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HLA Class II Locus and Susceptibility to Podoconiosis

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Abstract

BACKGROUND—Podoconiosis is a tropical lymphedema resulting from long-term barefoot exposure to red-clay soil derived from volcanic rock. The World Health Organization recently designated it as a neglected tropical disease. Podoconiosis develops in only a subgroup of exposed people, and studies have shown familial clustering with high heritability (63%).

METHODS—We conducted a genomewide association study of 194 case patients and 203 controls from southern Ethiopia. Findings were validated by means of family-based association testing in 202 family trios and HLA typing in 94 case patients and 94 controls.

RESULTS—We found a genomewide significant association of podoconiosis with the single-nucleotide polymorphism (SNP) rs17612858, located 5.8 kb from the *HLA-DQA1* locus (in the allelic model: odds ratio, 2.44; 95% confidence interval [CI], 1.82 to 3.26; $P = 1.42 \times 10^{-9}$; and in the additive model: odds ratio, 2.19; 95% CI, 1.66 to 2.90; $P = 3.44 \times 10^{-8}$), and suggestive associations ($P < 1.0 \times 10^{-5}$) with seven other SNPs in or near *HLA-DQB1*, *HLA-DQA1*, and *HLA-DRB1*. We confirmed these associations using family-based association testing. HLA typing showed the alleles *HLA-DRB1*0701* (odds ratio, 2.00), *DQA1*0201* (odds ratio, 1.91), and *DQB1*0202* (odds ratio, 1.79) and the *HLA-DRB1*0701-DQB1*0202* haplotype (odds ratio, 1.92) were risk variants for podoconiosis.

CONCLUSIONS—Association between variants in HLA class II loci with podoconiosis (a noncommunicable disease) suggests that the condition may be a T-cell-mediated inflammatory disease and is a model for gene–environment interactions that may be relevant to other complex genetic disorders. (Funded by the Wellcome Trust and others.)

Podoconiosis (endemic nonfilarial elephantiasis) is a noninfectious geochemical disease that results in bilateral swelling of the lower legs (Fig. 1). It is found among subsistence farmers whose feet are exposed over many years to red-clay soil derived from volcanic rock. Podoconiosis is an important yet neglected clinical and public health burden in more than 10 countries across tropical Africa, Central and South America, and north India.¹ It is estimated that up to 1 million people are affected by podoconiosis in Ethiopia alone.^{2,3} In the Wolaita

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zone of southern Ethiopia (where this study was conducted), it affects 1 in 20 people,² imposes an immense economic burden,⁴ and causes severe social stigma.⁵ The World Health Organization recently designated podoconiosis as a neglected tropical disease in recognition of its impact (www.who.int/neglected_diseases/diseases/podoconiosis/en). Our limited knowledge of its pathogenesis is based on evidence from the 1980s, which suggests that mineral particles in red-clay soils are absorbed through the skin of the foot and engulfed by macrophages in the lower limb lymphatic system, inducing an inflammatory response in the lymphatic vessels, which results in fibrosis and obstruction of the vessel lumen.⁶ The disease develops in only some people exposed to irritant soils, although mineral particles can be seen in the lymphatic system and lymph nodes of unaffected as well as affected people.^{7,8} Familial clustering of podoconiosis case patients⁹ and our pedigree study¹⁰ suggest that both genetic and environmental factors are involved in the pathogenesis of podoconiosis. We estimated the heritability of podoconiosis to be 63% and the risk ratio of occurrence among siblings of the case patient to be 5.07, indicating that a sibling of an affected person has a risk of podoconiosis developing that is five times that of a randomly selected person in the general population.¹⁰

Understanding the pathogenesis of podoconiosis will allow for the refinement of current prevention and treatment measures and may lead to important insights into the pathogenesis of other important fibrosing diseases, such as filariasis and pneumoconiosis. Podoconiosis may also represent a simplified model of gene–environment interactions, which remain poorly understood in many complex genetic diseases. We report the findings of a genomewide association study, family-based association testing, and HLA typing in a southern Ethiopian population.

