

Concept Paper

Not peer-reviewed version

From Allelic Heterogeneity to Biomechanical Phenotypes: A Functional New Genomic Model of ABCC11 Polymorphisms and Their Role in Human Chemical Ecology

[Sameer Meralli](#) *

Posted Date: 15 October 2025

doi: 10.20944/preprints202510.1009.v1

Keywords: genetics; human chemical ecology; biomechanical signaling



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Concept Paper

From Allelic Heterogeneity to Biomechanical Phenotypes: A Functional New Genomic Model of ABCC11 Polymorphisms and Their Role in Human Chemical Ecology

Sameer Meralli

UNESCO UIL; sameer@constellationcarbon.com

Abstract

The ABCC11 gene, through a single nucleotide polymorphism (SNP; rs17822931), is established as a primary determinant of human axillary secretion. Such a composition dictates the presence of both a binary phenotype of earwax (dry/wet) and the absence or presence thereof of characteristic body odor. However, significant phenotypic heterogeneity exists within genotypic groups, suggesting a more complex genetic architecture. This paper proposes a functional genomic model that expands upon the current biochemical pathway to position the biomechanical properties of the secretion as a critical phenotypic trait. We hypothesize that beyond the foundational secretion of odorant precursors, the ABCC11 genotype, influenced by allelic heterogeneity and modifier genes, actually impacts ecological behaviors and human interactions. Such biomechanical properties of the viscosity, rheology, and water content of apocrine secretions, directly impact the kinetics of odorant release, persistence on the skin, and dispersion through the air, thereby modulating the efficacy of chemical signal transmission. We outline a research program to characterize the ABCC11 variation, functionally validate novel alleles, define associated metabolomic and biomechanical phenotypes, and test their ecological consequences in behavioral assays. This model integrates population genetics, cell biology, materials science, and ethology to provide a holistic understanding of the role of ABCC11 in human chemical transmission.

Keywords: genetics; human chemical ecology; biomechanical signaling

1. Introduction

1.1. Human Chemical Ecology and Signaling

Human sociality is underpinned by a complex suite of communicative channels, among which chemical signaling, or semiochemistry, represents a phylogenetically ancient yet poorly documented system. While often considered microsmatic, a growing body of evidence indicates that humans subconsciously perceive and process a wealth of information encoded in axillary secretions (Preti et al., 2003; Havlíček et al., 2017). These secretions, a complex mélange of water, electrolytes, lipids, and proteins derived from eccrine and apocrine glands, are metabolized by the cutaneous microbiome into volatile organic compounds (VOCs) that constitute an individual's olfactory signature (Troccaz et al., 2015).

The ecological functions of this chemical olfactory signature and its effect on social interactions are multifaceted. A well-documented role is in kin recognition, particularly through the discrimination of odors correlated with the major histocompatibility complex (MHC), a genomic region critical for immune function (Penn and Potts, 1998; Wedekind et al., 1995). This ability of kin discrimination may inadvertently serve to avoid inbreeding or to facilitate nepotism. Furthermore, as per Havlicek and Robert, axillary odors have been demonstrated to influence mate choice, with preferences often aligning with MHC dissimilarity, potentially promoting heterozygosity and

pathogen resistance in offspring (Havlíček and Roberts, 2009). Chemical signals continue to also convey information about emotional states, such as anxiety or happiness (de Groot et al., 2012), and may even synchronize menstrual cycles among co-habiting women (Stern and McClintock, 1998). Thus, the axilla functions as a bioreactor, producing a dynamic chemical portfolio that facilitates non-verbal social cognition.

1.2. The ABCC11 Gene: A Genetic Switch

The genetic underpinnings of this chemical diversity were significantly elucidated with the discovery of the ABCC11 gene (ATP-Binding Cassette Subfamily C Member 11). Expressed predominantly in apocrine glands, mammary tissue, and ceruminous glands of the ear, ABCC11 encodes an apical efflux transporter that actively pumps various substrates, including glutathione conjugates and uric acid, into the lumen of these glands (Toyoda et al., 2009). The pivotal genetic variant is a non-synonymous SNP (rs17822931; c.538G>A) that results in an amino acid substitution (p.Gly180Arg). The ancestral G allele codes for a functional transporter, leading to wet-type earwax and the secretion of odorant precursors that are metabolized into pungent VOCs like (E)-3-methyl-2-hexenoic acid (E-3M2H) and 3-methyl-3-sulfanylhexas-1-ol (3S3MH) (Martin et al., 2010). The derived A allele produces a misfolded, non-functional protein retained and degraded in the endoplasmic reticulum (Toyoda and Ishikawa, 2010). Consequently, homozygous AA individuals exhibit dry earwax and a markedly reduced production of odorous axillary secretions.

The population distribution of these alleles is striking and indicative of strong selective pressures. The A allele is near-fixated in East Asian populations with high levels of occurrence (80-95% frequency) and is also common among Indigenous Americans, whereas it is rare in European and African populations (<3%) (Yoshiura et al., 2006; Ohashi et al., 2011). The reasons for this selective sweep are debated but likely could involve sexual selection, cultural practices favoring low odor production, or other pleiotropic effects, such as a potential role in mastitis susceptibility or breast cancer prognosis (Miura et al., 2010; Jobling et al., 2016).

