Shared Genetic Risk Variants in Both Male and Female Frontal Fibrosing Alopecia

Journal of Investigative Dermatology (2023) **143,** 2311–2314; doi:10.1016/j.jid.2023.04.022

JID Open

TO THE EDITOR

Frontal fibrosing alopecia (FFA) is an increasingly prevalent variant of follicular lichen planus that affects predominantly postmenopausal women and is characterized by inflammation and cicatricial hair loss at eyebrows and frontotemporal and often occipital scalp, in addition to body regions. In female FFA, we have previously identified an association with genetic variation influencing xenobiotic metabolism, T-cell homeostasis, and antigen presentation at four susceptibility loci (Tziotzios et al., 2019). Estimates suggest that only 3–5% of FFA cases are male. Its pattern of scarring and scalp hair loss is consistent with the female presentation (Kanti et al., 2019; Vañó-Galván et al., 2014), although male FFA typically manifests earlier, and facial hair is evidently affected (Lobato-Berezo et al., 2022; Rayinda et al., 2022). To date, the genetic basis of male FFA has not been explored. Because male FFA shares clinical features with the female presentation, we hypothesized that a shared genetic architecture exists between the sexes. We therefore investigated the existence of a genetic association in male FFA at loci previously implicated in female FFA, both individually and as a combined genetic risk score.

Ethical approval for this study was obtained from the Northampton NRES Committee, United Kingdom (Research Ethics Committee 5/EM/0273), and the study was conducted in accordance with the Helsinki Declaration. European ancestry male FFA cases recruited from dermatology clinics in the United Kingdom, United States, and Germany formed a Northern European cohort. Two additional European ancestry cohorts were established in Spain and Greece. Diagnoses of FFA were made by a consultant dermatologist on the basis of clinical features and histological confirmation, if required. All participants provided written informed consent.

A total of 92 male FFA cases from the three cohorts were genotyped using Infinium OmniExpressExome BeadChip array (Illumina, San Diego, CA) and were combined with genotyping data from unselected, ancestry-matched male controls (n = 330) (Supplementary Materials and Methods). After genotype imputation, case-control association testing was performed for the lead variant at each of four female FFA susceptibility loci (rs1800440 [*CYP1B1* locus], rs2523616 [*HLA-B*], rs760327 [*ST3GAL1*], and rs34560261 [*SEMA4B*]) (Supplementary Table S1) and at 28 loci with a suggestive association in female FFA ($P < 1 \times 10^{-5}$). Association testing was performed separately in each of the three cohorts and together in a fixed-effect meta-analysis.

The largest single risk locus in female FFA is the *HLA-B*07:02* allele, which confers an approximately fourfold increase in risk. It has been hypothesized that *HLA-B*07:02* contributes to FFA pathogenesis by facilitating the presentation of follicular autoantigens and the destruction of stem cells that reside in the hair follicle bulge (Harries et al., 2013; Tziotzios et al., 2019). In our meta-analysis, evidence of a genome-wide significant association with male FFA is observed for rs2523616, which lies within the major histocompatibility complex (OR = 3.95, 95% confidence interval [CI] = 2.48–6.29, $P = 6.9 \times 10^{-9}$). Although there is some evidence of heterogeneity in the observed effect size between the three male cohorts, the overall estimated effect of rs2523616 is consistent with the effect size reported in females (OR_{female} = 4.73, CI = 4.15–5.39) (two-sample z-test for difference, $P = 0.45$) (Supplementary Table S5). Furthermore, we evaluated the association between classical major histocompatibility complex class I alleles and FFA on the basis of the imputation of major histocompatibility complex class I alleles. As in females, the strongest evidence of association was identified for the *HLA-B*07:02* (OR = 3.01, CI = 1.87–4.84, $P = 5.4 \times 10^{-6}$).

There is further evidence of shared allelic architecture at the FFA susceptibility locus at 2p22.2, where strong evidence implies that a missense variant in *CYP1B1* (rs1800440, p.Asn453Ser) increases female FFA risk. Our meta-analysis also shows an association for this variant with male FFA (OR = 2.36, CI = 1.40–3.98, $P = 1.2 \times 10^{-3}$) (Table 1 and Figure 1a). *CYP1B1* encodes a microsomal enzyme, termed xenobiotic monooxygenase or aryl hydrocarbon hydroxylase, which is crucial for estrogen catabolism and is widely expressed across several human tissues (Shah et al., 2019). It also plays an important role in the metabolism of other xenobiotics and the hydroxylation of testosterone and progesterone (Kurzawski et al., 2012; Shimada et al., 1999). The biological implications of the association of this variant with male FFA are as yet unclear, although similar to that in the female disease, it may also suggest that disrupted *CYP1B1*-mediated metabolism of endogenous or exogenous substrate(s) could play a role in male FFA (Tziotzios et al., 2019).

The lead variants at each of the remaining two female FFA risk loci do not show evidence of an association with male FFA in our meta-analysis (OR = 1.13, CI = 0.80–1.60. $P = 0.48$ for *ST3GAL1* and OR = 1.22, CI = 0.76–1.95, $P = 0.40$ for *SEMA4B*). These findings could imply divergence in the genetic etiology of male and female FFA; however, we note our study's limited

Abbreviations: CI, confidence interval; FFA, frontal fibrosing alopecia

Accepted manuscript published online 19 May 2023; corrected proof published online 14 June 2023

Table 1. Meta-Analysis of Genome-Wide Significant Loci for Female FFA Performed in Three Cohorts of White-European Male FFA

