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Short communication

Identification of volatile organic compounds in human cerumen

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ABSTRACT

We report here the initial examination of volatile organic compounds (VOCs) emanating from human earwax (cerumen). Recent studies link a single nucleotide polymorphism (SNP) in the adenosine triphosphate (ATP) binding cassette, sub-family C, member 11 gene (*ABCC11*) to the production of different types of axillary odorants and cerumen. *ABCC11* encodes an ATP-driven efflux pump protein that plays an important function in ceruminous apocrine glands of the auditory canal and the secretion of axillary odor precursors. The type of cerumen and underarm odor produced by East Asians differ markedly from that produced by non-Asians. In this initial report we find that both groups emit many of the same VOCs but differ significantly in the amounts produced. The principal odorants are volatile organic C_2-to-C_6 acids. The physical appearance of cerumen from the two groups also matches previously reported ethnic differences, viz., cerumen from East Asians appears dry and white while that from non-Asians is typically wet and yellowish-brown.

1. Introduction

Ceruminous glands are specialized sweat glands located subcutaneously in the external ear canal. It has been estimated that there are between 1000 and 2000 ceruminous glands in the ear. The ceruminous gland is a modified apocrine gland, which together with sebaceous glands, produces cerumen, or earwax [1,2]. Cerumen keeps the eardrum pliable and lubricates, waterproofs, and cleans the external auditory canal. Cerumen also has antibacterial properties [3–5] and presents a barrier that traps foreign particles (dust, fungal spores, etc.). There are two distinct types of cerumen: wet, yellowish-brown wax, which is found in Caucasians and Africans; and a dry, white wax, which is most common in East Asians (e.g., Chinese, Korean and Japanese) and Native Americans [6–8].

The human adenosine triphosphate (ATP)-binding cassette (ABC) transporter gene *ABCC11* encodes an ATP-driven efflux pump protein that plays an important function in ceruminous apocrine glands of the auditory canal [9,10]. This protein is also expressed in axillary apocrine sweat glands and appears to play a role in regulating the secretion of axillary odor precursors [11,12]. A simple change in *ABCC11* results in the production of different types of axillary odorants and cerumen. Individuals who are homozygous for a single nucleotide polymorphism (SNP; 538G > A), which changes amino acid 180 in the resultant protein's polypeptide chain from glycine (G) to arginine (R; G180R), were found to have significantly less of the characteristic axillary odorants than either those who are heterozygous for this change or those who had the wild type gene [10,13]. Although many studies have focused on the chemistry of human axillary odorants, to date, there are no data regarding the volatile organic compounds (VOCs) associated with human cerumen. The composition of cerumen has been assessed by several techniques (e.g., gas-chromatography [GC], gas chromatography–mass spectrometry [GC/MS], high performance liquid chromatography, and thin layer chromatography), which have revealed long-chain fatty acids, alcohols, triacylglycerols, cholesterol, and squalene as the major components [14–23]. Herein, we provide the first analysis of the nature and relative abundance of VOCs present in cerumen. We also directly compare volatile cerumen profiles of individuals of East Asian vs. Caucasian descent.

2. Materials and methods

2.1. Collection of cerumen

All protocols were approved by the University of Pennsylvania Institutional Review Board (IRB) for Research Involving Human Subjects. For 7–10 days prior to collection, the subjects were instructed to bathe/shower with fragrance-free liquid soap/shampoo (Symrise, Inc., Teterboro, NJ) to reduce the influence

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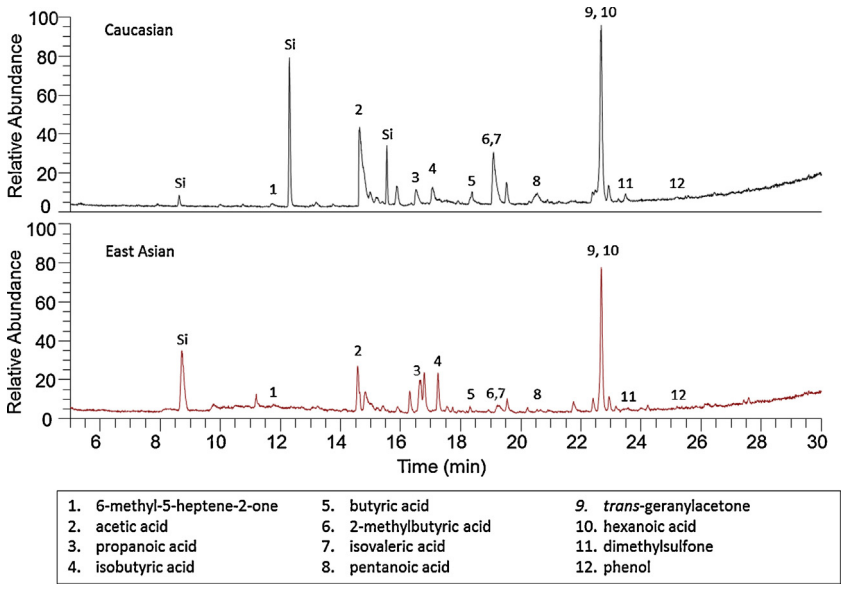