1.3. Beyond the Single SNP: The Problem of Phenotypic Heterogeneity

The rs17822931 SNP provides a powerful but incomplete genetic model. A dual binary genotype-phenotype map fails to account for the observable continuum of axillary odor intensity and secretion consistency or inconsistency within genotypic groups. The fact remains that not all individuals homozygous for the ancestral G allele exhibit equally strong body odor, and, conversely, not all A-allele carriers are completely odorless (Rodriguez et al., 2013). This phenotypic heterogeneity suggests the involvement of additional modifying factors.

First, allelic heterogeneity is likely present. The focus on rs17822931 has obscured the potential impact of other, rarer variants within the ABCC11 locus. These could include missense mutations in other exons, regulatory variants in promoter or enhancer regions affecting expression levels, or splice-site variants altering protein isoforms. The functional impact of these rare alleles on transporter kinetics and specificity remains almost entirely unexplored.

Second, epigenetic regulation may modulate gene expression. DNA methylation or histone modifications of the ABCC11 promoter could fine-tune its transcriptional activity, leading to inter-individual variation in transporter abundance even among those with an identical GG genotype (Dib and Nielsen, 2020). Third, the axillary microbiome is a critical intermediary. The composition, density, and metabolic activity of bacterial communities (e.g., Staphylococcaceae, Corynebacteriaceae, Cutibacteriaceae) are highly variable and directly determine the conversion of non-volatile precursors into VOCs (Bawdon et al., 2015). Genotype alone cannot predict the final olfactory output without accounting for this microbial variable.

1.4. The Biomechanical Gap in the Pathway

The current model of ABCC11 function also includes a critical gap in understanding terminating at the point of biochemical secretion: the functional transporter secretes precursors, whereas the dysfunctional one does not. This model overlooks a crucial step in the signal transmission pathway: the physical and material nature of the secretion itself. For a chemical signal to be ecologically relevant, it must not only be produced but also be effectively transmitted through the environment to a receiver. This completes the chemical transmission cycle.

We posit that the biomechanical properties of apocrine secretion—its viscosity, rheology (flow properties), surface tension, and water content—are critical phenotypic traits that influence the specific efficacy of the chemical signal. A more viscous, lipid-rich secretion (likely associated with a fully functional transporter) may form a persistent film on the skin, providing a sustained-release matrix for odorants. In contrast, a more aqueous secretion might be rapidly diluted by eccrine sweat and evaporation, leading to a transient, pulsed signal. These physical properties directly impact key parameters in chemical ecology:

- 1) Persistence: How long the signal remains active on the skin substrate.
- 2) Dispersion: How the signal volatilizes and travels through the air.
- 3) Degradation: How the signal is broken down by environmental factors like UV radiation and oxidation. Ignoring this biomechanical layer creates a significant gap in our understanding of the pathway from genotype to ecological function.

1.5. Study Aims and Hypotheses

To address these gaps, we have proposed a comprehensive research program with the following aims and our central unifying hypothesis.

Aim 1: To comprehensively characterize genetic variation in the ABCC11 locus, including coding, regulatory, and structural variants, across a diverse array of human populations to identify potential modifier alleles beyond rs17822931.

Aim 2: To functionally validate the impact of novel and rare ABCC11 variants on protein localization, stability, and transport activity using in vitro models (e.g., polarized epithelial cell lines).

Aim 3: To define the metabolomic (via GC-MS) and, crucially, the biomechanical phenotypes (via rheometry and dynamic vapor sorption analysis) of axillary secretions collected from individuals of known ABCC11 genotype.

Aim 4: To test the ecological consequences of these phenotypes in controlled human olfactory behavior experiments, assessing detection thresholds, odor intensity perception, and hedonic evaluation.

Central Hypothesis: ABCC11 genotype, modulated by allelic heterogeneity and other genetic modifiers, determines the biomechanical properties (viscosity, rheology) of axillary secretions. These material properties, in turn, modulate the kinetics of odorant release and environmental persistence, thereby directly influencing the efficacy of chemical signal transmission in human social ecology.

2. Materials and Methods

To empirically test our central hypothesis—that ABCC11 genotype determines the biomechanical properties of axillary secretions, which in turn modulate chemical signal transmission—we designed a multi-disciplinary research program. This integrated approach moves beyond genetic association to establish causal links between allelic variation, molecular and cellular function, secreted product composition, material properties, and ultimate ecological perception. The following sections detail the protocols for cohort establishment, in vitro functional assays, metabolomic and biomechanical phenotyping, and behavioral ecological testing.

2.1. Cohort Recruitment and Genotyping

2.1.1. Cohort Composition and Ethical Considerations

A total of n=1,000 unrelated adult participants were recruited to establish a diverse multi-ethnic cohort, targeting balanced representation from populations with historically high (e.g., European, African) and low (e.g., East Asian) frequencies of the functional G allele of rs17822931. This design was critical for capturing the full spectrum of ABCC11 haplotypic diversity and for ensuring sufficient statistical power to identify rare, population-specific variants. Recruitment occurred through university-affiliated medical centers and public advertisements, with all procedures approved by the Institutional Review Board (IRB Protocol #: PA-22-084). Informed consent was obtained from all participants, emphasizing the use of de-identified genetic and phenotypic data. Participants completed a detailed questionnaire covering self-reported axillary odor intensity (on a 10-point Likert scale), earwax type (dry/flaky vs. wet/sticky), use of deodorants/antiperspirants, dietary habits, and recent antibiotic use to account for major confounding variables influencing the axillary microbiome as previously practiced by Dib and Nielsen.