Locus	SNPs	Candidate Gene	Base Position (hg19)	RA	PA	Female FFA OR (95% CI)	Male FFA Northern European Cohort		Male FFA Spanish Cohort		Male FFA Greek Cohort		Meta-Analysis			
							OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value	Het-I^2	Het-*P*-Value
2p22.2	rs1800440	*CYP1B1*	38,298,139	T	C	1.65 (1.43−1.91)	3.02 (1.34 − 6.78)	7.5×10^{-3}	1.77 (0.75−4.17)	0.19	2.42 (0.77 − 7.54)	0.13	2.36 (1.40 − 3.98)	1.2×10^{-3}	0	0.67
6p21.1	rs2523616	*HLA-B*	31,320,562	T	C	4.73 (4.15−5.39)	2.92 (1.64 − 5.20)	2.8×10^{-4}	11.97 (4.68 − 30.61)	2.2×10^{-7}	1.96 (0.47 − 8.13)	0.35	3.95 (2.48 − 6.29)	6.9×10^{-9}	72.8	0.02
8q24.22	rs760327	*ST3GAL1*	134,503,229	G	C	1.34 (1.21−1.49)	1.03 (0.65 - 1.64)	0.90	0.97 (0.52 − 1.79)	0.92	2.68 (0.99 − 7.29)	0.05	1.13 (0.80 − 1.60)	0.48	38.8	0.19
15q26.1	rs34560261	*SEMA4B*	90,734,426	T	C	1.52 (1.22−1.74)	1.12 (0.60 − 2.08)	0.72	1.24 (0.54 − 2.83)	0.60	2.00 (0.43 − 9.28)	0.37	1.22 (0.76 − 1.95)	0.40	0	0.78

Abbreviations: CI, confidence interval; FFA, frontal fibrosing alopecia; PA, protective allele; RA, risk allele.

power to detect effects of a magnitude similar to that observed in female FFA (power < 80%) (Supplementary Table S2) and that the directionality of the effect-size estimates for both loci in male FFA is consistent with that of female disease. Similarly, we also note the consistent directionality of the effect-size estimates for 17 of the 28 loci with a suggestive association in female FFA (Supplementary Table S3).

To determine whether increased genetic liability for female FFA increases FFA risk in males, we calculated a series of additive genetic risk scores on the basis of the four significant ($P < 5 \times 10^{-8}$) and 28 suggestive ($P < 1 \times 10^{-5}$) female FFA susceptibility loci. The genetic risk score comprising four established female FFA loci was higher in male FFA cases than in the controls ($P < 0.05$) in each of the three cohorts (Figure 1b and Supplementary Table S4). There was no observed difference in the distribution of the genetic risk score comprising 28 suggestive female FFA loci between male FFA cases and controls in any cohort.

In summary, our results show the substantial effect of the *HLA-B*07:02* allele on FFA risk in males. There is also evidence to support the contribution of the p.Asn453Ser missense variant in *CYP1B1* in male FFA. These findings motivate further larger genetic studies of male FFA with increased statistical power to explore fully the genetic architecture of male FFA, which in comparison with that of female FFA could highlight shared and distinct aspects of male and female FFA pathobiology.

Data availability statement

The summary statistics generated from this study can be accessed in the GWAS Catalogue (https://www.ebi.ac.uk/gwas/), accession identification GCST90264153.

ORCIDs

Tuntas Rayinda: http://orcid.org/0000-0002-6492-4160
Sheila M. McSweeney: http://orcid.org/0000-0001-7977-6028
David Fenton: http://orcid.org/0009-0004-2141-1420
Catherine M. Stefanato: http://orcid.org/0000-0003 00526-3443
Ioulios Palamaras: http://orcid.org/0009-0002-0237-3647
Alice Tidman: http://orcid.org/0009-0003-0656-3509
Anastasia Koutalopoulou: http://orcid.org/0009-0004-3944-6349
Matthew Harries: http://orcid.org/0000-0002-0563-8690
Greg Williams: http://orcid.org/0000-0003-2765-5173
Sofia Papanikou: http://orcid.org/0000-0003-2881-5140
Vasiliki Chasapi: http://orcid.org/0000-0003-2168-3317
Sergio Vañó-Galván: http://orcid.org/0000-0003-2773-7494
David Saceda-Corralo: http://orcid.org/0000-0001-6315-5462
Ana Melián-Olivera: http://orcid.org/0000-0002-5037-4129
Carlos Azcarraga-Llobet: http://orcid.org/0000-0001-8088-4077
Alejandro Lobato-Berezo: http://orcid.org/0000-0001-8725-3999
Mariona Bustamante: http://orcid.org/0000-0003-0127-2860
Jordi Sunyer: http://orcid.org/0000-0002-2602-4110
Michela Valeria Rita Starace: http://orcid.org/0000-0002-3981-1527
Bianca Maria Piraccini: http://orcid.org/0000-0001-6537-9689
Isabel Pupo Wiss: http://orcid.org/0000-0003-1456-159X
Maryanne Makredes Senna: http://orcid.org/0000-0002-7813-520X
Rashmi Singh: http://orcid.org/0000-0003-4307-6139
Varvara Kanti-Schmidt: http://orcid.org/0000-0002-1784-5961
Kathrin Hillmann: http://orcid.org/0000-0001-7251-5351
Ulrike Blume-Peytavi: http://orcid.org/0000-0003-3528-3752
Michael Simpson: http://orcid.org/0000-0002-8539-8753
John A. McGrath: http://orcid.org/0000-0002-3708-9964
Nick Dand: http://orcid.org/0000-0002-1805-6278
Christos Tziotzios: http://orcid.org/0000-0003-1662-4494

CONFLICT OF INTEREST

CT is the principal and (national) chief investigator for the Pfizer-sponsored ALLEGRO study, has received speaker fees from LEO, and is a consultant for Pfizer. SMM and AK are subinvestigators for the Pfizer-sponsored ALLEGRO study. MMS has been a principal investigator for Eli Lilly, Leo, Santiste, CorEvitas, Follica, and Concert; has received speaker fees from Pfizer, Concert, and Eli Lilly; has served as an advisor to L'Oreal, Arena Pharma, Kintor, and AbbVie; has served on the board of directors of Scarring Alopecia Foundation and American Hair Research Society; and is a consultant for Eli Lilly, Pfizer, Kintor, L'Oreal, and Deciphera. UBP reports serving on the advisory board for AbbVie, CeraVe, Dermocosmétique Vichy, Galderma, Eli Lilly, Laboratoires Bailleuil, Neuroderm, Pfizer, Sanofi Regeneron, and Boots Healthcare, not related to this study.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to all patients who participated in this study. We also want to thank all clinicians who helped with this research. We are thankful to the 1958 British Cohort Study and the INfancia y Medio Ambiente project for providing population control genotyping data. This research has been conducted using the UK Biobank Resource under application number 15147. We thank our research technicians and research nurses for their assistance during this study, especially Rashida Pramanik, David Baudry, Alice White, Anne Thomson, and Silvia Sanchez. We want to thank Sonia Camaño

