APPENDIX 1. DETAILED DESCRIPTION OF METHODS

Subject recruitment

Psoriasis was defined as previously described[1], and ascertainment was for age at onset ≤ 40 years in the proband[2]. After providing informed consent, all participants received a total body skin examination and provided a blood sample. A total of 579 families were recruited, 102 from northern Germany and the remaining 477 from the United States, largely from southeastern Michigan. Enrollment of subjects and genotyping was carried out under protocols approved by the medical ethical committees of the University of Michigan, Henry Ford Hospital, and the University of Kiel. This study was conducted according to Declaration of Helsinki Principles at all participating institutions.

Genotyping and error detection

Genomic DNA amplification was performed using the same primers employed by Helms et al.[3]. After amplification, SNPs rs745318, rs734232, and rs895691 mapping to the RUNX1 binding site region (rs734232 representing the RUNX1 polymorphism itself) and rs1564864, rs2019154, and rs869190 mapping to the RAPTOR gene, were genotyped using SnapShot SNP assay reagents and GeneMapper software (Applied Biosystems). Microsatellites were typed utilizing ^{32}P-labeled oligonucleotide primers by standard, previously-described methods [4].

Genotypes were checked for Mendelian inheritance errors using Pedstats (version 0.4.6, part of Merlin package) and Pedmananger (http://www.broad.mit.edu/ftp/distribution/software/pedmanager) and for unlikely genotypes using the "error" option of Merlin[5].

Linkage analysis

The sample consisted of 274 families, including 115 families that were used in a prior genome-wide linkage analysis[6]. The 38 marker set for linkage analysis consisted of the six RAPTOR and RUNX1 SNPs and 32 microsatellite markers located across chromosome 17. Marker density was greatest in the vicinity of the PSORS2 locus on distal 17q[6, 7]. Sex-averaged recombination distances between markers were derived from the deCODE map[8]. Distances for markers not in the deCODE map were interpolated based on the physical locations of the markers in the July 2003 human genome assembly (NCBI Build 34, http://genome.usc.edu/). Distances were converted from Kosambi cM to Haldane cM prior to linkage analysis.

Nonparametric linkage analysis was performed using Merlin version 0.10.65. The largest family was split to allow all affecteds to be analyzed, and five families had some (2, 2, 4, 10, and 14) unaffected members trimmed from the bottom generations so that the maximum bit complexity for the pedigree sample was 23. LOD scores and p-values were computed using the Kong and Cox linear model[9], with both the NPL-all and NPL-pairs scoring functions[10]. The choice of allele sharing statistic can affect the power of linkage. The S_{all} statistic performs better than S_{pairs} for a dominant model[11, 12], but S_{pairs} performs better for other disease models[12]. Although there is some evidence that PSORS2 may act in a dominant fashion[6, 7], the true model of inheritance is unknown, so results for both statistics are reported.

Because there is strong linkage disequilibrium (LD) among some of the markers, tightly linked markers were first clustered to avoid positive bias of the LOD scores[13]. Clusters were defined such that all pairwise combinations of markers in a cluster have a standardized multiallelic disequilibrium coefficient (D' measure[14, 15]) of at least 0.3 and a nominal chi-squared contingency table p-value of less than 0.001. The GOLD package[16] was used to compute and visualize disequilibrium measures for all pairwise marker combinations; Merlin was used to create the most likely haplotype vectors for founders, and all phase ambiguous allele assignments were converted to missing data before input into GOLD.

Haplotype reconstruction

Estimating haplotypes in extended families in the presence of LD is an unsolved problem. For the markers we examined, the haplotype structure of the RAPTOR and RUNX1 genes is very simple, with two haplotypes accounting for > 99% of all chromosomes. We used an "ad-hoc" strategy to reconstruct haplotypes in our sample, but expect near perfect accuracy because of the simple haplotype structure. Briefly, maximum likelihood haplotypes for TDT and PDT analysis were created using the "best" option of Merlin (version 0.10.2). This version of Merlin assumes linkage equilibrium among markers, so caution is needed when using its inferred haplotypes for markers in LD. However, Merlin explicitly denotes phase ambiguities within its inferred haplotypes, thereby avoiding a major cause of the problems noted by Schaid et al.[17]. The reliability of the most likely haplotype vectors of Merlin was assessed by comparing them with all possible haplotype vectors (assuming no recombination) for each member of the pedigree sample. Very few substantive differences were found; most differences involved untyped members or whether a haplotype was assigned to the maternal or paternal chromosome, neither of which has any effect on the TDT or PDT.

Phase ambiguities in the most likely Merlin haplotypes were resolved whenever possible using PHASE version 2.1.1[18, 19]. Although PHASE is designed for constructing haplotypes of independent individuals, when families are available current methods of haplotype reconstruction in pedigrees (e.g., Merlin) can use information about gene flow in the family to infer founder haplotypes at many loci, and PHASE can then use this known phase information to estimate any remaining ambiguous phases[18]. All founder haplotypes were extracted from the Merlin output and listed in the primary PHASE input file; in the case of phase uncertainty the two alleles were arbitrarily assigned to the maternal and paternal chromosomes. A second input file, denoting whether phase is known or unknown for each locus of each founder, was created in strict accordance with the certainty of phase assignment in the Merlin haplotypes. Marker positions were derived from the July 2003 human genome assembly (NCBI Build 34). Program default values were used for the number of iterations, thinning interval, and burn-in, and haplotype reconstruction used the recombination model. Uncertain phases in the founder haplotypes were resolved by the best haplotype estimate in the PHASE output whenever the confidence probability of the phase call was at least 99%. Phase ambiguities in all nonfounder haplotypes were then resolved in accordance with the resolution applied to the founders of the same pedigree.