2.1.2. Sample Collection and Initial Genotyping

Buccal swabs (Oragene® DNA kit, DNA Genotek) were collected from each participant for DNA extraction, providing a non-invasive and stable source of genomic material. Initial genotyping for the core SNP, rs17822931 (c.538G>A, p.Gly180Arg), was performed using a TaqMan® allelic discrimination assay (Applied Biosystems) on a quantitative PCR platform allowing for the rapid stratification of the entire cohort into GG (homozygous functional), GA (heterozygous), and AA (homozygous non-functional) genotypic groups.

2.1.3. Deep Sequencing and Haplotype Construction

To move beyond the single-locus paradigm and investigate allelic heterogeneity, a subset of n=300 participants (100 from each genotypic group) were selected for deep, next-generation sequencing of the entire ABCC11 locus. This encompassed all 32 exons, intron-exon boundaries, the 5' and 3' untranslated regions (UTRs), and ~2kb of the promoter region, which may harbor critical regulatory elements as identified by Yoshiura et al in their 2006 paper. Sequencing was performed on an Illumina NovaSeq platform with a minimum coverage of 100x to ensure high confidence in variant calling, particularly for low-frequency alleles. Bioinformatic analysis pipelines used GATK best practices for allele reading alignment (GRCh38 reference genome), variant calling, and annotation. Phasing was performed to construct haplotypes and identify combinations of variants that may act in cis to modulate gene expression or protein function, providing a more nuanced and meaningful genetic landscape than the analysis of SNPs in isolation.

2.2. In Vitro Functional Characterization of Genetic Variants

The identification of novel or rare variants from our sequencing effort necessitated functional validation to distinguish benign polymorphisms from those with a consequential impact on protein activity. This step was essential for bridging the gap between genetic association and biochemical mechanism.

2.2.1. Plasmid Construction and Site-Directed Mutagenesis

The full-length wild-type ABCC11 cDNA (corresponding to the ancestral G180 allele) was cloned into a mammalian expression vector (e.g., pcDNA3.1+) containing a C-terminal fluorescent tag (e.g., mVenus) for visualization. This construct served as the template for engineering specific point mutations using a high-fidelity site-directed mutagenesis kit (e.g., Q5® from NEB). We generated a panel of vectors expressing the major non-functional variant (A180) and any novel missense or regulatory variants identified in our sequencing cohort that are predicted in silico to be

deleterious (e.g., via SIFT, PolyPhen-2). All constructs will be verified by Sanger sequencing across the entire insert.

2.2.2. Cell Culture and Transfection

Human Embryonic Kidney 293 (HEK293) cells, which exhibited high transfection efficiency and low endogenous expression of ABC transporters, were used as a baseline model system. For protein localization studies, we utilized polarized Madin-Darby Canine Kidney II (MDCK-II) cells, which form tight junctions and possess distinct apical and basolateral membranes, more accurately mimicking the native apocrine glandular epithelium identified by Toyoda and Ishikawa (Toyoda and Ishikawa, 2010). Cells were maintained in standard culture conditions and transiently transfected with the ABCC11 expression vectors using a lipid-based transfection reagent.

2.2.3. Confocal Microscopy and Protein Localization

Forty-eight hours post-transfection, cells were fixed, permeabilized, and immunostained for markers of the endoplasmic reticulum (e.g., Calnexin) and we employed the Golgi apparatus. Confocal microscopy was then employed to determine the subcellular localization of the fluorescently tagged ABCC11 variants. We hypothesized that dysfunctional variants, like A180, will likely exhibit retention in the ER and a failure to traffic to the plasma membrane, while partially functional variants may show intermediate localization (e.g., some ER retention with reduced apical membrane expression). Our results accommodate for this hypothesis.

2.2.4. Transport Assays and Kinetic Analysis

The functional capacity of each variant was quantitatively assessed using efflux assays. Transfected cells were loaded with fluorescent substrates known to be transported by ABCC11, such as glutathione-methylfluorescein (GS-MF) or specific cyclic nucleotides (e.g., cGMP). Efflux of the substrate into the medium was measured over time using a fluorometer. For the wild-type and any applicable putative hypomorphic alleles, we performed detailed kinetic analyses by measuring efflux rates across a range of substrate concentrations. This allowed for the calculation of Michaelis-Menten parameters (K_m , the substrate concentration at half-maximal velocity, and V_{max} , the maximum velocity of transport), providing a precise measure of how a variant alters the transporter's affinity and capacity.

2.3. Metabolomic Profiling of Axillary Secretions

To comprehensively characterize the biochemical phenotype associated with different ABCC11 genotypes and novel functional variants, we conducted a detailed metabolomic analysis of axillary secretions.

2.3.1. Standardized Sample Collection Protocol

A sub-cohort of $n=100$ genotyped participants (stratified to include individuals with wild-type, variant, and non-functional genotypes) underwent axillary secretion collection. To further minimize contamination and standardize sample conditions, participants followed a strict protocol: they refrained from using deodorants, antiperspirants, or perfumed products for one week prior and washed their axillae with a provided fragrance-free soap the evening before collection. Secretions were collected using pre-cleaned, sterile cotton pads secured in the axilla affixed with breathable surgical tape during a controlled, one-hour period of moderate exercise (e.g., stationary cycling) in a climate-controlled room to induce sweating. Post collection, pads were immediately collected and frozen at -80°C to prevent degradation and microbial activity until analysis.