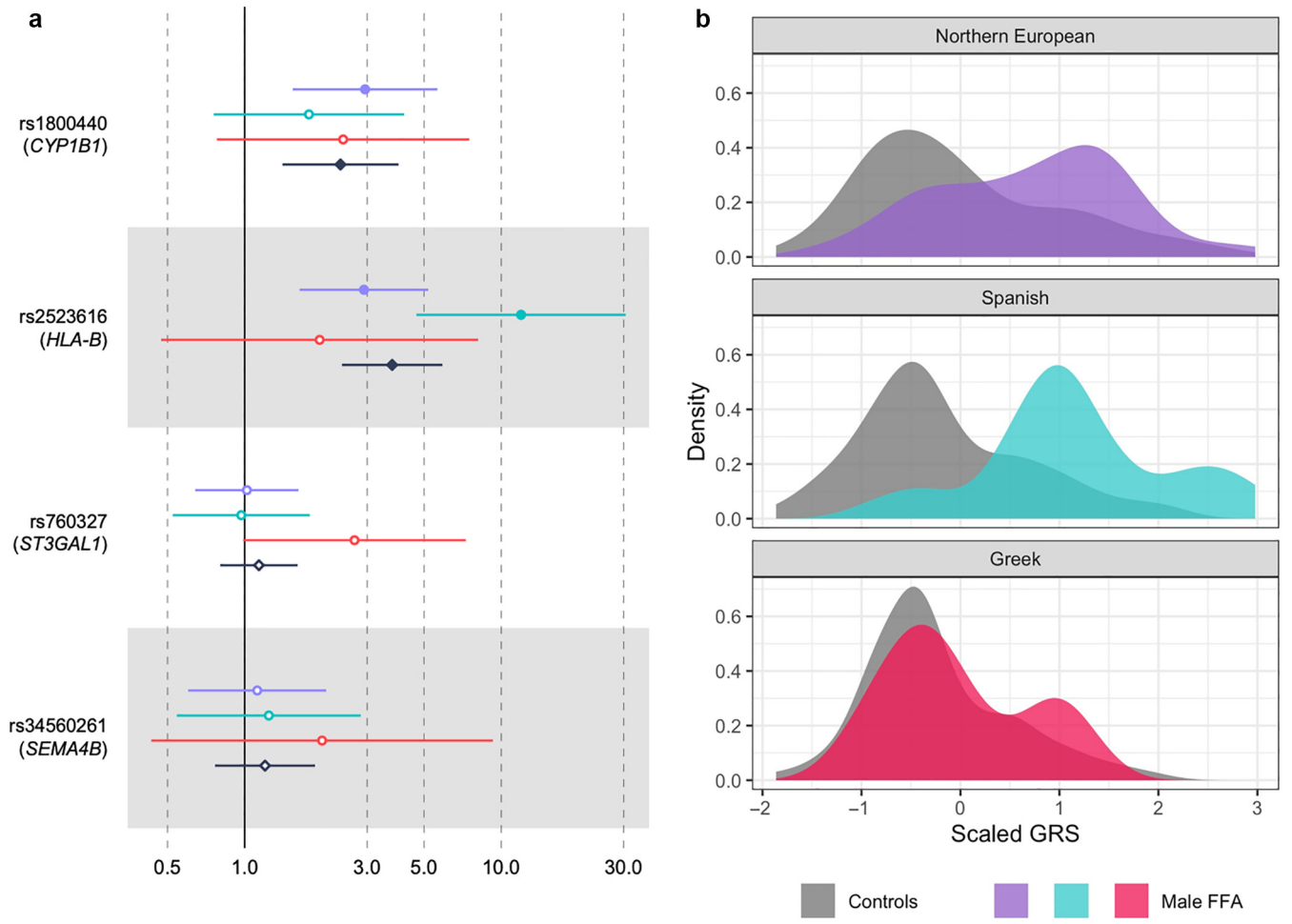

Figure 1. Shared allelic architecture at the FFA susceptibility loci. (**a**) Forest plot of candidate genes-association tests meta-analysis and (**b**) GRSs of male FFA calculated on the basis of four genome-wide significant associated loci for female FFA in three cohorts of White-European male FFA. FFA, frontal fibrosing alopecia; GRS, genetic risk score.

Páez and Ana Maria Torres from the Hospital Universitario Ramón y Cajal-IRYCIS Biobank (B.0000678), integrated in the Platform ISCIII Biobanks and Biomodels (PT20/00045) for their collaboration. We would also like to thank FFA Research Consortium, NIHR Rare Disease Translational Research Collaboration for their initial help with the frontal fibrosing alopecia study. This study is supported in part by the British Skin Foundation Young Investigator Award to CT and Small Research Grants by Alopecia UK to TR and CT. TR is funded by the Beasiswa Pendidikan Pascasarjana Luar Negeri DIKTI Scholarship, awarded by the Ministry of Education and Culture, Directorate General of Higher Education, Republic of Indonesia. MH is supported by the NIHR Manchester Biomedical Research Centre (NIHR2 03308).

AUTHOR CONTRIBUTIONS

Conceptualization: CT, JAM; Formal Analysis: TR, ND; Funding Acquisition: CT; Resources: DF, CMS, MH, IP, AT, SH, AK, MAJ, GW, SP, VC, SVG, DSC, AMO, CAL, ALB, MB, JS, MVRS, BMP, IPW, MMS, RS, KH, VKS, UBP; Investigation: TR, ND; Supervision: CT, JAM; Writing - Original Draft Preparation: TR; Writing – Review and Editing: SMM, ND, CT, JAM, MS.