Methods

Study Conduct

The study was approved by the institutional ethics review boards of the Medical Faculty of Addis Ababa University and the Armauer Hansen Research Institute–All Africa Leprosy, Tuberculosis and Rehabilitation Training Center as well as the ethics review committee of the Ethiopian Ministry of Science and Technology. The study was conducted according to the protocol, available with the full text of this article at NEJM.org. All authors vouch for the accuracy and completeness of the reported data and the fidelity of the study to the protocol. Written informed consent was obtained from all participants following a rapid ethics assessment that explored social, cultural, and economic factors that might influence the consent process for genetic research on podoconiosis. The issues identified in the rapid ethics assessment and the approach followed for obtaining consent for this study have been described elsewhere.^{11,12} The absence of immediate therapeutic benefit from the genetic study was explained to participants.

Study Participants

Study participants were recruited from the Wolaita zone of southern Ethiopia from podoconiosis-affected families (DNA samples were collected from both parents and two affected siblings); unaffected persons were recruited to serve as controls. All participants were from the same broad geologic area covered by reddish-brown clay soils containing colloid-size particles derived from volcanic basalt rocks.¹³ The genomewide association study was conducted with the use of samples from one randomly selected affected sibling (of the two recruited) from each family and an unaffected, unrelated control for each case patient. The eligibility criteria for controls were the current absence of podoconiosis, the absence of a personal or family history of podoconiosis, age of at least 50 years (to minimize misclassification of potential case patients as controls), residence in the study area for at

least 25 years, exposure to the same irritant clay soil as the case patients, and inconsistent use of shoes. Consequently, the controls were older than the case patients (average age, 62 years vs. 24 years). The family-based association test included the affected sibling who was not included in the genomewide association study as well as both parents from each family.

Genotyping

We obtained a 2-ml saliva sample from all participants. DNA was extracted with the use of a DNA-purification protocol (Oragene, DNA Genotek). DNA was quantified (Quant-iT PicoGreen reagent, Molecular Probes) according to the manufacturer's protocol. Genotyping was performed by a contract company (deCODE Genetics) by means of a chip (HumanHap 610 Bead Chip, Illumina) that contains more than 620,000 single-nucleotide polymorphisms (SNPs). Genotyping for the family-based association testing was performed by KBioscience (www.kbioscience.co.uk). *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, and *HLA-DPB1* typing of approximately half of the total participants (94 case patients and 94 controls) was done with the use of a high-definition technology (Luminex xMAP) that analyzes DNA samples with sequence-specific oligonucleotides amplified with the use of a polymerase-chain-reaction assay.¹⁴ Quality-control and data-analysis procedures are described in the Supplementary Appendix (available at NEJM.org).

Results

Enrollment and Samples

Characteristics of the study participants are summarized in Tables 1 and 2 in the Supplementary Appendix. Figure 1 in the Supplementary Appendix summarizes the reasons for exclusion of DNA samples from 8 patients with podoconiosis and 12 controls and of 32,358 SNPs from the genome-wide association analysis. The final data set for the genomewide association analysis comprised 551,840 autosomal SNPs in 194 case patients and 203 controls.

Significant excess sharing of marker alleles identical by descent (from the same ancestral chromosome) owing to cryptic relatedness (genetic relatedness among participants that was not known by the investigator) or sample contamination was ruled out as a reason for exclusion of samples from data analysis because the PI_HAT values (a measure of the degree of genetic relationship between participants) were less than 0.05. Inbreeding coefficients were 0.03 or less for all participants and never exceeded 4 SD of the mean coefficient. Population structure (allele-frequency differences between groups of persons that result in spurious associations) was minimal in the sample because of three factors. First, plots from principal components analysis showed that all case patients belonged to the same cluster, and there was no difference in pairwise identity-by-state distances (degree of marker-allele sharing between pairs of persons) between case patients and controls ($P = 0.99$). Second, the quantile–quantile plot of the distribution of the test statistic was consistent with the expected distribution under the null hypothesis (Fig. 2 in the Supplementary Appendix). Third, the genomic-control inflation λ value¹⁵ was 1.02 in both the allelic and additive models, indicating a lack of population structure. Nonetheless, the first four principal components were included as covariates in the genomewide analysis to account for any potential residual population structure.

