**Genome-wide meta-analysis reveals an epistatic interaction between MHC Class I genes and *ERAP1* in frontal fibrosing alopecia**

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**Funding**

We acknowledge financial support by the British Skin Foundation via the Young Investigator Award to CT. TR is funded by BPPLN 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 (NIHR203308). CT is supported by a research grant (MR/X030466/1) by the Medical Research Council (MRC).

**Conflicts of interest:**

CT is principal and (national) chief investigator for the Pfizer-sponsored ALLEGRO study; has received speaker fees from LEO; and is consultant for Pfizer Ltd. SMS and CYU are sub-investigators for the Pfizer-sponsored ALLEGRO study.

**Words count:** 2,006

**Keywords**: frontal fibrosing alopecia, meta-analysis, genome-wide association study, epistasis

**Abstract**

**Background:** Frontal fibrosing alopecia (FFA) is an inflammatory and scarring form of hair loss or increasing prevalence that most commonly affecting women. Identifying susceptibility loci may shed light on FFA pathogenesis and therapeutic targets.

**Objective**: To identify novel genomic loci at which common genetic variation influences FFA susceptibility.

**Methods:** In this case-control study of genetic susceptibility to FFA, comprising 1,585 European females and 5,083 European controls, standard error-weighted meta-analysis was performed, followed by statistical fine-mapping and colocalization. In the HLA region, stepwise conditional analysis was undertaken to determine whether there were independent classical HLA allele associations. An epistatic interaction test between the lead variant at *ERAP1* (rs10045403) and risk alleles of the Major Histocompatibility Complex (MHC) Class I genes was undertaken using a logistic regression model separately in each cohort and combined in meta-analysis.

**Results:** We identify genome-wide significant associations at four genomic loci. The locus with the largest effect on FFA risk lies within the MHC, where we delineate statistically independent associations with HLA-A\*11:01, HLA-A\*33:01, HLA-B\*07:02 and HLA-B\*35:01. We identify a novel susceptibility locus at 5q15 and fine-map the association signal to a single nucleotide substitution in the 5’ untranslated region of *ERAP1*. We demonstrate evidence of epistasis between risk alleles of the MHC Class I genes and the *ERAP1* locus (5q15) in susceptibility to FFA.

**Conclusions:** Our findings implicate the processes of peptide trimming and antigen presentation in disease pathogenesis and highlight FFA as a potential indication for emerging therapeutic approaches that modulate ERAP-mediated processes.

**Key Messages**

* Common genetic variation at four loci influences genetic susceptibility to frontal fibrosing alopecia, with the largest association in MHC Class 1 region.
* The contribution of the newly discovered ERAP1 locus to FFA risk through its interaction with specific MHC Class I alleles implicates the processes of peptide trimming and antigen presentation in disease pathogenesis, which is underpinned by the collapse of the follicular immune privilege in FFA.

**Capsule Summary**

This genome-wide meta-analysis identifies a novel susceptibility locus associated with FFA at 5q15 and detects independent associations at four HLA-alleles. The interaction between *ERAP1* and MHC class 1 alleles implicates the processes of peptide trimming and antigen presentation in disease pathogenesis, which is underpinned by the collapse of the follicular immune privilege.

**Abbreviations**

FFA frontal fibrosing alopecia

GWAS genome-wide association study

HLA human leucocyte antigen

IMID immune mediated-inflammatory diseases

MHC major histocompatibility complex

OR odds ratio

UK United Kingdom

**Introduction**

Major histocompatibility complex (MHC) class I proteins are expressed on the cell surface of all nucleated cells where they mediate the presentation of antigenic peptides to CD8+ cytotoxic T cell receptors and killer-cell immunoglobulin-like receptors1. Following proteolytic degradation of cellular proteins, peptide fragments pass through the endoplasmic reticulum where they are subjected to the activity of endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1 and ERAP2), which trim the N-termini to create smaller fragments. The resulting peptide fragments are loaded onto the MHC class I proteins and exported to the cell surface for presentation to immune surveillance systems2.