Fig. 1. Representative total ion chromatograms (TICs) from solid-phase microextraction (SPME) collection of cerumen samples from a Caucasian (top, 2.7 mg sample) and East Asian male (bottom, 2.2 mg sample) with endogenous VOCs labeled. Compounds labeled Si represent exogenous silicon contaminants. See Table 1 for full list of identified compounds.

of exogenous VOCs from consumer products during analysis. The subjects were also instructed not to use colognes and perfumed sprays during this time.

Cerumen was collected from both ears of 16 healthy males ($N = 8$ Caucasians, average age = 35 ± 5; and $N = 8$ East Asians, average age = 28 ± 3). Cerumen was collected on sterile, 6-in., cotton-tipped wooden applicators (Fisher Scientific). The cotton applicator was inserted approximately 10–15 mm into the subject's external auditory canal and gently swabbed. The applicator was removed from the ear and cerumen was transferred to a pre-weighed 4 mL clear glass vial (Supelco Corp., Bellefonte, PA) by rotating the cotton tip for 20 s on the bottom and sides of the vial. Collections were performed on at least three separate occasions, on non-consecutive days. The cerumen sample weight was recorded after each collection.

Following cerumen collection, the sample vial was tightly capped with a white silicone/TFE septum-containing screw cap and incubated in a 37 °C water bath for 30 min. Solid-phase microextraction (SPME) was performed using a 2 cm, 50/30 μm divinylbenzyene/carboxen/polydimethylsiloxane 'Stableflex' fiber (Supelco Corp., Bellefonte, PA). The fiber was introduced into the vial and the headspace VOCs were collected for an additional 30 min at 37 °C. The SPME fiber was then inserted into the injection port of a GC/MS and the VOCs were desorbed for 1 min at 230 °C.

2.2. GC/MS

A Thermo Scientific ISQ single quadrupole GC/MS (Waltham, MA) with Xcalibur software (ThermoElectron Corp.) was used for separation and analysis of the desorbed VOCs. The GC/MS was equipped with a Stabilwax column, 30 m × 0.32 mm with 1.0 μm film thickness (Restek Corp., Bellefonte, PA). The injection port was set at 230 °C. The oven temperature was held at 60 °C for 4 min, raised to 230 °C at 6 °C min^{-1}, and maintained at 230 °C for 40-min. Helium carrier gas constantly flowed at 2.5 mL min^{-1}.

The mass spectrometer was operated at an ionizing energy of 70 eV with a 2 s^{-1} scan rate over a scan range of m/z 40–400 and an ion source temperature of 200 °C. Identification of structures/compounds was performed using the National Institute of Standards and Technology Library, as well as comparisons with known literature compounds and commercially available standards. All standards were purchased from either Sigma-Aldrich or Alfa Aesar at the highest available purity and used as received. Relative retention times were obtained by comparison of sample VOCs to authentic samples with a series of C_2–C_{16} fatty acid ethyl esters (International Flavors and Fragrances Inc.) to obtain their "ethyl ester retention index" (EERI) [24].

2.3. Data analysis

The mass spectra of all peaks ~1% above the baseline with a retention time between 5 and 35 min were examined to eliminate large, exogenous components arising from unwanted sources. These exogenous compounds can be attributed to liquid soap and cosmetic products (e.g., siloxanes, dodecanol), solvents (e.g., traces of acetone and chlorinated solvents), as well as compounds arising from septa and column bleed. The peaks of interest were normalized by both sample weight and an external standard (methyl stearate). Compounds consistently seen in all subjects were subjected to multivariate analyses of variance with IBM SPSS Statistics (v. 20).

3. Results and discussion

The cerumen samples from the Caucasian and East Asian donors exhibited notable differences. While the Caucasian samples were yellow and sticky in nature, cerumen collected from the East Asian donors was consistently drier and colorless. As the pH of cerumen is weakly acidic (~5.4; [25]) we assumed that many of the odoriferous VOCs would be volatile acids. Freshly collected cerumen was qualitatively rated by three odor judges: descriptors used were "acidic/pungent" "fecal" "sweaty feet." Addition of a small amount (~30 μL) of saturated bicarbonate solution to the collected cerumen eliminated the odor, further suggesting an odor profile dominated by organic acids.

The SPME-collected VOCs were analyzed by GC/MS and representative chromatograms from a Caucasian and East Asian cerumen donor are shown in Fig. 1. A full list of identified compounds with retention times and characteristic ions can be found in Table 1. As noted, a number of the compounds can be attributed to exogenous