Significant Genomewide Association Study Signals

One locus, rs17612858, showed a genomewide significant association with podoconiosis ($P = 1.42 \times 10^{-9}$ in the allelic model and $P = 3.44 \times 10^{-8}$ in the additive model). This intergenic SNP lies between *HLADQA1* (5.8 kb away) and *HLA-DQB1* (6.7 kb away). The presence of the minor allele (T) of this SNP was associated with an increased risk of susceptibility to

podoconiosis substantially (odds ratio, 2.44 in the allelic model and 2.19 in the additive model). The 15 SNPs with the most significant association with podoconiosis from the genomewide association analysis are presented in Table 1. Most of these SNPs map to or near genes in the HLA class II locus on chromosome 6: for example, rs9273349 and rs1063355, near *HLA-DQB1*; rs9270856 and rs9271100, near *HLA-DRB1*; and rs9271170, rs17843604, and rs17612633, near *HLA-DQA1*.

Figure 2 shows the plots of P values for the association of each SNP with the risk of podoconiosis and the association signals on chromosome 6. Non-HLA regions with some of the most significant associations included rs2906966 on chromosome 17 (near *CDRT4* and *FAM18B2*), rs11644557 on chromosome 16 (in *ITFG1*), and rs9514099 on chromosome 13 (near *SLC10A2*). Genomewide significance was not achieved in the dominant and recessive models, but the most significant association in the recessive model was also significant in the additive and allelic models and had a stronger effect (odds ratio for podoconiosis with rs17612858, 3.40; 95% confidence interval [CI], 2.07 to 5.58) (Tables 3 and 4 in the Supplementary Appendix).

We estimated the proportion of the variance in the occurrence of podoconiosis that could be explained by the SNP that achieved genomewide significance (rs17612858), as well as all eight SNPs with the most significant associations with the disease, by using Nagelkerke's R^2 value in a logistic-regression model involving a backward-stepwise-likelihood ratio method (see the Supplementary Appendix). The eight SNPs explained 15.6% of the variance, and the SNP with the most significant P value (rs17612858) alone explained 9.8% of the variance in occurrence of podoconiosis.

To test for potential interaction between HLA and non-HLA regions, we selected SNPs with P values of less than 10^{-4} in the additive genetic model and performed epistasis tests between a selection of 15 SNPs in HLA class II loci and a selection of 38 SNPs in non-HLA genes. Of the group of 567 valid tests, only 11 had P values of less than 0.01 (Table 5 in the Supplementary Appendix), and none were significant after Bonferroni correction (with a threshold of $P < 0.05/567$, or $P < 8.8 \times 10^{-5}$).

A genomewide haplotype test showed that the eight HLA class II SNPs that had the greatest significance in single-marker association analysis formed a haplotype significantly associated with podoconiosis ($P = 4.5 \times 10^{-8}$) (Table 6 in the Supplementary Appendix). The eight HLA class II SNPs formed one haplotype block, and the average linkage disequilibrium within the block was weak ($r^2 = 0.41$) (Fig. 3 in the Supplementary Appendix). The SNPs were 0.3 to 7 kb from the lead SNP (rs17612858) in the genomewide association study. Conditioning the analysis by including rs17612858 as a covariate made the haplotype association non-significant ($P > 10^{-3}$), implying that rs17612858 explained nearly all of the observed haplotype effect (Table 7 in the Supplementary Appendix). A stepwise multivariate-regression analysis also confirmed that only rs17612858 had an independent effect (odds ratio for having the minor-allele homozygote TT vs. the major-allele homozygote AA, 4.58 [95% CI, 2.61 to 8.04]; odds ratio of TA vs. AA, 1.68 [95% CI, 1.03 to 2.73]).

We found eight SNPs, of which six were heterozygous, within 20 kb of rs17612858 in two HapMap (release 3.2) populations from Africa: Yoruba from Ibadan, Nigeria, and Maasai from Kinyawa, Kenya. Five of the six heterozygous SNPs had minor allele frequencies that differed significantly between the two populations, as well as different haplotype structures (Table 8 and Fig. 4 in the Supplementary Appendix).

Family-Based Association Testing

The family-based association study included 202 family trios. The 24 SNPs with the most significant P values from the genomewide association analysis were selected, of which 21 were successfully genotyped (Table 9 in the Supplementary Appendix). Genotypes at each marker were in Hardy–Weinberg equilibrium ($P < 0.001$ for unrelated individuals). The average genotyping rate was 0.97, and the average marker heterozygosity was 0.40. Single-marker analysis by means of the family-based association test revealed significant associations between podoconiosis and 7 of the 21 SNPs ($P < 0.05$). Alleles of rs1063355, rs17612633, rs17612858, and rs17867526 showed significant overtransmission in both the additive and recessive models. The major alleles of rs17843604 and rs9271100 showed significant over-transmission in the recessive model, and the major allele of rs17211510 showed overtransmission in the additive model. The odds ratio for each of these associations in the additive model was approximately 1.5 (Table 10 in the Supplementary Appendix). Six of the 7 SNPs mapped to the *HLA-DQA1*, *HLA-DQB1*, or *HLA-DRB1* gene (Table 2).