Genetic variation at 6p21.1 and 5q15, where the genes encoding MHC class I proteins and ERAP1 and ERAP2 are respectively located, influence the selection and presentation of antigenic peptides, and contribute to susceptibility to a broad range of immune mediated-inflammatory diseases (IMID)3. Notably, epistatic effects – non-additive effects of allelic combinations across the two loci – have been demonstrated in the susceptibility to four IMIDs: psoriasis, ankylosing spondylitis, Behcet's disease, and birdshot uveitis4–7.

Frontal fibrosing alopecia (FFA) is an inflammatory and scarring form of hair loss that has shown a dramatic increase in prevalence over the last 30 years8,9. Hair follicle loss in FFA is underpinned by loss of the immune privilege at the level of the follicular isthmus, the upper portion of the follicular unit, which is home to epithelial hair follicle stem cells.10,11 A previous genome-wide association study of female FFA identified four susceptibility loci, with the largest single contribution to FFA risk arising from *HLA-B\*07:02* allele12. Recent investigation of the contribution of these female FFA risk loci in the less prevalent male form of the disease also identified an elevated frequency of the *HLA-B\*07:02* allele in males with FFA, consistent with an effect of *HLA-B\*07:02* on FFA risk that is independent of sex13.

In the current study, we further investigate the genetic contribution to FFA risk among females. In a series of four female FFA case-control cohorts, systematically ascertained within the UK and Spain and including newly assembled FFA case collections, we have performed a series of genetic analyses that identify common alleles that influence susceptibility to FFA and interact to modulate individual risk profiles.

**Results**

**Genome-wide Association Meta-analysis of FFA**

We performed an inverse variance-weighted fixed effect GWAS meta-analysis, combining summary statistics across 8,181,782 biallelic variants from four independent genome-wide association studies of female FFA. In aggregate, these four studies comprised a total of 1,585 females with FFA and 5,083 controls, arising from two studies that formed the previous GWAS meta-analysis of female FFA12 and two additional cohorts ascertained in the UK (426 cases and 748 controls), and Spain (143 cases and 190 controls). Evaluation of the distribution of test statistics across genetic variants in each of the four GWAS and the resulting meta-analysis indicated that potential sources of systematic bias were well controlled (λGC = 1.04; **Supplementary Figure 1**).

Genome-wide significant evidence of association was observed at three of the four previously reported FFA susceptibility loci (2p22.2, 6p21.1, and 15q26.1, **Figure 1, Table 1**). The previously reported FFA risk locus at 8q24.22 was not supported by genome-wide significant evidence of association in the meta-analysis, though a nominally significant association with a consistent direction and magnitude of effect as previously reported was observed (rs760327, PMETA = 1.5 × 10-4, OR = 1.17, 95%CI =1.07 – 1.28).

In addition to the three previously reported susceptibility loci, we observed genome-wide significant evidence of association with FFA risk for genetic variation at chromosome 5q15 (rs10045403, ORMETA: 1.30, 95%CI: 1.19 – 1.43, p-value: 3.6 × 10-8). Functional variation at the *ERAP1* locus includes both *ERAP1* missense alleles that have a demonstrated impact on the trimming efficiency of specific substrates and regulatory variation that impacts the expression of the gene14. Statistical fine-mapping of the association signal indicated that the variant with strongest support was rs10045403 (n = 41 variants in the 95% credible set, posterior probability = 0.21). This variant is located in the 5′ untranslated region of *ERAP1* but has been previously reported as tagging an *ERAP1* haplotype that is associated with susceptibility to ankylosing spondylitis in individuals carrying *HLA-B\*27* 15.

**Fine-mapping of the FFA susceptibility locus within the MHC**

To refine the association signal within the MHC on chromosome 6p21.1, we undertook statistical imputation of four-digit classical MHC class I and class II alleles and tested each for association with FFA in each of the individual case-control cohorts before combining the evidence through a fixed effect meta-analysis.