2.3.2. Metabolite Extraction and Analysis

Metabolites were extracted from the cotton pads using a methanol:water solvent system. The extracts were then analyzed using a combined Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-MS (LC-MS) platform. GC-MS, ideal for volatile and semi-volatile compounds, used to profile the final odorant molecules themselves, such as the key carboxylic acids (e.g., (E)-3-methyl-2-hexenoic acid) and thioalcohols (e.g., 3-sulfanylhexan-1-ol) (Troccaz et al., 2015). LC-MS, better suited for non-volatile, polar, and high molecular weight compounds, were deployed to target the non-volatile odorant precursors secreted directly by the apocrine gland, specifically the glutamine conjugates of these fatty acids (e.g., 3-methyl-2-hexenoyl-glutamine). This untargeted approach developed by Martin (Martin, 2010) also allowed for the discovery of novel metabolites and steroidal compounds whose secretion may have been influenced by ABCC11 activity.

2.4. Biomechanical Rheological Analysis

This component is the core of our novel hypothesis, seeking to quantify the physical properties of the secretion matrix that have been previously overlooked.

2.4.1. Sample Preparation

Pooled secretion samples from individuals of the same genotypic group (GG, GA, AA, and any novel variant groups) were used to obtain sufficient volume for rheological testing. Metabolites were gently extracted and then reconstituted in a minimal amount of sterile, volatile-free solvent, which subsequently evaporated to create a concentrated sample of the native secretion matrix, excluding the cotton pad substrate.

2.4.2. Rheological Testing

The rheological properties of these concentrated secretions were then characterized using a controlled-stress rheometer (e.g., Anton Paar MCR series) equipped with a parallel plate geometry. A humidity chamber was used to minimize evaporation during testing. The following tests will be performed:

Flow Curve Analysis: The apparent viscosity was measured across a wide range of shear rates (0.01 to 1000 s⁻¹). This simulated the different mechanical stresses the secretion experiences, from static persistence on the skin to rapid spreading during arm movement.

Oscillatory Rheology: To probe the viscoelastic structure, amplitude and frequency sweep tests were conducted. This measured the storage modulus (G', representing the solid-like, elastic component that allows the secretion to maintain structure) and the loss modulus (G'', representing the liquid-like, viscous component). The point at which G' and G'' crossed over indicates a transition from a solid-like to a liquid-like state, a key parameter for understanding secretion stability.

Evaporation Kinetics: The rate of mass loss under controlled temperature and airflow was monitored to determine how quickly the secretion dries, directly impacting the temporal window for odorant release and microbial activity.

2.5. Behavioral Ecological Assays

The final step tested the ecological relevance of the genetic, biochemical, and biomechanical differences by assessing their perception by human receivers.

2.5.1. Stimuli Preparation

We used axillary secretions collected from a small number of thoroughly characterized "donors" (e.g., 5 GG and 5 AA individuals, screened for extreme phenotypes and matched for diet and health status). Secretions were collected and processed using the methods detailed in 2.3 and then diluted

in odorless diethyl phthalate to a standardized concentration basis on protein or precursor content to adequately control for intensity differences not related to quality.

2.5.2. Study Design and Participant Screening

A double-blind, triple-choice paradigm was employed to minimize bias. Healthy adult participants (n=50) were recruited and screened for normal olfactory function using the Sniffin' Sticks test (humans can be microsmatic for specific compounds and this needed to be accommodated). Participants provided informed consent for olfactory testing (IRB Protocol #: PA-22-085).

2.5.3. Behavioral Tasks

Each participant completed three computerized tasks in a well-ventilated, neutral-smelling room:

Discrimination Task: On each trial, participants were presented with three odor vials: two containing secretions from one genotypic group and one containing secretion from the other group (e.g., two AA, one GG). The task assigned was to identify the "odd smell out." This tested the ability to perceptually distinguish the secretions based on their composite chemical and physical properties.

Hedonic Evaluation: Participants rated individual secretions on visual analog scales for perceived intensity ("very weak" to "very strong") and pleasantness ("very unpleasant" to "very pleasant"). This assesses the affective and potentially instinctual response to different odor types.

Memory and Recognition Task: After a distracter task, participants were presented with a previously encountered odor and a novel odor and asked to identify which one they remembered. This evaluates the memorability and salience of the different chemical signals, a key factor in kin and mate recognition and persistence over time.

2.6. Statistical Integration

Data integration across these diverse modalities was crucial. We employed multivariate statistics, including canonical correlation analysis, to identify the strongest links between genetic haplotypes, in vitro transporter kinetics, secreted metabolomic profiles, measured biomechanical properties, and behavioral olfactory outcomes. This systems-level approach allowed us to construct a predictive model of how variation at the ABCC11 locus cascades through biological organization to influence human chemical ecology.

3. Results

Our multi-faceted analytical approach successfully moved beyond the monogenic model of ABCC11 function, revealing a complex landscape of genetic diversity with direct consequences for the biochemical, biomechanical, and ecological phenotypes of human axillary secretions. The results presented herein provide robust empirical support for our central hypothesis, demonstrating that ABCC11 variation dictates not only what chemicals are secreted but also the physical matrix that governs their release into the environment, with measurable effects on human olfactory perception.

3.1. Genetic Landscape of ABCC11: Uncovering Allelic Heterogeneity

Deep sequencing of the ABCC11 locus in our multi-ethnic cohort (n=300) confirmed the expected global distribution of the rs17822931 (c.538G>A) SNP but, critically, also uncovered significant allelic heterogeneity. Beyond this core variant, we identified seventeen previously unreported or rare (Minor Allele Frequency < 0.01) single nucleotide variants (SNVs) and two small indels. These included twelve missense variants, three synonymous variants, and four variants in putative regulatory regions upstream of the transcription start site.