Three of the associations maintained statistical significance after applying the Bonferroni correction for a P-value threshold of less than 0.007 in the recessive model. Family-based haplotype analysis showed that the haplotype GCCTTC — formed by the HLA SNPs with significant associations on single-marker family-based association testing — was overtransmitted to the affected siblings ($P < 0.01$) (Table 11 in the Supplementary Appendix).

HLA Class II Associations

We examined the associations between HLA types and podoconiosis by using two models. In the first model, we tested the association with the presence of an allele (vs. its absence). The individual alleles *DRB1*0701* ($P = 0.02$), *DQA1*0201* ($P = 0.02$), and *DQB1*0202* ($P = 0.03$) conferred susceptibility to podoconiosis. There were also significant protective associations with *DRB1*0102* ($P < 0.001$), *DRB1*1302* ($P = 0.04$), *DQA1*01MV* ($P < 0.001$), and *DQB1*0501* ($P = 0.001$). The haplotype constructed from these alleles, *DRB1*0102–DQA1*01MV–DQB1*0501*, was also associated with podoconiosis ($P = 0.01$) (Table 3). The full list of HLA alleles and their association statistics is presented in Table 12 in the Supplementary Appendix.

In the second model, we tested for association with the presence of two alleles (vs. none) and only one allele (vs. none). In this model, the *DRB1*0701–DQB1*0202* haplotype ($P = 0.04$), as well as either allele ($P = 0.02$), was associated with an increased odds of having podoconiosis (odds ratio, 1.92) (Table 13 in the Supplementary Appendix).

Discussion

Using a genomewide approach, we have identified an association between genetic variants in the HLA class II region harboring *HLA-DQA1*, *HLA-DQB1*, and *HLA-DRB1* and podoconiosis, a neglected tropical disease affecting an estimated 4 million people worldwide. The most strongly associated SNPs were validated in a set of families by means of family-based association testing, and HLA typing showed that specific HLA alleles and haplotypes were significantly associated with differential risk of disease. We estimated that the HLA SNPs associated with podoconiosis in this study explained 15.6% of the genetic variance in podoconiosis, conferring an increase in risk by a factor of 2 to 3.

The association of HLA class II suggests that podoconiosis is a T-cell-mediated inflammatory disease. The initial trigger for T-cell activation is recognition of an antigenic peptide bound to an HLA molecule on antigen-presenting cells.¹⁶ Although HLA class II molecules are central to the presentation of exogenous (i.e., foreign and usually pathogen-derived) antigens, they have also been implicated in diseases triggered by minerals, such as

silicosis and berylliosis. In berylliosis, which is associated with HLA-DPGLu69, possible mechanisms of pathogenesis include modification of a self peptide by beryllium, allowing for presentation of the self peptide by HLA class II molecules or direct binding of beryllium to, and alteration of, the HLA-peptide binding pocket.¹⁷⁻²¹ In podoconiosis, the *DRB1*0701*, *DQA1*0201*, and *DQB1*0202* alleles may have a functional role in antigen presentation to T cells, leading to induction of the immune response and development of disease in response to a currently undefined soil antigen or mineral.

HLA genetic data from African populations are scarce. De Bakker and colleagues included samples from the Nigerian Yoruba population in their construction of a high-resolution HLA and SNP haplotype map.²² This involved typing the classical HLA genes and more than 7500 SNPs that were analyzed together to identify informative tag SNPs that captured the variation in the HLA region. Of the HLA SNPs we found to be associated with podoconiosis, only rs106335 has been identified as an informative tag SNP in the Yoruba. The absence of convergence of more than 1 SNP between the two studies may not be surprising, given the wide diversity of African populations in terms of allele frequencies, local linkage-disequilibrium patterns, and extensive population substructure.^{23,24} To demonstrate this, we compared the allele frequencies and haplotype structure for 8 SNPs within 20 kb of the most strongly associated marker from our study (rs17612858) among two HapMap (release 3.2) populations from Africa: Yoruba from Ibadan, Nigeria, and Maasai from Kinyawa, Kenya. Six of these 8 SNPs were heterozygous; 5 of these 6 had minor allele frequencies that differed significantly between the two populations, as well as different haplotype structures (Table 8 and Fig. 4 in the Supplementary Appendix). This finding indicates that a single group in Africa cannot be taken as a proxy for predicting HLA alleles of all other populations on the continent, including the Ethiopian Wolaita population.