Consistent with our previous study of the MHC association signal in FFA, the strongest evidence of association was observed for *HLA-B\*07:02* (PMETA = 8.1 × 10-149, OR = 5.02 (95%CI = 4.44 – 5.67). Stepwise conditional association analyses revealed independent evidence of association with FFA risk for a further three classical MHC class I alleles, including alleles of both *HLA-A* and *HLA-B*. The magnitude of effect of each MHC class I risk allele estimated in a joint model demonstrates that the *HLA-A\*33:01* allele confers the greatest risk at *HLA-A* and *HLA-B\*07:02* at *HLA-B* (**Figure 2**)*.* FFA risk alleles of both *HLA-A* and *HLA-B* can be observed together on haplotypes circulating in the population. It is therefore possible for individuals to carry up to four FFA risk-increasing MHC class I alleles across the two genes, with a corresponding increase in risk with each additional allele carried **(Figure 2**).

**Epistasis between MHC Class I alleles and ERAP1 in FFA susceptibility**

We investigated potential non-additive genetic effects between the FFA risk alleles at the MHC and *ERAP1* loci. Specifically, we performed a series of interaction analyses for each of the four female FFA cohorts independently and combined through a standard error-weighted meta-analysis.

The analysis revealed evidence of a statistical interaction between the MHC Class I allele strongly associated with FFA risk, *HLA-B\*07:02*, and the lead variant at 5q15 (rs10045403), with an estimated additive per-allele ORinteraction of 1.29 (95%CI= 1.07 – 1.57, PMETA = 0.007). This interaction odds ratio can be interpreted as the degree to which FFA risk conferred by the 5q15 risk allele increases for each copy of *HLA-B\*07:02* carried. Evidence of the interaction effect is more clearly demonstrated when considering the effect of carrying one or more copies of any FFA HLA risk allele (*HLA-A\*11:01, HLA-A\*33:01, HLA-B\*07:02* and *HLA-B\*35:01*) on rs10045403-attributable risk (additive per-allele ORinteraction= 1.40, 95%CI= 1.06 – 1.85, PMETA = 0.01) (**Figure 3**). This analysis clearly demonstrates an effect on FFA risk at rs10045403 that is specific to the stratum of individuals that carry one or more FFA HLA risk allele (OR= 1.36, 95% CI= 1.21 - 1.52, PMETA= 2.15 × 10-7), with no evidence of an effect at rs10045403 in individuals without (OR= 1.01, 95% CI= 0.78 - 1.30, PMETA= 0.92).

**Discussion**

Examples of epistatic interactions underlying disease risk in human populations are rare. However, association of *ERAP1* alleles that is limited to individuals carrying specific MHC Class I alleles has been previously reported in four immune-mediated inflammatory diseases (IMIDs)4–7. The observed interaction between *ERAP1* and MHC Class I alleles in FFA follows a similar pattern, with the association of rs10045403 limited to individuals carrying at least one of four identified FFA risk alleles of *HLA-A and HLA-B; HLA-A\*11:01, HLA-A\*33:01, HLA-B\*07:02* and *HLA-B\*35:01*. Whilst our fine-mapping analysis of the *ERAP1* association signal indicates that rs10045403 is the most plausible causal variant, the *ERAP1* locus is highly polymorphic and previous investigations indicate that rs10045403 tags a haplotype of *ERAP1* containing several missense variants with demonstrated impact on the aminopeptidase activity towards specific substrates15,16. MHC Class I risk alleles have previously been reported to be associated with a series of other immune-mediated traits including Behçet's disease (HLA-B\*51) psoriasis (HLA-C\*06:02), and birdshot uveitis (HLA-A29), though no other disease or trait has been previously reported to be associated with this specific combination of alleles across *HLA-A* and *HLA-B*4,17,18.