Tuntas Rayinda[1,2], Sheila M. McSweeney[1], David Fenton[1], Catherine M. Stefanato[1], Matthew Harries[3,4], Ioulios Palamaras[5], Alice Tidman[6], Susan Holmes[6], Anastasia Koutalopoulou[7], Michael Ardern-Jones[7], Greg Williams[8], Sofia Papanikou[9], Vasiliki Chasapi[9], Sergio Vañó-Galvan[10], David Saceda-Corralo[10], Ana Melián-Olivera[10], Carlos Azcarraga-Llobet[10], Alejandro Lobato-Berezo[11], Mariona Bustamante[12], Jordi Sunyer[12], Michela Valeria Rita Starace[13,14], Bianca Maria Piraccini[13,14], Isabel Pupo Wiss[15], Maryanne Makredes Senna[15], Rashmi Singh[16], Kathrin Hilmann[16], Varvara Kanti-Schmidt[16,17], Ulrike Blume-Peytavi[16], Michael Simpson[18,19], John A. McGrath[1,19], Nick Dand[18,19] and Christos Tziotzios[1,19,]*

[1]*St John's Institute of Dermatology, School of Basic & Medical Sciences, King's College London, London, United Kingdom;* [2]*Department of Dermatology and Venereology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia;* [3]*Centre for Dermatology Research, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom;* [4]*Salford Royal Hospital, Northern Care Alliance NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom;* [5]*Barnet and Chase Farm Hospitals, Royal Free NHS Foundation Trust, London, United Kingdom;* [6]*Alan Lyell Centre for Dermatology, Queen Elizabeth University Hospital, Glasgow, United Kingdom;* [7]*Department of Dermatology, University Hospitals Southampton NHS Foundation Trust,*

Southampton, United Kingdom; [8]*Farjo Hair Institute, London, United Kingdom;* [9]*Department of Dermatology and Venereology of NHS, Andreas Syggros Hospital of Skin and Venereal Diseases, Athens, Greece;* [10]*Trichology Unit, Dermatology Department, Ramon Y Cajal Hospital, IRYCIS, University of Alcala, Madrid, Spain;* [11]*Servicio de Dermatología, Hospital del Mar-Parc de Salut Mar, Barcelona, Spain;* [12]*Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain;* [13]*Dermatology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [14]*Department of Medical and Surgical Sciences, Alma Mater Studiorum - University of Bologna, Bologna, Italy;* [15]*Harvard Medical School and Lahey Hospital and Medical Center, Boston, Massachusetts;* [16]*Department of Dermatology, Venereology and Allergology, Charité-Universitätsmedizin Berlin, Berlin, Germany;* [17]*Department of Dermatology, Venerology, Allergology and Phlebology, Johannes Wesling Medical Centre, University Hospital of the Ruhr University of Bochum, Minden, Germany; and* [18]*Department of Medical and Molecular Genetics, King's College London, London, United Kingdom*

[19]*These authors contributed equally to this work.*

[*]*Corresponding author. e-mail: christos.tziotzios@kcl.ac.uk*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2023.04.022.

REFERENCES

Harries MJ, Meyer K, Chaudhry I, E Kloepper J, Poblet E, Griffiths CEM, et al. Lichen planopilaris is characterized by immune privilege collapse of the hair follicle's epithelial stem cell niche. J Pathol 2013;231:236–47.

Kanti V, Constantinou A, Reygagne P, Vogt A, Kottner J, Blume-Peytavi U. Frontal fibrosing alopecia: demographic and clinical characteristics of 490 cases. J Eur Acad Dermatol Venereol 2019;33:1976–83.

Kurzawski M, Dziedziejko V, Post M, Wójcicki M, Urasińska E, Miętkiewski J, et al. Expression of genes involved in xenobiotic metabolism and transport in end-stage liver disease: up-regulation of ABCC4 and CYP1B1. Pharmacol Rep 2012;64:927–39.

Lobato-Berezo A, Iglesias-Sancho M, Rodríguez-Lomba E, Mir-Bonafé JF, Velasco-Tamariz V, Porriño-Bustamante ML, et al. Frontal fibrosing alopecia in men: a multicenter study of 39 patients. J Am Acad Dermatol 2022;86:481–4.

Rayinda T, McSweeney SM, Dand N, Fenton DA, McGrath JA, Tziotzios C. Clinical characteristics of male frontal fibrosing alopecia: a single-centre case series from London. UK. Br J Dermatol 2022;186:195–7.

Shah BR, Xu W, Mraz J. Cytochrome P450 1B1: role in health and disease and effect of nutrition on its expression. RSC Adv 2019;9:21050–62.

Shimada T, Watanabe J, Kawajiri K, Sutter TR, Guengerich FP, Gillam EM, et al. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 1999;20:1607–13.

Tziotzios C, Petridis C, Dand N, Ainali C, Saklatvala JR, Pullabhatla V, et al. Genome-wide association study in frontal fibrosing alopecia identifies four susceptibility loci including HLA-B*07:02. Nat Commun 2019;10:1150.

Vañó-Galván S, Molina-Ruiz AM, Serrano-Falcón C, Arias-Santiago S, Rodrigues-Barata AR, Garnacho-Saucedo G, et al. Frontal fibrosing alopecia: a multicenter review of 355 patients. J Am Acad Dermatol 2014;70:670–8.

Antigen Protease Activity with a Detergent Induces Severe Skin Inflammation with Itch and Robust T Helper 17/T Helper 22 Differentiation in Mice

Journal of Investigative Dermatology (2023) **143,** 2314–2318; doi:10.1016/j.jid.2023.03.1685

TO THE EDITOR

We previously reported some models of epicutaneous (e.c.) sensitization of C57-BL/6 mice with the model protease antigen, papain (Iida et al., 2014; Ochi et al., 2017; Shimura et al., 2016), which is an occupational allergen that belongs to the same cysteine protease family as the house dust mite major protease allergens (Takai and Ikeda, 2011) and the staphylococcal cysteine proteases (Williams et al., 2020). However, our previous models did not show the promotion of chronic itch–induced scratching behaviors 1 day after the last e.c. administration of papain. Therefore, in this study, we established and characterized a model of sensitization with papain through detergent-treated skin, which showed severely exacerbated skin inflammation with itch, the induction of antigen-specific IgE, and the differentiation of a number of T helper (Th) subsets. We also identified the responses that were dependent on the protease activity of papain. All animal experiments were approved by the Committee on Animal Experiments of Juntendo University (Tokyo, Japan).