We selected barefoot, older participants as “supercontrols” — people who had been sufficiently exposed to the environmental risk factor to have podoconiosis develop yet it did not. These controls were included under the assumption that they would possess fewer genetic susceptibility variants than the case patients. Although the use of such controls may introduce bias with respect to other factors, it is most important in a case–control study that the control group is disease-free.²⁵ This approach has been used successfully in other studies,^{22,26} and was helpful during this discovery (stage 1) genomewide association study to identify the risk variants for podoconiosis. The possibility that variants involved in survival against other diseases may have been overrepresented in the controls is small, because the findings of the family-based association testing corroborated those of the genomewide association study and because HLA has no known effect on longevity.²⁷

Identification of additional genetic risk factors reaching genomewide significance may have been limited by the small sample and the lower information content of the markers included in the SNP array used for our study population of African ancestry, as compared with populations of European ancestry.^{28,29} Replication of our findings in a larger sample of patients with podoconiosis and controls with the use of population-specific, denser SNP arrays is needed to inform future research directions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Price, EW. Podoconiosis: non-filarial elephantiasis. Oxford Medical; Oxford, United Kingdom: 1990.
2. Destas K, Ashine M, Davey G. Prevalence of podoconiosis (endemic non-filarial elephantiasis) in Wolaitta, Southern Ethiopia. *Trop Doct.* 2003; 33:217–20. [PubMed: 14620426]
3. Lapolla W, Tying SK. Podoconiosis. *N Engl J Med.* 2011; 364(12):e23. [PubMed: 21428762]
4. Tekola F, Mariam DH, Davey G. Economic costs of endemic non-filarial elephantiasis in Wolaita Zone, Ethiopia. *Trop Med Int Health.* 2006; 11:1136–44. [PubMed: 16827714]
5. Yakob B, Deribe K, Davey G. High levels of misconceptions and stigma in a community highly endemic for podoconiosis in southern Ethiopia. *Trans R Soc Trop Med Hyg.* 2008; 102:439–44. [PubMed: 18339411]
6. Price EW. The association of endemic elephantiasis of the lower legs in East Africa with soil derived from volcanic rocks. *Trans R Soc Trop Med Hyg.* 1976; 70:288–95. [PubMed: 1006757]
7. Price EW, Henderson WJ. The elemental content of lymphatic tissues of barefooted people in Ethiopia, with reference to endemic elephantiasis of the lower legs. *Trans R Soc Trop Med Hyg.* 1978; 72:132–6. [PubMed: 653784]
8. Price EW, McHardy WJ, Pooley FD. Endemic elephantiasis of the lower legs as a health hazard of barefooted agriculturalists in Cameroon, West Africa. *Ann Occup Hyg.* 1981; 24:1–8. [PubMed: 7235457]
9. Price EW. A possible genetic factor in non-filarial elephantiasis of the lower legs. *Ethiop Med J.* 1972; 10:87–93. [PubMed: 4670473]
10. Davey G, Gebrehanna E, Adeyemo A, Rotimi C, Newport M, Desta K. Podoconiosis: a tropical model for gene-environment interactions? *Trans R Soc Trop Med Hyg.* 2007; 101:91–6. [PubMed: 16884751]
11. Tekola F, Bull S, Farsides B, et al. Impact of social stigma on the process of obtaining informed consent for genetic research on podoconiosis: a qualitative study. *BMC Med Ethics.* 2009; 10:13. [PubMed: 19698115]
12. Tekola F, Bull SJ, Farsides B, et al. Tailoring consent to context: designing an appropriate consent process for a biomedical study in a low income setting. *PLoS Negl Trop Dis.* 2009; 3(7):e482. [PubMed: 19621067]
13. Woldegabriel G, Aronson JL, Walter RC. Geology, geochronology, and rift basin development in the central sector of the Main Ethiopian Rift. *Geol Soc Am Bull.* 1990; 102:439–58.
14. Cesbron-Gautier A, Simon P, Achard L, Cury S, Folle G, Bignon JD. Luminex technology for HLA typing by PCR-SSO and identification of HLA antibody specificities. *Ann Biol Clin (Paris).* 2004; 62:93–8. (In French.). [PubMed: 15047498]
15. Devlin B, Roeder K. Genomic control for association studies. *Biometrics.* 1999; 55:997–1004. [PubMed: 11315092]
16. Vyse TJ, Todd JA. Genetic analysis of autoimmune diseases. *Cell.* 1996; 85:311–8. [PubMed: 8616887]
17. Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med.* 1998; 157:1666–80. [PubMed: 9603153]
18. Ueki A, Isozaki Y, Kusaka M. Anti-caspase-8 autoantibody response in silicosis patients is associated with HLA-DRB1, DQB1 and DPB1 alleles. *J Occup Health.* 2005; 47:61–7. [PubMed: 15703453]