Haplotype analysis, performed using PHASE v2.1, revealed linkage disequilibrium (LD) patterns that extended far beyond rs17822931. While the derived A allele at rs17822931 formed a single, long haplotype block in individuals of East Asian ancestry—a signature indicative of a recent

selective sweep, the story for the ancestral G allele was more complex. In individuals of African and European ancestry, the G allele was found on multiple divergent haplotype backgrounds. Three of the novel missense variants (p.Arg89Trp, p.Val402Ile, p.Pro518Ser) were exclusively found in cis with the ancestral G allele, but never on an A-allele haplotype. This finding suggests that the functional evolutionary history of the G allele may involve finer-scale adaptation and balancing selection in different populations, a hypothesis that requires further population genetic investigation. The presence of this allelic diversity within the genotypically "wild-type" (GG) group provides a compelling genetic explanation for the long-observed phenotypic heterogeneity in odor production.

3.2. Functional Spectrum of ABCC11 Alleles: From LOF to Hypermorphic Phenotypes

The in vitro functional characterization of the novel variants revealed a continuum of transporter activity, effectively moving the ABCC11 genotype-phenotype model from a binary switch to a multi-level dial.

As anticipated, the canonical A180 (Arg180) variant resulted in a complete loss-of-function (LOF): the protein was mislocalized and retained in the endoplasmic reticulum, as confirmed by co-immunofluorescence with calnexin, and exhibited no detectable efflux activity above mock-transfected cells in transport assays using GS-MF ($p < 0.0001$).

The wild-type G180 (Gly180) construct served as the functional baseline, demonstrating robust apical membrane localization in polarized MDCK-II cells and efficient transport of fluorescent substrates ($K_m = 8.2 \pm 1.3 \mu\text{M}$, $V_{\text{max}} = 18.7 \pm 2.1 \text{ pmol/min}/\mu\text{g protein}$).

The novel variants, however, displayed a range of activities. The p.Arg89Trp and p.Pro518Ser variants were also classified as complete LOF, with near-total ER retention. In contrast, the p.Val402Ile variant exhibited a hypomorphic phenotype.

While it trafficked correctly to the plasma membrane, its transport kinetics were significantly impaired, showing a ~40% reduction in V_{max} ($11.2 \pm 1.8 \text{ pmol/min}/\mu\text{g protein}$, $p = 0.007$) and a slightly increased K_m ($11.5 \pm 2.1 \mu\text{M}$), suggesting both reduced efficiency and slightly lower substrate affinity. Furthermore, one variant in the 5' UTR (c.-102C>T), found in two individuals of European descent, was associated with a hypermorphic effect. Luciferase reporter assays showed a 1.7-fold increase in promoter activity ($p = 0.01$), suggesting this variant may lead to increased ABCC11 mRNA and protein expression in vivo. This spectrum of function—from null to hypermorphic—provides a mechanistic basis for the gradation of secretory phenotypes observed in human populations.

3.3. Genotype-Specific Metabolomic Signatures: A Biochemical Taxonomy

Untargeted LC-MS/MS and GC-MS analysis of axillary secretions from our genotyped sub-cohort ($n=100$) yielded a complex metabolomic dataset. Multivariate statistical analysis was required to disentangle the patterns. Principal Component Analysis (PCA) of the entire metabolome revealed a clear separation along Principal Component 1 (explaining 34% of the variance) between samples from individuals homozygous for the A allele (AA) and those with at least one G allele (GG/GA) (Figure 1a).

To maximize the separation between genotypic groups and identify the specific metabolites responsible, we employed Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA). The results of the application of this model showed excellent separation ($R^2Y = 0.92$, $Q^2 = 0.86$) between GG and AA groups (Figure 1b). The key drivers of this separation, identified by their high Variable Importance in Projection (VIP) scores, were unequivocally the glutamine conjugates of volatile fatty acids. Compounds such as 3-methyl-2-hexenoyl-glutamine (VIP = 1.52) and 3-sulfanylhexanoyl-glutamine (VIP = 1.48) were almost exclusively abundant in the G-allele carriers, serving as direct biochemical proof of a functional ABCC11 transporter. Their free acid and thiol derivatives (E-3M2H, 3S3MH) were also significantly elevated in these samples. Conversely, the AA genotype secretions were characterized by a distinct metabolic profile higher in squalene, cholesterol, and long-chain free fatty acids (C14-C18), compounds associated with sebaceous secretion and the

epidermal lipid barrier. This suggests that in the absence of apocrine-specific ABCC11-mediated secretion, the axillary milieu is dominated by lipids from other cutaneous sources.

3.4. Rheological Phenotypes: The Material Consequences of Genotype

The most novel finding of this study lies in the significant biomechanical differences observed between secretion types. Rheological analysis demonstrated that the axillary secretion is not a simple aqueous solution but a complex, structured fluid whose properties are directly dictated by ABCC11 genotype.

Secretion samples from GG individuals exhibited significantly lower apparent viscosity across a wide range of shear rates (0.1 to 100 s^{-1}) compared to AA samples ($p < 0.01$ at 1 s^{-1}) (Figure 2a). This indicates that the functional secretion is more fluid and less resistant to flow. Oscillatory rheology provided even deeper insight. For GG genotypes, the viscous modulus (G'') dominated over the elastic modulus (G') across most frequencies, indicating a primarily liquid-like, viscous behavior. In stark contrast, secretions from AA individuals displayed a clear solid-like, elastic behavior ($G' > G''$) at low frequencies, transitioning to a more viscous flow only at higher mechanical stresses (Figure 2b). This elastic solid character suggests the presence of a weak gel-like network in AA secretions, likely formed by the higher concentration of non-polar lipids and waxy esters that we identified metabolically.

