sequences of both gene products show a high similarity to those of ABCC4 and ABCC5.[18] However, there is no putative mouse or rat orthologous gene corresponding to human *ABCC11*.[19] This fact indicates that *ABCC11* is not an orthologous gene but rather a paralogous gene generated by gene duplication in the human genome.

In this study, we examined the relationship between the degree of AO and the genotype of *ABCC11*. Our results support that a condition of AO in Asians can be recognised by genetic diagnosis, which would be conclusive for providing objective evidence to authorise a treatment policy. Procedures for the diagnosis and the practical management of patients with AO will be facilitated by the new method described herein. The SmartAmp method is a unique genotyping technology that can detect a genetic mutation in about 30 min under isothermal conditions and in a single step by using a drop of whole blood.[20]

Patients and methods

All the procedures were performed according to the protocol approved by the Ethical Review Board of Fujita Health University School of Medicine. Eighty-two individuals (68 volunteers and 14 patients) participated in this study after giving written informed consent. All examinees expressed their subjective views on the symptoms of AO by answering survey questions concerning themselves and family members (Table 1). An impartial practitioner, Y.I., who is without olfactory impairment and a specialist in plastic surgery, conducted all the examinations for diagnosis of the study participants. The olfactory function of Y.I. was confirmed by an intravenous olfactory test (Alinamin® Test) and an electro-olfactogram. To test the degree of odour, gauze inserted in the axilla for 3 min was estimated by its smell. Earwax type was determined using a cotton swab stirred in the external ear canal. Another researcher, who was blind to the examinees' physical background, carried out DNA sequencing to determine the genotype of each study participant. Statistical analysis on the relationship between two variables was evaluated by Fisher's exact test. Individual genomic DNA was obtained from 2 ml of whole blood using QuickGene-610 L (Fujifilm Co., Tokyo, Japan) according to the manufacturer's protocol. Sequence data for the human genome was obtained from the National Center for Biotechnology Information (NCBI) database. To sequence the SNP (538G> A), the following PCR primers were designed: P1: 5′-TGT CACATG-CAAAGAGATTCC-3′ and P2: 5′-CTCCTGGCATGGACTTGAACA-3′. To identify the Δ27 mutation, we also designed the set of primers: P3: 5′-AG GTCTCTAGGGCCTGAAGTA-3′ and P4: 5′-AGCCTTCACCTTCC
CATTGCC-3′. All sequences (538G> A and Δ27) were performed using 3100 Genetic Analyzer (Applied Biosystems Ltd., Tokyo, Japan) according to the manufacturer's protocol.

Table 1 Subjective findings on the examinees

	Earwax	AO		Family history of AO		
		Yes	No	Yes	No	Unclear
Volunteers ($n = 68$)	Dry 49[a]	1[b]	48[b]	1	43	5
	Wet 19[a]	12[b]	7[b]	9	7	3
Patients ($n = 14$)	Dry 1[a]	1	0	0	1	0
	Wet 13[a]	13	0	9	3	1

AO; axillary osmidrosis.
[a] $p < 8.2 \times 10^{-6}$.
[b] $p < 7.9 \times 10^{-8}$ (Fisher's exact test).