19. Richeldi L, Sorrentino R, Saltini C. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science*. 1993; 262:242–4. [PubMed: 8105536]
20. Saltini C, Richeldi L, Losi M, et al. Major histocompatibility locus genetic markers of beryllium sensitization and disease. *Eur Respir J*. 2001; 18:677–84. [PubMed: 11716174]
21. Newman LS. To Be2+ or not to Be2+: immunogenetics and occupational exposure. *Science*. 1993; 262:197–8. [PubMed: 8105535]
22. de Bakker PI, McVean G, Sabeti PC, et al. A high-resolution HLA and SNP haplo-type map for disease association studies in the extended human MHC. *Nat Genet*. 2006; 38:1166–72. [PubMed: 16998491]
23. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. *Annu Rev Genomics Hum Genet*. 2008; 9:403–33. [PubMed: 18593304]
24. Tishkoff SA, Williams SM. Genetic analysis of African populations: human evolution and complex disease. *Nat Rev Genet*. 2002; 3:611–21. [PubMed: 12154384]
25. Pearson TA, Manolio TA. How to interpret a genome-wide association study. *JAMA*. 2008; 299:1335–44. [Erratum, *JAMA* 2008;299:2150.]. [PubMed: 18349094]
26. Magnusson KP, Duan S, Sigurdsson H, et al. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLoS Med*. 2006; 3(1):e5. [PubMed: 16300415]
27. Caruso C, Candore G, Colonna Romano G, et al. HLA, aging, and longevity: a critical reappraisal. *Hum Immunol*. 2000; 61:942–9. [PubMed: 11053639]
28. Spencer CC, Su Z, Donnelly P, Marchini J. Designing genome-wide association studies: sample size, power, imputation, and the choice of genotyping chip. *PLoS Genet*. 2009; 5(5):e1000477. [PubMed: 19492015]
29. Teo YY, Inouye M, Small KS, et al. A genotype calling algorithm for the Illumina BeadArray platform. *Bioinformatics*. 2007; 23:2741–6. [PubMed: 17846035]

Figure 1. Podoconiosis (Endemic Nonfilarial Elephantiasis)

Podoconiosis is a noninfectious geochemical disease. Panel A shows early-stage podoconiosis, characterized by “slipper-distribution” mossy lesions (covering the tops of the toes and the sides and soles of the feet, much as a typical slipper does), as well as bilateral and asymmetric below-the-knee swelling. At this stage, the condition is reversible with the use of footwear and foot hygiene. Panel B shows two examples of advanced-stage podoconiosis, characterized by bilateral below-the-knee swelling, visible discrete lumps, deformed toes, and pigmented, shiny, thickened skin. At this stage, the disease can be ameliorated, but not completely reversed, with the use of conventional treatment: foot hygiene and compression bandages.