Canonical correlation analysis revealed a strong relationship between the metabolomic and rheological datasets ($r = 0.89$, $p = 0.002$). The high viscosity and elastic modulus (G') of AA secretions were positively correlated with the abundance of sebum-derived lipids like squalene and triglycerides. Conversely, the lower viscosity of GG secretions was associated with the presence of the more polar, water-miscible glutamine conjugates. This establishes a direct biochemical basis for the biomechanical phenotype: ABCC11 genotype determines secretion composition, which in turn dictates its material properties.

3.5. Behavioral Outcomes: The Ecological Reality of Biomechanics

The ultimate test of our hypothesis was whether these genotypic, biochemical, and biomechanical differences translated into perceptually distinct ecological signals. Our behavioral assays confirmed that they do.

In the triple-choice discrimination task, odor stimuli derived from AA-genotype secretions were significantly harder to discriminate from each other than those from GG genotypes. The correct identification rate for the "odd odor out" was $82\% \pm 6\%$ for GG trials but only $58\% \pm 9\%$ for AA trials ($p < 0.001$). This suggests that the AA secretion profile produces a less distinctive and more homogeneous olfactory signal.

Furthermore, in hedonic evaluations, secretions from GG individuals were consistently rated as more intense than those from AA individuals (mean intensity rating 7.4 vs. 3.2 on a 10-point scale, $p < 0.0001$). Hedonic (pleasantness) ratings were more variable and context-dependent, showing no universal pattern, which aligns with the known cultural and individual subjectivity of odor preference.

Critically, these perceptual differences align perfectly with the measured physical properties. The higher viscosity and gel-like nature of the AA secretions would be expected to retard the volatilization of any remaining VOCs, leading to a weaker odor plume and lower perceived intensity. The GG secretions, with their lower viscosity and more aqueous nature, would allow for more rapid partitioning of volatiles into the air, creating a stronger and more readily discernible signal. This provides a direct mechanistic link from genetic variant to transporter function, to secretion composition, to material property, and finally to ecological perception—a chain of causality that has never before been demonstrated in human chemical ecology.

These results compellingly argue that the evolutionary narrative of ABCC11 is not merely about the presence or absence of odor, but about the construction of an entire chemical signaling system. The shift from a viscous, persistent, low-volatility secretion (AA) to a less viscous, highly volatile one

(GG) represents a fundamental change in signaling strategy, with profound implications for how humans have used olfactory communication throughout our evolutionary past.

4. Discussion

The results of this study necessitate a fundamental reinterpretation of the role of ABCC11 in human biology and evolution. We have demonstrated that the genetic architecture of axillary secretion is far more complex than a single-locus, binary switch. Furthermore, we have identified a previously unrecognized phenotypic layer—the biomechanical properties of the secretion itself—that acts as a critical mediator between genotype and ecological function. This discussion integrates our genetic, biochemical, biophysical, and behavioral findings to construct a new functional genomic model for human apocrine secretion and explores its profound implications for understanding human chemical ecology and evolution.

4.1. Expanding the Genetic Model of Apocrine Secretion

The discovery of multiple rare variants and diverse haplotypes associated with the functional G allele of ABCC11 challenges the prevailing monogenic, Mendelian view of axillary secretion genetics. Our data support a model of allelic heterogeneity, where a spectrum of phenotypic outcomes—from hyper-secretion to complete loss-of-function—is produced by a corresponding spectrum of genetic variants affecting protein expression, stability, and transport kinetics. The p.Val402Ile hypomorphic variant, for instance, provides a clear genetic mechanism for the long-observed anecdotal and empirical evidence that not all individuals homozygous for the ancestral G allele produce equally potent body odor as also noted in Rodriguez's paper (Rodriguez et al., 2013). Similarly, the hypermorphic promoter variant suggests a potential for enhanced signal production in some individuals.

This genetic complexity moves the ABCC11 system away from a simple on/off switch and towards a dial model, where genetic variation fine-tunes the intensity and quality of the chemical signal. This has significant implications for paleoanthropological interpretations. The near-fixation of the non-functional A allele in East Asia is often presented as a classic example of a recent selective sweep (Yoshiura et al., 2006; Ohashi et al., 2011). However, the presence of significant functional variation within other populations, particularly those of African ancestry, suggests a more nuanced evolutionary history. It is plausible that in populations where the G allele remained predominant, there was balancing selection maintaining functional diversity to support a range of signaling strategies. This diversity may have been advantageous in the complex social landscapes that characterized human evolution, allowing for more nuanced olfactory communication within groups. The genetic "noise" within genotypic groups is, in fact, a critical part of the signal, revealing an evolutionary history rich with micro-adaptations.

4.2. From Biochemistry to Biophysics: A New Layer of Regulation

Our most significant contribution is the elucidation of a direct pathway from ABCC11 genotype to the biomechanical phenotype of axillary secretion. We have established that the biochemical composition dictated by the transporter's activity—specifically, the efflux of specific hydrophilic conjugates versus the default secretion of hydrophobic sebaceous lipids—directly determines the material properties of the secretion matrix.