Antigen presentation is an essential process that enables cells infected with a pathogen or otherwise producing foreign proteins to be recognised and eliminated by CD8+ T cells19. The generation of antigens for presentation by MHC class I epitopes typically results from the action of proteasomes in the cytosol, which generate precursors that are extended by several N-terminal amino acids20,21. Within the endoplasmic reticulum, ERAP1’s length-specific aminopeptidase activity trims N-extended precursors longer than ~8 amino acid residues but spares shorter peptides22,23. The process produces fragments that are of optimal length for binding to MHC class I proteins before export to the cell surface2.

Within the hair follicle bulge and outer root sheath, restricted auto-antigen presentation conferred by MHC Class I downregulation, alongside the presence of an immuno-inhibitory milieu of cytokines, have previously been proposed as potential mechanisms through which follicular immune privilege is maintained24,25. More recently, skin regulatory T cells (Tregs) have been found to immunologically protect the hair follicle stem cell (HFSC) niche and act as a final safeguard of follicular immune privilege26. In FFA, immune privilege is lost, leading to destruction of epithelial stem cells within the hair follicle bulge through an auto-inflammatory process involving lymphocytic autoaggression14. The identified interaction of variation in *ERAP1* and MHC Class I genes in the susceptibility to FFA is consistent with a hypothesis that variation at these loci may exert their effects on FFA through variation in antigen processing combined with the specific affinities of FFA risk alleles of *HLA-A* and *HLA-B* to specific peptides. This combination impacts the processing and presentation of antigens and auto-antigens and may influence the immunodominance hierarchy leading to the immune privilege collapse of the hair follicle and resulting hair loss and scarring. *ERAP1* deficiency has been associated with reduced Tregs activity and ankylosing spondylitis-related inflammatory features in mice27 and our findings are consistent with the recently proposed role of skin Tregs as a final safeguard against autoaggression and self-tolerance evasion in hair follicles28.

The association of genetic variation impacting *ERAP1* and *ERAP2* with immune-mediated diseases has triggered interest in the targeting of this process as a potential therapeutic approach28. Indeed, a series of small molecule inhibitors of ERAP1 have been identified that inhibit processing of virally encoded antigenic peptides for presentation onto MHC Class I molecules in cellular systems29. The development of these therapeutic approaches has attracted intense interest, but remains in its infancy. Our findings, implicating ERAP1-mediated antigen processing in FFA designate this disease as a potential indication for such emerging treatments.

In summary, we demonstrate that the risk of females developing the scarring hair loss observed in FFA is largely driven by alleles *HLA-A\*11:01*, *HLA-A\*33:01*, *HLA-B\*07:02* and *HLA-B\*35:01*; and through an epistatic interaction, by variation at ERAP1 when at least one of these MHC Class I alleles are present. The established role of these genes in antigen processing and presentation indicates that the collapse of the immune privilege of the hair follicle, which is central to FFA pathogenesis, may be influenced by this genetic landscape. Our findings identify FFA as a candidate for developing therapeutic approaches that target ERAP1 and motivate further studies of the relevance of this antigen processing in the male form of the disease.

**Acknowledgement**

The authors wish to express their gratitude to all patients and clinicians who participated in this study. We are thankful to the 1958 British Cohort Study and the INfancia y Medio Ambiente (INMA) project for providing population control genotyping data. We thank our research technicians and research nurses for their assistance during this study, especially Rashida Pramanik, David Baudry, Alice White, Anne Thomson, and Ruth Joslyn. We want to thank Sonia Camaño Páez and Ana Maria Torres from the Hospital Universitario Ramón y Cajal-IRYCIS Biobank (B.0000678), for their collaboration. We would also like to thank the FFA Research Consortium and the NIHR Rare Disease Translational Research Collaboration for their initial help with the FFA study. Special thanks go to Dr. Dimitra Dritsa for her continuous support and input throughout this work.