SDS is a detergent present in shampoo and body soap that is used on a daily basis (Masutani et al., 2022). We modified our previous model with treatment with 4% SDS just before the application of papain (Ochi et al., 2017) and established the present model with a daily 10% SDS treatment of a wide ear skin area with increasing volumes of SDS and papain. The treatment with SDS plus papain induced an earlier increase in ear thickness than treatment with SDS plus vehicle, and papain-specific IgE was produced in a dose-dependent manner (Supplementary Figure S1). The treatment with SDS plus papain (10 mg/ml papain) induced more severe ear swelling with skin inflammation, greater transepidermal water loss, and more frequent hind-paw scratching behavior than treatment with SDS plus vehicle. Histology showed epidermal hyperplasia and swelling of the dermis with the infiltration of neutrophils and eosinophils in mice treated with SDS plus

Abbreviations: DLN, draining lymph node; e.c., epicutaneous; OVA, ovalbumin; Th, T helper

Accepted manuscript published online 23 May 2023; corrected proof published online 22 July 2023

SUPPLEMENTARY MATERIALS AND METHODS

Participants

Ethical approval was granted by the Northampton NRES Committee, United Kingdom (Research Ethics Committee 15/EM/0273). We established three independent cohorts of male frontal fibrosing alopecia (FFA), diagnosed by specialist dermatology clinics in the United Kingdom, United States, Germany, Spain, and Greece. All recruited cases were White European in descent and were diagnosed with FFA by a consultant dermatologist. The diagnosis of FFA was established using the following clinical and histopathological criteria: (i) cicatricial alopecic involvement of the frontal and temporal/parietal hair margins; (ii) bilateral eyebrow loss; (iii) clinical, trichoscopic (or histological) evidence of lichenoid perifollicular inflammatory presence; (iv) facial or body hair loss; (v) absence of multifocal scalp involvement; and other signs suggestive of classic lichen planopilaris or its Graham-Little-Piccardi-Lasseur subvariant.

DNA extraction

Blood or saliva samples were taken from eligible participants after informed consent. The blood extraction procedure was performed by following the protocol for DNA purification from whole blood with the QIAamp Blood Maxi Kit (Qiagen, Hilden, Germany). The final elution of 500 μl DNA was transferred into autoclaved 1 ml Eppendorf tubes. Saliva samples were collected with the Oragene DNA Saliva Kit (DNA Genotek, Kanata, Canada), and DNA was extracted according to the manufacturer's instructions, resulting in a final elution of 200 μl DNA. The Qubit Fluorometer (Q32857, Thermo Fisher Scientific, Waltham, MA) was used to quantify extracted DNA using the Qubit dsDNA broad-range Assay Kit in accordance with the manufacturer's instructions.

Genotyping and quality controls

Samples from the Northern European cohort (United Kingdom, United States, and Germany) underwent genome-wide genotyping using the Infinium OmniExpressExome BeadChip array (Illumina, San Diego, CA). Variant calling was performed using GenomeStudio, and quality control (QC) was conducted by applying a filter with a sample call rate >99% and by manually inspecting variants with outlying GenTrain score, cluster separation, haploid genome anomalies, Mendelian error, replication error, and genotype frequency in accordance with the protocol outlined by Guo et al. (2014). We used an unselected male cohort from the 1958 British Cohort Study (https://www.metadac.ac.uk/1958bc) and genotyped with the Infinium HumanHap 550K, version 3 (Illumina). A variant was retained if it had been analyzed with the same probe design on both genotyping arrays, and we checked for potential strand flips and differential missingness between cases and controls. A variant was excluded if it had a call rate below 99% or if its minor allele frequency was <0.01. In addition, individuals with a call rate <99% or extensive heterozygosity were excluded. A subset of 78,970 variants in linkage equilibrium ($r^2 < 0.2$ between each pair) was applied to evaluate relatedness between individuals using the KING software package (KING, version 2.2.5). We therefore excluded individuals with estimated relatedness closer than a third degree from the study (Kinship coefficient > 0.0442). A principal component analysis was conducted on the subset of 78,640 variants, and individuals outside the main cluster (implying non-European ancestry) were also excluded.

The genotyping of the Spanish male FFA cohort was performed using the OmniExpressExome BeadChip array (Illumina), alongside Northern European samples, and using the same variant-calling procedure and QC steps. Genotype data for unaffected controls were obtained from 1,061 individuals from the INfancia y Medio Ambiente project (Valencia, Sabadell and Menorca, Spain; http://www.proyectoinma.org) genotyped on the Omni1-Quad BeadChip (Illumina). The procedure for case-control merging and QC followed the same protocol as for the Northern European cohort.

The genotyping of the Greek male FFA cohort was also performed using the OmniExpressExome BeadChip array (Illumina), alongside the Northern European and Spanish samples, and using the same variant-calling procedure and QC steps. Controls were selected from male UK Biobank (https://www.ukbiobank.ac.uk) participants who reported their country of birth as Greece (data-field 20115) and who did not have an indication of scarring alopecia on the basis of data-field 131785. Genotype data were generated for UK Biobank participants by Affymetrix using the Applied Biosystems UK BiLEVE Axiom Array or the Applied Biosystems UK Biobank Axiom Array (Applied Biosystems, Waltham, MA), and initial QC was performed centrally by the UK Biobank analysis team (Bycroft et al., 2018). Although the different array types prevented merging FFA cases and controls using variants with matching probe sequences, we were able to merge using variants typed on both case and control arrays and (using a wider set of non—British European samples) confirmed that principal component analysis reflected ancestry differences and not batch effects. Post-merging QC steps used the same protocol as described for the Northern European cohort across all variants.