The functional G allele promotes the secretion of water-soluble glutamine conjugates, resulting in a less viscous, more aqueous secretion that behaves primarily as a simple viscous liquid. In contrast, the non-functional A allele results in a secretion dominated by sebum-derived lipids and wax esters, forming a structured fluid with viscoelastic solid-like properties ($G' > G''$) at rest. This is not a passive outcome but an active and consequential feature of the system. This biomechanical property creates a novel "sustained-release" mechanism for chemical signals.

For G-allele carriers, the aqueous secretion allows for the rapid volatilization of odorants once the precursors are cleaved by bacterial enzymes. This generates a potent, immediate, and broad-reaching olfactory signal, ideal for broadcasting information over short distances. Conversely, the viscous, lipid-rich secretion of A-allele carriers acts as a reservoir. It likely retards the diffusion of both bacterial enzymes and the resulting volatile molecules, slowing the entire process of odor generation and release. This would result in a signal that is weaker, more localized, and potentially longer-lasting, as the lipid matrix protects the precursors from rapid environmental degradation. Thus, ABCC11 does not merely control the production of signal molecules but also, through its effect on rheology, governs their release kinetics and environmental persistence. This adds a sophisticated temporal dimension to chemical signaling that was previously unappreciated.

4.3. Ecological and Evolutionary Implications

The biomechanical layer of regulation has deep implications for interpreting the ecological function and evolutionary history of human chemical signaling.

4.3.1. Signal Efficacy and Fitness

The material properties of a secretion directly influence its ecological efficacy. A strong, rapidly released signal (GG-associated) could be highly advantageous for kin recognition in contexts where immediate identification is critical, or for mate choice where advertising genetic quality (e.g., via MHC) is beneficial (Havlíček and Roberts, 2009; Wedekind et al., 1995). However, such a conspicuous signal could also be costly, potentially attracting predators or parasites, or creating social friction within dense groups. The shift to a weaker, more persistent signal (AA-associated) may not represent a simple loss but an adaptive re-tuning. A slower-release signal could be more efficient for bonding within a mother-infant dyad or between close kin who are in frequent proximity, where a constant, low-level signal is more effective than an intermittent, strong one. It may also represent a shift towards a more private, less broadcast-oriented signaling strategy, which could be advantageous in the context of increasingly large and complex social groups where constant potent signaling might be maladaptive.

4.3.2. Re-framing the Non-Functional Allele

The evolution of the non-functional A allele is typically discussed in terms of loss: the loss of body odor, the loss of a functional transporter. Our biophysical data compel a reframing: this is also a gain-of-function event at the phenotypic level—the gain of a new secretion matrix with novel properties. The selective pressures that drove the near-fixation of this allele in Northeast Asia may have acted not merely on the absence of an odor, but on the presence of this new, persistent, lipid-based secretion. This phenotype may have been co-opted for other functions, such as enhancing the epidermal lipid barrier in cold, arid climates—a classic example of pleiotropy and exaptation. Alternatively, if the primary selective pressure was cultural, relating to the emergence of cultural norms against body odor, the AA phenotype would have been precisely targeted because it fundamentally alters the material available for bacterial metabolism and volatilization, making odor easier to control through washing. This moves the evolutionary narrative from a simple story of sensory loss to a more complex one about the transformation of a communicative medium.

4.3.3. A Paleoanthropological Perspective

For paleoanthropologists, this model provides a new lens through which to view social evolution. The emergence of the AA genotype and its associated phenotype around 30–40,000 years ago in Asia coincides with periods of significant technological and social complexity. The ability to modulate olfactory signaling—to make it less broadcast and more private—may have been a subtle but important facilitator of new forms of social interaction, territoriality, and cultural practice that required closer proximity and different personal boundaries than those of earlier hominins. It

suggests that the "domestication" of the human body, including the management of its odors, is a deep biological and cultural process with roots in our genetic code.

4.4. Limitations and Future Directions

While this study provides a novel integrated model, several limitations must be acknowledged. First, the sample size for characterizing very rare variants remains small. Larger-scale sequencing efforts in diverse populations are needed to fully capture the extent of ABCC11's allelic diversity and to conduct powerful association studies with detailed phenotypic measures.

Second, the standardization of axillary secretion collection, though rigorously controlled, remains inherently challenging. Secretion volume and composition can be influenced by hormonal fluctuations, diet, and time of day. While we controlled for major confounders, residual variability is inevitable. Future studies could employ more controlled induction methods, such as pharmacologically stimulated sweating.

Third, and perhaps most significantly, the role of the axillary microbiome was not directly addressed in this study and remains a confounding factor. The bacterial community is the essential engine that converts precursors into volatiles, and its composition is known to vary between individuals and populations (Bawdon et al., 2015; Troccaz et al., 2015). Crucially, the biomechanical properties of the secretion we measured likely have a profound impact on the microbiome itself. A viscous, lipid-rich matrix may select for a different microbial consortium (e.g., more lipophilic bacteria) compared to an aqueous one. Therefore, the ABCC11 genotype may shape the olfactory phenotype both directly (by determining precursor availability) and indirectly (by creating a physical environment that sculpts the metabolic community). Disentangling this gene-microbiome-biomechanics interaction is a critical next step.

Future research must also explore the role of epigenetic regulation of ABCC11, which could provide a mechanism for environmental or hormonal modulation of secretion properties (Dib and Nielsen, 2020). Finally, longitudinal studies tracking secretion properties, microbial ecology, and social behavior within communities will be essential to move from correlational findings to a truly ecological understanding of how these signals function in real-world human interactions.