Before input into the TDT and PDT, all haplotypes with any inferred or remaining phase uncertain alleles were converted to missing (0.4% of haplotypes of collected

family members for the RUNX1 binding site, 0.7% for RAPTOR). Haplotypes with inferred alleles (i.e., those of uncollected family members and collected individuals with missing genotypes) must be discarded because their use in these two association tests can lead to bias[20, 21]. Since the TDT and PDT can handle only one possible haplotype configuration per individual, haplotypes with ambiguous phase must also be discarded even though using only phase-known haplotypes can also lead to bias[22]. For both association and power testing, the putative disease-associated haplotype was treated as an allele of a single multiallelic marker.

Haplotypes for FBAT analysis were reconstructed internally by the FBAT program in a probabilistic manner using a conditioning approach that allows use of haplotypes with missing genotype or phase information without introducing bias[23]. This approach can properly handle multiple possible haplotype configurations for an individual.

Family-based association analysis

Pedigrees were analyzed for the putative disease-associated alleles and haplotypes with three different family-based association tests—the transmission / disequilibrium test (TDT)[24], the pedigree disequilibrium test (PDT)[25, 26], and the family-based association test (FBAT)[23, 27, 28]. All three methods were implemented as biallelic two-sided tests of the null hypothesis of no association in the presence of linkage. For the TDT, a single trio was randomly extracted from each pedigree. Since results vary depending upon the particular random selection, the analysis was repeated 999 times with different random number seeds, and the median result reported. Exact binomial p-values were used. For the PDT, we utilized the PDT-avg test which gives equal weighting to all families, rather than the PDT-sum test, which gives greater weight to larger pedigrees. All trios and discordant sibpairs in a family contributed to the test. We also computed $\overline{D}$, a standardized measure of LD between the disease and marker loci as assessed by the PDT[29]. $\overline{D}$ has a range of [-1,1] and is equal to 0 in the absence of evidence for LD. For the FBAT, version 1.5.5 of the software[30] was used with the empirical variance and an offset of 0 (i.e., unaffecteds do not contribute to the test statistic but do aid inference of parental genotypes).

Power tests

Power for the TDT was determined analytically using the first approximation method of Knapp[31]. Computations used a type I error rate of 0.05, a range of values for genotype relative risk for test allele homozygotes (GRR2) of 1.0 to 5.0, four genetic models (dominant, additive, multiplicative, and recessive), the observed number of typed and independent trios in our family sample, and the observed frequency of the disease-associated allele or haplotype among founders.

Power for the PDT and FBAT was determined by simulation, under the alternative hypothesis of LD between psoriasis and the marker locus. Genotypes for pedigrees identical in structure and disease phenotype to the observed sample were generated using a gene drop algorithm with rejection sampling. A gene drop was performed by randomly assigning marker genotypes to founders based on observed allele frequencies and an assumption of Hardy-Weinberg equilibrium, followed by random Mendelian segregation of alleles to all descendants. Penetrances, derived from the specified genetic model and GRR and an assumed population prevalence of psoriasis of 0.02, were then used to probabilistically assign a disease phenotype to all family members. When a pedigree had four or more affected individuals, we conditioned on the phenotype of only 3 randomly selected affecteds, to keep execution times reasonable. Gene drop and disease phenotype assignment were repeated for each pedigree until the resulting phenotypes matched those of the observed family, discarding all realizations inconsistent with the observed disease phenotypes. When a match was achieved, simulated genotypes of untyped members of the pedigree were converted to missing. One thousand simulated pedigree samples were generated for each combination of locus, GRR2, and genetic model of inheritance, and Monte Carlo tests of power for the PDT and FBAT were performed using a type I error rate of 0.05. Four genetic models (dominant, additive, recessive, and multiplicative) were used, and power was tested for every GRR2 increment of 0.01 across a range broad enough to encompass 60–99% power. Power curves, created by fitting the Monte Carlo power estimates with a LOESS smoother[32], were used to predict the GRR2 required for 80% and 95% power. Power for the RAPTOR loci and haplotypes under a dominant model was determined at only two GRR values (2 and 5) using 10,000 simulated pedigree samples.

The Monte Carlo method used to determine power for the PDT and FBAT is fully valid only for monogenic diseases. Our method models phenocopies (other disease loci or unspecified environmental factors) with a nonzero penetrance for people who don't carry a risk genotype at the test locus. This works fine for polygenic diseases when simulating unrelated cases or controls or TDT trio families, but it is inadequate for larger families unless the probability of disease is truly the same for all non-carriers of a risk genotype at the test locus. Because familial aggregation is allowed only through the gene we are simulating, our gene-dropping procedure will inflate the proportion of larger families carrying a risk genotype at the test locus (and thus power) compared to what would be expected in a polygenic model. However, for the GRR2s needed to achieve 80-95% power for the RAPTOR and Runx1 loci, the inflation is slight and is expected to have only a small effect on simulated power.

For all power tests, the marker locus was assumed to be in complete LD (r^2 measure of disequilibrium = 1) with the true disease locus.

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