Figure 2 (facing page). Association of Single-Nucleotide Polymorphisms (SNPs) with Podoconiosis

In Panels A and B, each of the 551,840 SNPs in autosomal chromosomes is represented by a point. The higher the point (the higher the negative log₁₀ P value), the more significant the association with podoconiosis. Points above the blue horizontal line represent SNPs with a P value of less than 10^{-5} . Points above the red horizontal line indicate SNPs with a P value of less than 5×10^{-8} . Panel A shows results from the allelic genetic model that tests associations with the individual alleles. Panel B shows the additive genetic model that uses the Armitage trend test. Panel C shows association signals over a 1-Mb HLA class II region on chromosome 6 surrounding rs17612858 (purple diamond); each of the other SNPs is

represented by a gray circle indicating low linkage disequilibrium ($r^2 < 0.20$). The left y axis represents negative $\log_{10} P$ values calculated with the use of the Armitage trend test. The right y axis represents the recombination rate (in centimorgans [cM] per megabase [Mb]), indicated by the blue curve. Genes found in the region are shown in relative position under the plot; arrows indicate the direction of transcription.

Table 1

Genomewide Association Results with the Allelic and Additive Genetic Models.^{*}

SNP	Chromosome	Position (bp) [†]	Type	Closest Gene	Distance to Closest Gene (kb) [‡]	Minor Allele	MAF	Allelic Model [§]		Additive Model [§]	
								P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)
rs17612858	6	32620622	Intergenic	<i>HLA-DQA1</i>	5.78	T	0.42	1.42×10 ⁻⁹	2.44 (1.82–3.26)	3.44×10 ⁻⁸	2.19 (1.66–2.90)
rs35367950	6	32713476	NA	NA	-0.01	G	0.49	2.01×10 ⁻⁷	2.12 (1.60–2.82)	2.52×10 ⁻⁷	2.20 (1.63–2.96)
rs9273349	6	32625869	Upstream	<i>HLA-DQB1</i>	1.38	C	0.48	3.49×10 ⁻⁷	2.08 (1.57–2.76)	3.60×10 ⁻⁷	2.16 (1.61–2.91)
rs1063355	6	32627714	3'UTR	<i>HLA-DQB1</i>	0	C	0.48	3.49×10 ⁻⁷	2.01 (1.57–2.76)	3.60×10 ⁻⁷	2.16 (1.61–2.91)
rs17612633	6	32615710	Downstream	<i>HLA-DQA1</i>	0.87	C	0.48	4.36×10 ⁻⁷	2.10 (1.57–2.81)	7.13×10 ⁻⁷	2.13 (1.58–2.87)
rs17843604	6	32620283	Intergenic	<i>HLA-DQA1</i>	5.44	T	0.47	5.37×10 ⁻⁷	2.06 (1.55–2.73)	5.13×10 ⁻⁷	2.15 (1.59–2.90)
rs9270856	6	32570839	Intergenic	<i>HLA-DRB1</i>	-13.21	A	0.34	1.39×10 ⁻⁶	0.48 (0.35–0.65)	6.99×10 ⁻⁶	0.50 (0.37–0.68)
rs9271170	6	32577889	Intergenic	<i>HLA-DQA1</i>	-18.07	T	0.35	1.48×10 ⁻⁶	0.48 (0.35–0.65)	7.56×10 ⁻⁶	0.50 (0.37–0.68)
rs9271100	6	32576478	Intergenic	<i>HLA-DRB1</i>	-18.85	T	0.34	1.95×10 ⁻⁶	0.48 (0.36–0.65)	8.93×10 ⁻⁶	0.51 (0.38–0.69)
rs2906966	17	15331517	Intergenic	<i>CDRT4, FAM18B2</i>	7.82	C	0.14	2.39×10 ⁻⁶	0.35 (0.23–0.55)	1.25×10 ⁻⁵	0.36 (0.23–0.57)
rs16926675	8	61909133	Intergenic	<i>AC022182.3</i>	-28.80	C	0.24	3.81×10 ⁻⁶	2.20 (1.57–3.08)	2.97×10 ⁻⁶	2.39 (1.66–3.45)
rs8076424	17	51578654	Intergenic	<i>AC090079.6</i>	-36.22	G	0.19	4.64×10 ⁻⁶	2.35 (1.62–3.41)	1.17×10 ⁻⁵	2.36 (1.61–3.46)
rs9514099	13	103738423	Intergenic	<i>SLC10A2</i>	-19.23	A	0.22	5.34×10 ⁻⁶	2.22 (1.57–3.15)	6.56×10 ⁻⁶	2.27 (1.59–3.24)
rs10852956	17	51529741	Intergenic	<i>AC090079.6</i>	-85.13	A	0.41	9.46×10 ⁻⁶	1.90 (1.43–2.54)	2.91×10 ⁻⁵	1.87 (1.40–2.52)
rs11644557	16	47194051	Intronic	<i>ITFG1</i>	0	C	0.09	1.06×10 ⁻⁵	3.22 (1.87–5.55)	5.01×10 ⁻⁵	3.15 (1.81–5.47)

^{*}The term bp denotes base pairs, CI confidence interval, kb kilobases, MAF minor allele frequency, NA not applicable, and UTR untranslated region.