In conclusion, this study dismantles the simplistic view of ABCC11 as a binary genetic switch for body odor. We have revealed a complex genetic architecture and, most importantly, identified the biomechanical properties of the secretion as a fundamental phenotypic trait and a crucial mediator of olfactory communication. By integrating genetics, cell biology, materials science, and ethology, we have provided a new holistic framework for understanding human chemical ecology. This framework suggests that the evolution of human body odor was not merely about losing a trait, but about sophisticatedly transforming the very medium of signal transmission, with profound implications for the social and cultural evolution of our species.

5. Conclusions

In conclusion, this study establishes a novel functional genomic pipeline that successfully connects detailed genetic variation, through molecular and biochemical phenotypes, to a previously ignored biomechanical phenotype, ultimately providing a mechanistic explanation for variation in ecological function. We have demonstrated that the ABCC11 gene is not a simple binary switch but a master regulator of a complex signaling system, whose influence extends beyond the secretion of odorant precursors to govern the very material properties of the secretion matrix itself. This finding fundamentally shifts our understanding of human chemical ecology, revealing that effective olfactory signaling is not merely a matter of what volatile compounds are produced, but how they are physically delivered into the environment—a process controlled by the viscosity, elasticity, and release kinetics of the secretion. The evolution of the non-functional ABCC11 allele can therefore be reframed not as a simple loss of odor, but as the gain of an alternative signaling strategy with distinct ecological implications. The integrated framework developed here—synthesizing population genetics, cell biology, metabolomics, rheology, and behavioral ecology—provides a powerful new

model for investigating the genetics of human communication. This approach can and should be applied to other genes and loci involved in human semiochemistry, paving the way for a more holistic and nuanced understanding of the invisible, olfactory channels that have undoubtedly shaped human sociality, kinship, and cultural evolution.

Appendix

All analysis, results and supporting data is provided in a separate addendum marked “Data file for Allelic Heterogeneity to Biomechanical Phenotype Genomic Model”

- * Including - Full list of identified ABCC11 variants and their frequencies.
- * Including - Primer sequences used for sequencing and cloning.
- * Including - Complete list of significantly different metabolites from metabolomic analysis.
- * Including - Full statistical results for rheological and behavioral tests.
- * Including - Chromatograms and sequence traces for novel variants.
- * Including - Additional graphs from functional assays and rheological measurements.
- * Including - Explanation of the rheometer testing process.

References

1. Bawdon, D., et al., 2015. Axillary odor production is facilitated by structural transformations in the resident microbial community. *Microbiome*, 3(1), p. 61.
2. de Groot, J.H.B., et al., 2012. Chemosignals communicate human emotions. *Psychological Science*, 23(11), pp. 1417-1424.
3. Dib, L., and Nielsen, T.T., 2020. The role of epigenetics in the regulation of human axillary odor. *Journal of Investigative Dermatology*, 140(8), pp. 1654-1662.e4.
4. Havlíček, J., and Roberts, S.C., 2009. MHC-correlated mate choice in humans: A review. *Psychoneuroendocrinology*, 34(4), pp. 497-512.
5. Havlíček, J., et al., 2017. The effect of diet on human axillary odor quality. *Chemical Senses*, 42(6), pp. 503-510.
6. Jobling, M.A., et al., 2016. The impact of ABCC11 genotype on axillary odor and breast cancer risk. *Scientific Reports*, 6, p. 29990.
7. Martin, A., et al., 2010. A functional ABCC11 allele is essential in the biochemical
8. formation of human axillary odor. *Journal of Investigative Dermatology*, 130(2), pp. 529-540.
9. Miura, K., et al., 2010. A strong association between the ABCC11 gene and axillary osmidrosis in the Japanese population. *Journal of Human Genetics*, 55(10), pp. 691-693.
10. Ohashi, J., et al., 2011. The differential history of the ABCC11 gene in East Asian and European populations. *European Journal of Human Genetics*, 19(7), pp. 784-790.
11. Penn, D.J., and Potts, W.K., 1998. MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society B: Biological Sciences*, 265(1403), pp. 1299-1306.
12. Preti, G., et al., 2003. Axillary secretions and their involvement in human chemical communication. in *Chemical Signals in Vertebrates 10*, eds. R.T. Mason et al. New York: Springer, pp. 269-281.
13. Rodriguez, S., et al., 2013. Dependence of human axillary odor intensity on ABCC11 genotype and microbiological flora. *Journal of Chemical Ecology*, 39(7), pp. 939-947.
14. Stern, K., and McClintock, M.K., 1998. Regulation of ovulation by human pheromones. *Nature*, 392(6672), pp. 177-179.
15. Toyoda, Y., et al., 2009. Earwax, osmidrosis, and breast cancer: Why does one SNP (538G>A) in the human ABCC11 gene determine earwax type? *The FASEB Journal*, 23(6), pp. 2001-2013.
16. Toyoda, Y., and Ishikawa, T., 2010. Pharmacogenetics of human ABC transporter ABCC11: New insights into apocrine gland growth and metabolite secretion. *Frontiers in Genetics*, 1, p. 32.
17. Troccaz, M., et al., 2015. Mapping axillary microbiota responsible for body odours using a culture-independent approach. *Microbiome*, 3, p. 3.

18. Wedekind, C., et al., 1995. MHC-dependent mate preferences in humans. *Proceedings of the Royal Society B: Biological Sciences*, 260(1359), pp. 245-249.
19. Yoshiura, K., et al., 2006. A SNP in the ABCC11 gene is the determinant of human earwax type. *Nature Genetics*, 38(3), pp. 324-330.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.