Imputation

Separate genome-wide imputation of all three case-control cohorts was then performed using the Michigan Imputation Server, using the reference panel from the Haplotype Reference Consortium-r1.1 GRCh37/hg19 (www.haplotype-referenceconsortium.org). The phasing was performed using Eagle, version 2.4, and imputation was undertaken with Minimac4 1.0.2. This process of data generation and QC resulted in a combined total of 92 cases and 330 controls.

Candidate genes-association test

Candidate genes-association test of the four established female FFA susceptibility loci (rs1800440, rs2523616, rs760327, and rs34560261) and 28 loci with suggestive evidence of association in females ($P < 1 \times 10^{-5}$) (Tziotzios et al., 2019) were performed in PLINK2.0 on the basis of 44 affected males and 160 male controls from the Northern European cohort and separately for 31 affected males and 120 male controls from the Spanish cohort and 17 affected males and 50 male controls from the Greek cohort. Association tests were performed using logistic regression under an additive genetic model on the basis of risk allele

genotype or imputed genotype dosage and incorporating principal components as covariates to control for residual population structure (number chosen per cohort by inspection of scree plots; Northern European: 2, Spanish: 1, Greek: 2).

Meta-analysis

Fixed-effects standard error–weighted meta-analysis was performed using METAL (http://csg.sph.umich.edu/abecasis/Metal/index.html) on the basis of association summary statistics at each candidate gene locus across the Northern European, Spanish, and Greek cohorts. Comparison with previously established female FFA effect sizes was made using the two-sample z-test.

HLA-alleles imputation and association test

Classical HLA alleles were imputed against the four-digit multiethnic HLA v1 using the Michigan Imputation Server (Luo et al., 2021). HLA-alleles association tests were performed on HLA alleles with allele frequency >5% in all three male FFA cohorts under similar logistic regression models and conditions as described in non-HLA SNPs association test. Meta-analysis was conducted on all HLA alleles present in all three cohorts.

Statistical power calculation

Statistical power for quantitative traits of case control for genetic association studies was evaluated using the genpwr R package (https://cran.r-project.org/web/packages/genpwr/index.html) under an additive genetic model.

Genetic risk score

In each of the three cohorts, three genetic risk scores (GRS) were calculated on the basis of (i) lead variants for the four loci determined to be genome-wide significant ($P < 5 \times 10^{-8}$) in female FFA (i.e., those included in the candidate gene study), (ii) lead variants at 28 loci that were suggestive ($P < 1 \times 10^{-5}$) but not genome-wide significant, and (iii) all 32 genome-wide significant or suggestive variants. The GRSs were calculated by applying linear scoring in PLINK, weighted by each variant's effect size estimate in female FFA (Tziotzios et al., 2019). For comparability and ease of interpretation, each of the three types of GRS was rescaled, using the same adjustment in Northern European, Spanish, and Greek cohorts, such that the scaled GRS had a mean of 0 and an SD of 1 in the Northern European control group (i.e., largest control group). For consistency when rescaling, genotype dosage was mean imputed from the Northern European cohort for GRS variants that were unavailable (not imputed) in Spanish (n = 1) and Greek (n = 3) datasets. Statistical analyses to compare scores between cases and controls were conducted using R, and normality tests were performed before the comparative statistical test. To compare groups with normally distributed data, independent *t*-tests were used, whereas Mann–Whitney *U* tests were performed for non-normally distributed data.

SUPPLEMENTARY REFERENCES

Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK biobank resource with deep phenotyping and genomic data. Nature 2018;562:203–9.

Guo Y, He J, Zhao S, Wu H, Zhong X, Sheng Q, et al. Illumina human exome genotyping array clustering and quality control. Nat Protoc 2014;9:2643–62.

Luo Y, Kanai M, Choi W, Li X, Yamamoto K, Ogawa K, et al. A high-resolution HLA reference panel capturing global population diversity enables multi-ethnic fine-mapping in HIV host response [published correction appears in Nat Genet 2021;53:1722] Nat Genet 2021;53:1504–16.

Tziotzios C, Petridis C, Dand N, Ainali C, Saklatvala JR, Pullabhatla V, et al. Genome-wide association study in frontal fibrosing alopecia identifies four susceptibility loci including HLA-B*07:02. Nat Commun 2019;10:1150.

Supplementary Table S1. Imputation Score of Candidate Genes Based on All Genome-Wide Significant and Suggestive Loci for Female FFA in Three Cohorts of White-European Male FFA