**Data availability**

Full meta-analysis summary statistics are available at GWAS catalogue under the accession ID GCST90310136.

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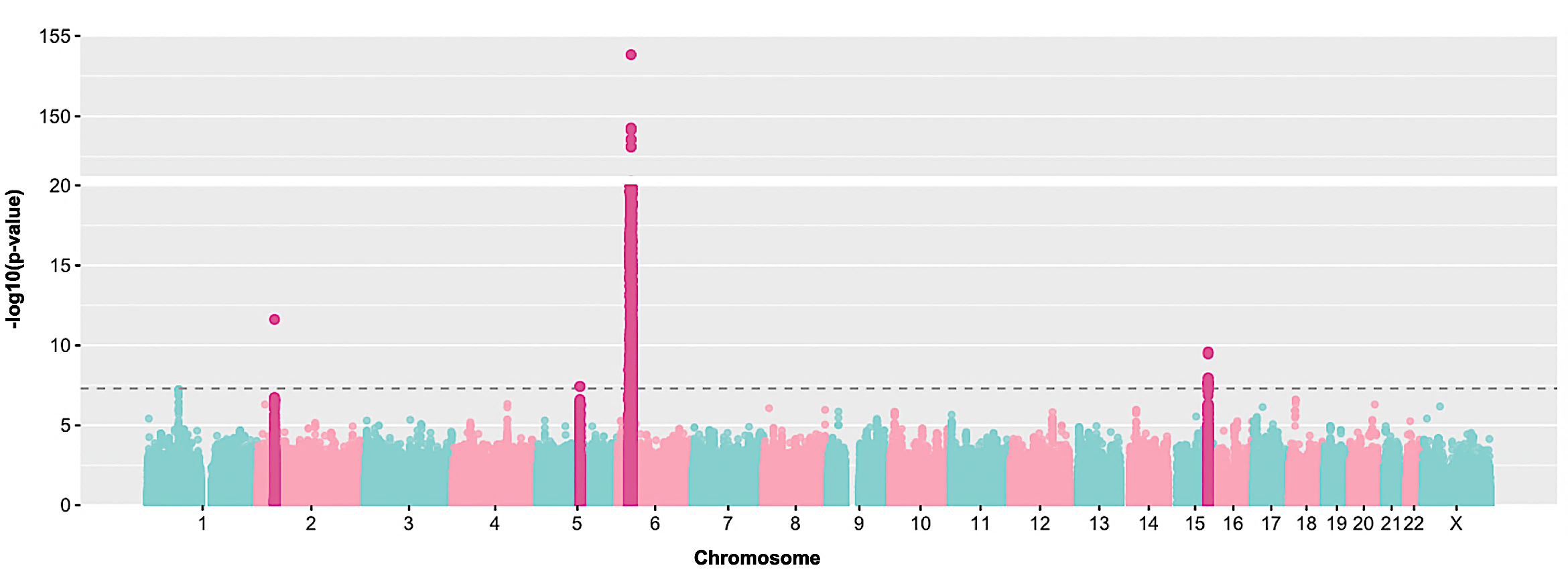
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**Table 1**. Association results for lead variants at genome-wide significant FFA susceptibility loci

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Locus | Position (hg 19) | rsID | Candidate genes | RA | PA | RAF cases | RAF controls | Meta-analysis | | P-Het |
| OR (95% CI) | *P*-value |
| 2p22.2 | 38,298,139 | rs1800440 | *CYP1B1* | T | C | 0.87 | 0.81 | 1.55 (1.37 - 1.75) | 2.4 × 10-12 | 0.34 |
| 5q15 | 96,147,733 | rs10045403 | *ERAP1* | G | A | 0.31 | 0.26 | 1.30 (1.19 - 1.43) | 3.6 × 10-8 | 0.43 |
| 6p21.1 | 31,320,562 | rs2523616 | *HLA-A, HLA-B* | T | C | 0.47 | 0.18 | 4.62 (4.15 - 5.20) | 1.5 × 10-154 | 0.41 |
| 15q26.1 | 90,734,627 | rs36034702 | *SEMA4B* | T | C | 0.20 | 0.17 | 1.46 (1.29 – 1.64) | 2.6 × 10-10 | 0.34 |