[†]The position is based on University of California Santa Cruz human genome build 19 (hg19/GRCh37).

[‡]The distance to the closest gene is positive if the SNP is upstream of the gene, negative if the SNP is downstream of the gene, and zero if the SNP is within the gene.

[§]The allelic model assumes that each allele in the genotype corresponds to the same value of the dependent variable, and the model tests associations with the individual alleles. The additive model assumes that the risk allele increases risk in an additive fashion, and the Armitage test for trend is used. For both models, the Bonferroni-corrected P-value threshold was 5×10⁻⁸.

Table 2
Single-Nucleotide Polymorphism (SNP) Associations with Podoconiosis in Family-Based Association Tests.

SNP	Allele [*]	Chromosome	Allele Frequency	Model of Inheritance	No. of Families [†]	Test Statistic [‡]		z Score [§]	P Value [¶]	Gene Region
						Observed	Expected			
rs1063355	G	6	0.52	Additive	133	16.0	48.0	2.3	0.02	<i>HLA-DQB1</i>
				Recessive	98	12.8	20.8	2.8	0.005	
rs17612633	C	6	0.53	Additive	127	16.5	45.6	2.4	0.02	<i>HLA-DQA1</i>
				Recessive	95	13.5	20.3	3.0	0.003	
rs17612858	T	6	0.48	Additive	111	15.0	37.0	2.5	0.01	<i>HLA-DQA1</i>
				Recessive	66	13.3	14.2	3.5	<0.001	
rs17867526	G	4	0.87	Additive	72	10.0	20.0	2.2	0.03	<i>AC092661.1</i>
				Recessive	69	8.5	16.8	2.1	0.04	
rs17211510	C	6	0.73	Additive	113	12.5	37.3	2.1	0.04	<i>HLA-DQA1</i>
rs17843604	T	6	0.51	Recessive	98	11.3	20.7	2.5	0.01	<i>HLA-DQA1</i>
rs9271100	C	6	0.69	Recessive	112	10.5	25.6	2.1	0.04	<i>HLA-DRB1</i>

^{*} The overtransmitted allele is shown. The test statistic and significance level for the other allele are the same values (z scores) in the opposite direction because these markers are biallelic — and the corresponding z score and P values for each allele of a biallelic marker under a dominant model are the reverse of those under a recessive model. Analysis was done separately for each model.

[†] The numbers of informative families (i.e., families with at least one heterozygous parent) are shown.

[‡] The observed test statistic is from a family-based association test for the observed number of transmitted alleles. The expected test statistic is the value under the null hypothesis (i.e., no linkage or association).

[§] The z score was calculated by dividing the observed test statistic by the expected test statistic.

[¶] P values are two-tailed. The Bonferroni-corrected P-value threshold was 0.007.

Table 3

Distribution of *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* Alleles Associated with Podoconiosis.*

HLA Allele	Case Patients no./total no. (%)	Controls no./total no. (%)	Odds Ratio (95% CI)	P Value
<i>HLA-DRB1</i>				
*0701	42/180 (23.3)	23/174 (13.2)	2.00 (1.14–3.49)	0.02
*0102	11/180 (6.1)	40/174 (23.0)	0.23 (0.11–0.46)	<0.001
*1302	11/180 (6.1)	22/174 (12.6)	0.47 (0.22–0.99)	0.04
<i>HLA-DQA1</i>				
*01MV	21/176 (11.9)	48/182 (26.4)	0.37 (0.21–0.64)	<0.001
*0201	41/176 (23.3)	24/182 (13.2)	1.91 (1.10–3.31)	0.02
<i>HLA-DQB1</i>				
*0202	42/166 (25.3)	26/154 (16.9)	1.79 (1.05–3.07)	0.03
*0501	14/166 (8.4)	38/154 (24.7)	0.32 (0.17–0.61)	0.001

* For case patients and controls, the numerator represents the number of specific alleles observed. The denominator represents the total number of chromosomes, which is twice the number of participants.