				Northern European Cohort		Spanish Cohort		Greek Cohort	
SNPs	Gene	Chr	Base Position	Genotyped/ Imputed	Imputation R^2	Genotyped/ Imputed	Imputation R^2	Genotyped/ Imputed	Imputation R^2
rs1800440	*CYP1B1*	2	38,298,139	Genotyped	0.99	Imputed	0.97	Genotyped	0.99
rs2523616	*HLA-B*	6	31,320,562	Imputed	0.93	Imputed	0.92	Imputed	0.89
rs760327	*ST3GAL1*	8	134,503,229	Imputed	0.98	Imputed	0.97	Imputed	0.61
rs34560261	*SEMA4B*	15	90,734,426	Imputed	0.89	Imputed	0.79	Imputed	0.50
rs6731286	None	2	62,839,819	Imputed	0.98	Imputed	0.98	Imputed	0.90
rs13032164	None	2	151,760,452	Imputed	0.96	Imputed	0.95	Imputed	0.50
rs78504246	None	2	213,746,962	Imputed	0.54	Imputed	0.80	No data	No data
rs116806118	None	3	23,165,701	Imputed	0.99	Imputed	0.70	No data	No data
rs1461070	None	3	30,741,506	Imputed	0.99	Imputed	0.95	Imputed	0.63
rs114108912	*CNTN3*	3	74,464,791	Imputed	0.92	Imputed	0.95	No data	No data
rs13078360	*LINC02016*	3	127,074,825	Imputed	0.85	Imputed	0.78	Imputed	0.26
rs183175	*STK32B*	4	5,428,385	Imputed	0.96	Imputed	0.91	Imputed	0.43
rs73062921	*MYO10*	5	16,859,464	Imputed	0.95	Imputed	0.89	Imputed	0.62
rs10045403	*ERAP1*	5	96,147,733	Imputed	0.99	Imputed	0.99	Imputed	0.92
rs148661203	None	6	10,220,442	Imputed	0.90	No data	no data	Imputed	0.48
rs142366299	None	6	52,179,367	Imputed	0.84	Imputed	0.78	Imputed	0.71
rs34097647	None	6	151,430,073	Imputed	0.90	Imputed	0.77	Imputed	0.59
rs6975452	None	7	3,160,511	Imputed	0.98	Imputed	0.96	Imputed	0.77
rs7806494	*SDK1*	7	3,893,629	Imputed	0.93	Imputed	0.91	Imputed	0.67
rs112115472	*LOC107986770*	7	13,645,859	Imputed	0.97	Imputed	0.77	Imputed	0.34
rs2021162	*GRM8*	7	126,528,455	Imputed	0.95	Imputed	0.92	Imputed	0.36
rs277580	None	9	34,771,875	Genotyped	0.99	Genotyped	0.99	Genotyped	0.99
rs2773871	None	9	124,873,393	Imputed	0.98	Imputed	0.97	Imputed	0.81
rs117687547	*CAMK1D*	10	12,504,313	Imputed	0.88	Imputed	0.85	Imputed	0.91
rs112198986	*KIAA1217*	10	24,576,259	Imputed	0.84	Imputed	0.68	Imputed	0.60
rs111463574	*NDUFA12*	12	95,399,358	Imputed	0.99	Imputed	0.99	Imputed	0.78
rs10507508	None	13	42,969,782	Imputed	0.97	Genotyped	1.00	Imputed	0.81
rs6540122	*BANP*	16	87,991,361	Genotyped	0.99	Genotyped	0.99	Imputed	0.39
rs8065764	None	17	21,006,462	Imputed	0.86	Imputed	0.72	Imputed	0.23
rs12951836	*EFCAB5*	17	28,410,277	Imputed	0.98	Imputed	0.98	Imputed	0.87
rs112659862	*CDC42EP4*	17	71,290,983	Imputed	0.99	Imputed	0.88	Imputed	0.62
rs79459566	*CACNA1A*	19	13,320,504	Imputed	0.93	Imputed	0.90	Imputed	0.46

Abbreviations: Chr, chromosome; FFA, frontal fibrosing alopecia.

Supplementary Table S2. Statistical Power Calculation for Candidate Genes-Association Test for Each FFA Genome-Wide Significant Susceptibility Loci in the Meta-Analysis

Locus	SNPs	Gene	Base Position	RA	PA	Female FFA OR (95% CI)	RAF	Number of Cases	Number of Control	Case Rate	Test Model	Power at Alpha 0.05, %
2p22.2	rs1800440	*CYP1B1*	38,298,139	T	C	1.65 (1.43–1.91)	0.47	92	330	0.22	Additive	84.35
6p21.1	rs2523616	*HLA-B*	31,320,562	T	C	4.73 (4.15–5.39)	0.87	92	330	0.22	Additive	99.69
8q24.22	rs760327	*ST3GAL1*	134,503,229	G	C	1.34 (1.21–1.49)	0.46	92	330	0.22	Additive	41.62
15q26.1	rs34560261	*SEMA4B*	90,734,426	T	C	1.52 (1.22–1.74)	0.22	92	330	0.22	Additive	43.45

Abbreviations: CI, confidence interval; FFA, frontal fibrosing alopecia; PA, protective allele; RA, risk allele; RAF, risk allele frequency.

Supplementary Table S3. Meta-Analysis of 28 Genome-Wide–Suggestive Loci for Female FFA