The association of each SNP (single nucleotide polymorphisms) was tested by logistic regression using an additive regression model. Abbreviations, CI, confidence interval, NA, not available; OR, odds ratio, PA, protective allele, P-Het, p-value for heterogeneity of effect sizes (Cochran’s Q test), RA, risk allele, RAF, risk allele frequency.



**Figure 1.** Manhattan plot illustrating the –log10 P-values for the meta-analysis of genome-wide association studies of FFA comprising 1,585 affected females and 6,668 controls. The dashed line indicates the significance threshold for genome-wide significance (*P*-value= 5.0×10-8); the y axis has been interrupted to enable representation of the genetic association signal on 6p21.1

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**Figure 2**. Forest plots of FFA risk estimates at the MHC locus. (A) Joint per-allele effect size estimates under an additive model for the four classical HLA alleles identified via stepwise conditional meta-analysis. (B) Cumulative effect size estimates per number of FFA risk alleles present (*HLA-A\*11:01*, *HLA-A\*33:01*, *HLA-B\*07:02* and *HLA-B\*35:01*). At most two risk alleles can be carried for *HLA-A* and two for *HLA-B*, meaning an individual can carry up to four in total; participants carrying three or four risk alleles are collapsed into one group due to low numbers of participants carrying four risk alleles. Effect estimates are pooled across studies. Bars represent 95% confidence intervals.

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**Figure 3.** Statistical interaction between *ERAP1* genotypes (rs10045403: AA/AG/GG) and presence of one or more FFA HLA risk alleles (*HLA-A\*11:01, HLA-A\*33:01, HLA-B\*07:02* and *HLA-B\*35:01)* in combined cohorts. Odds ratio estimates per two-locus genotype combination. The odds are calculated relative to the two-locus genotype expected to be most protective based on marginal effect estimates (which, by definition, has an odds ratio of 1). The bars represent 95% confidence intervals calculated based on the standard errors derrived from genotype counts

**FIGURE LEGENDS**

**Figure 1**. Manhattan plot illustrating the –log10 P-values for the meta-analysis of genome-wide association studies of FFA comprising 1,585 affected females and 6,668 controls. The dashed line indicates the significance threshold for genome-wide significance (P-value= 5.0×10-8); the y axis has been interrupted to enable representation of the genetic association signal on 6p21.1

**Figure 2**. Forest plots of FFA risk estimates at the MHC locus. (A) Joint per-allele effect size estimates under an additive model for the four classical HLA alleles identified via stepwise conditional meta-analysis. (B) Cumulative effect size estimates per number of FFA risk alleles present (*HLA-A\*11:01*, *HLA-A\*33:01*, *HLA-B\*07:02* and *HLA-B\*35:01*). At most two risk alleles can be carried for *HLA-A* and two for *HLA-B*, meaning an individual can carry up to four in total; participants carrying three or four risk alleles are collapsed into one group due to low numbers of participants carrying four risk alleles. Effect estimates are pooled across studies. Bars represent 95% confidence intervals

**Figure 3.** Statistical interaction between *ERAP1* genotypes (rs10045403: AA/AG/GG) and presence of one or more FFA HLA risk alleles (*HLA-A\*11:01, HLA-A\*33:01, HLA-B\*07:02* and *HLA-B\*35:01)* in combined cohorts. Odds ratio estimates per two-locus genotype combination. The odds are calculated relative to the two-locus genotype expected to be most protective based on marginal effect estimates (which, by definition, has an odds ratio of 1). The bars represent 95% confidence intervals calculated based on the standard errors derrived from genotype counts