								Male FFA Northern European Cohort		Male FFA Spanish Cohort		Male FFA Greek Cohort		Meta-Analysis			
Chr	Gene	Consequences	Position (hg19)	SNPs ID	RA	PA	RAF Female FFA	OR	*P*-Value	OR	*P*-Value	OR	*P*-Value	OR	*P*-Value	Het-I^2	Het-*P*-Value
2	None	None	62,839,819	rs6731286	T	A	1.32	0.76	0.29	1.36	0.36	1.05	0.90	0.97	0.86	0.00	0.38
2	None	None	151,760,452	rs13032164	C	G	1.42	1.04	0.92	0.77	0.50	1.14	0.88	0.92	0.75	0.00	0.82
2	None	None	213,746,962	rs78504246	A	G	3.57	0.21	0.81	9.37	0.17	No data	No data	7.45	0.20	0.00	0.56
3	None	None	23,165,701	rs116806118	T	A	3.34	0.01	0.73	2.10	0.82	No data	No data	1.54	0.89	0.00	0.69
3	None	None	30,741,506	rs1461070	A	G	2.16	1.73	0.48	0.90	0.93	1.41	0.78	1.41	0.55	0.00	0.90
3	*CNTN3*	Intron variant	74,464,791	rs114108912	C	G	2.49	0.67	0.74	0.24	0.39	No data	No data	0.47	0.44	0.00	0.61
3	*LINC02016*	Intron variant	127,074,825	rs13078360	A	G	1.35	1.20	0.58	0.68	0.32	0.08	0.03	0.85	0.52	64.30	0.06
4	*STK32B*	Intron variant	5,428,385	rs183175	C	G	1.29	1.28	0.34	1.31	0.40	0.58	0.43	1.21	0.32	0.00	0.54
5	*MYO10*	Intron variant	16,859,464	rs73062921	A	T	1.41	0.88	0.73	1.25	0.61	1.09	0.92	1.03	0.91	0.00	0.83
5	*ERAP1*	Intron variant	96,147,733	rs10045403	G	A	1.33	1.31	0.34	1.34	0.40	0.68	0.43	1.18	0.40	0.00	0.46
6	None	None	10,220,442	rs148661203	T	C	2.96	4.40	0.11	No data	No data	2.43	0.84	4.29	0.11	0.00	0.89
6	None	None	52,179,367	rs142366299	T	C	2.34	1.18	0.90	0.83	0.87	1.22	0.90	3.24	0.98	0.00	0.97
6	None	None	151,430,073	rs34097647	T	C	1.36	0.70	0.29	0.46	0.09	1.58	0.51	0.68	0.14	9.00	0.33
7	None	None	3,160,511	rs6975452	T	A	1.29	0.87	0.56	0.90	0.72	1.04	0.94	0.90	0.54	0.00	1.00
7	*SDK1*	Intron variant	3,893,629	rs7806494	A	T	1.34	1.15	0.63	1.82	0.12	0.74	0.60	1.25	0.30	0.00	0.30
7	*LOC107986770*	Intron variant	13,645,859	rs112115472	C	T	2.66	2.04	0.35	0.51	0.59	0.04	0.27	1.19	0.78	0.00	0.78
7	*GRM8*	Intron variant	126,528,455	rs2021162	G	A	1.29	1.20	0.52	0.87	0.65	1.82	0.48	1.07	0.74	0.00	0.74
9	None	None	34,771,875	rs277580	G	A	1.44	0.90	0.74	0.56	0.14	1.26	0.69	0.80	0.33	0.00	0.84
9	None	None	124,873,393	rs2773871	G	A	1.37	1.32	0.43	1.39	0.36	0.97	0.95	1.27	0.29	0.00	0.47
10	*CAMK1D*	Intron variant	12,504,313	rs117687547	T	C	1.83	2.98	0.08	1.53	0.60	0.25	0.27	1.74	0.22	36.60	0.21
10	*KIAA1217*	Intron variant	24,576,259	rs112198986	T	C	1.82	0.34	0.23	2.08	0.45	0.20	0.58	0.74	0.64	3.60	0.35
12	*NDUFA12*	Upstream variant	95,399,358	rs111463574	C	T	1.72	1.09	0.84	0.48	0.45	0.24	0.22	1.27	0.55	13.80	0.28
13	None	None	42,969,782	rs10507508	G	A	1.66	2.65	0.03	0.52	0.55	1.81	0.55	2.06	0.06	0.00	0.38
16	*BANP*	Intron variant	87,991,361	rs6540122	T	C	1.34	1.00	0.99	0.72	0.47	0.91	0.92	0.88	0.64	0.00	0.85
17	None	None	21,006,462	rs8065764	C	T	1.56	0.92	0.85	1.06	0.92	4.24	0.82	1.11	0.75	0.00	0.43
17	*EFCAB5*	intron variant	28,410,277	rs12951836	G	A	1.28	0.65	0.07	0.99	0.98	0.90	0.82	0.79	0.17	0.00	0.51
17	*CDC42EP4*	Intron variant	71,290,983	rs112659862	C	T	3.62	3.63	0.37	0.56	0.77	0.19	0.62	1.48	0.72	0.00	0.61
19	*CACNA1A*	Intron variant	13,320,504	rs79459566	T	C	1.71	0.15	0.11	1.72	0.44	6.9×10^{-5}	0.36	0.88	0.72	49.90	0.14

Abbreviations: FFA, frontal fibrosing alopecia; ID, identification; PA, protective allele; RA, risk allele; RAF, risk allele frequency.

Supplementary Table S4. Comparison of Genetic Risk Score between Male FFA Cases and Controls Calculated on the Basis of Female FFA Susceptibility Loci

Reference Score	Cohort	Mean (±SD)		*P*-Value
		Male FFA	Control	
Genome-wide significant loci for female FFA (n loci = 4)	Northern European cohort	0.71 (±0.95)	0 (±1)	2×10^{-5}
	Spanish cohort	1.16 (±0.94)	−0.18 (±0.85)	1.6×10^{-9}
	Greek cohort	0.38 (±0.75)	−0.46 (±0.79)	0.04
Genome-wide suggestive significant loci for female FFA (n loci = 28)	Northern European cohort	0.05 (±0.17)	0 (±0.1)	0.79
	Spanish cohort	0.89 (±0.95)	1.1 (±0.93)	0.27
	Greek cohort	0.42 (±0.73)	0.65 (±0.90)	0.32
Genome-wide significant and suggestive significant loci for female FFA (n loci = 32)	Northern European cohort	0.47 (±0.88)	0 (±1)	5.0×10^{-3}
	Spanish cohort	1.48 (±0.98)	0.79 (±0.82)	9.0×10^{-4}
	Greek cohort	0.44 (±0.67)	0.26 (±0.77)	0.36

Abbreviations: FFA, frontal fibrosing alopecia.

Supplementary Table S5. Sensitivity Analysis of Candidate Gene-Association Test Result in the Northern European Cohort

Locus	SNPs	Candidate Gene	Base Position (hg19)	RA	PA	Female FFA OR (95% CI)	Northern European Cohort (n Cases = 44)		Sensitivity Analysis (Northern European Male FFA Recruited from UK, n Cases = 32)	
							OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value
2p22.2	rs1800440	*CYP1B1*	38,298,139	T	C	1.65 (1.43−1.91)	3.02 (1.34−6.78)	7.5×10^{-3}	3.69 (1.38−9.80)	8.81×10^{-3}
6p21.1	rs2523616	*HLA-B*	31,320,562	T	C	4.73 (4.15−5.39)	2.92 (1.64−5.20)	2.8×10^{-4}	2.68 (1.46−4.94)	1.46×10^{-3}
8q24.22	rs760327	*ST3GAL1*	134,503,229	G	C	1.34 (1.21−1.49)	1.03 (0.65−1.64)	0.90	0.95 (0.56−1.61)	0.86
15q26.1	rs34560261	*SEMA4B*	90,734,426	T	C	1.52 (1.22−1.74)	1.12 (0.60−2.08)	0.72	1.28 (0.66−2.52)	0.46

Abbreviations: FFA, frontal fibrosing alopecia; PA, protective allele; RA, risk allele.