

ORIGINAL ARTICLE

Analysis of RUNX1 binding site and RAPTOR polymorphisms in psoriasis: no evidence for association despite adequate power and evidence for linkage

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Background: A previous study identified two peaks of allelic association between psoriasis and single nucleotide polymorphisms (SNPs) mapping to distal chromosome 17q, including a disease associated SNP that leads to loss of a RUNX1 transcription factor binding site, and additional SNPs in the third intron of the RAPTOR gene. Another study found an association with SNPs in the RAPTOR gene, but not with the RUNX1 binding site polymorphism.

Methods: In an effort to confirm these observations, we genotyped 579 pedigrees containing 1285 affected individuals for three SNPs immediately flanking and including the RUNX1 binding site, and for three SNPs in the RAPTOR gene.

Results: Here we report further evidence for linkage to distal chromosome 17q, with a linkage peak mapping 1.7 cM distal to the RUNX1 binding site (logarithm of the odds 2.26 to 2.73, depending upon statistic used). However, we found no evidence for association to individual SNPs or haplotypes in either of the previously identified peaks of association. Power analysis demonstrated 80% power to detect significant association at genotype relative risks of 1.2 (additive and multiplicative models) to 1.5 (dominant and recessive models) for the RUNX1 binding site, and 1.3 to 1.4 for the RAPTOR locus under all models except dominant.

Conclusions: Our data provide no support for the previously identified RUNX1 binding site or for the RAPTOR locus as genetic determinants of psoriasis, despite evidence for linkage of psoriasis to distal chromosome 17q.

Psoriasis is a common, immunologically mediated, hyperproliferative skin disease that is influenced by multiple genes, including a major gene in the major histocompatibility complex.^{1–3} Because the penetrance of the disease allele at this locus is only about 10%, and based on recurrence risk⁴ and linkage⁵ analysis, it is apparent that additional loci also influence susceptibility to psoriasis. An early candidate for such a locus was identified by linkage analysis on distal human chromosome 17q,⁶ and designated psoriasis susceptibility 2 (PSORS2). While we and others have provided some confirmatory evidence for genetic linkage to this locus,^{7–9} the PSORS2 gene itself has remained unidentified for a decade.

A recent study by Helms *et al* of single nucleotide polymorphisms (SNPs) and microsatellite markers in the distal 17q region identified allelic association between psoriasis and SNPs mapping in and between the SLC9A3R1 and NAT9 genes on distal chromosome 17q.¹⁰ The disease associated allele of one of these polymorphisms was shown to inactivate a binding site for RUNX1, which is a haematopoietic transcription factor implicated in leukaemogenesis.¹¹ Variant RUNX1 binding sites have also been genetically implicated in systemic lupus erythaematosus¹² and rheumatoid arthritis.^{13 14} Thus, the RUNX1 binding site polymorphism is an attractive candidate for PSORS2. However, only one marker in this peak exceeded the $p = 0.05$ level of significance after the most stringent level of correction for multiple testing,¹⁰ making independent confirmation critical.

The studies of Helms *et al* also identified a second peak of association 6 Mb distal to the RUNX1 binding site, which

mapped to the third intron of the RAPTOR gene. A second study of an independent set of subjects found a weak ($p = 0.027$) association with one SNP in the RAPTOR gene, but not with the RUNX1 binding site polymorphism.¹⁵ A third, independent study found no association with the RUNX1 binding site in any of three independent German cohorts.¹⁶

In this study, we genotyped 579 pedigrees of various structures for three SNPs mapping to the 3' end of SLC9A3R1 and the interval between SLC9A3R1 and NAT9, including the implicated RUNX1 binding site. We also typed the three SNPs in the RAPTOR gene that were previously reported to be associated with psoriasis.^{10–15} Our pedigree sample allowed us to refine and extend our previous linkage analysis of chromosome 17q⁷ by adding 159 pedigrees informative for linkage to our original cohort of 115 pedigrees. We found further evidence for linkage to distal chromosome 17q, with a linkage peak mapping quite close to the RUNX1 binding site. However, we found no evidence for association to individual SNPs or haplotypes in either of the previously identified peaks of association. Simulations demonstrated excellent

Abbreviations: FBAT, family based association test; GRR, genotype relative risk; GRR1, genotype relative risk for test allele heterozygotes; GRR2, genotype relative risk for test allele homozygotes; LD, linkage disequilibrium; LOD, logarithm of the odds; PDT, pedigree disequilibrium test; PSORS2, psoriasis susceptibility 2; SNP, single nucleotide polymorphism; %T, percent transmission; TDT, transmission/disequilibrium test

power of our 517 informative families to detect significant association at realistic genotype relative risks (GRRs) for both regions, with the exception of the RAPTOR locus under the dominant model. Taken together, our data provide no support for either region as genetic determinants of psoriasis, despite demonstration of evidence for linkage of psoriasis to distal chromosome 17q.

METHODS

A detailed description of the methods used, including marker primers and the composition of our pedigree sample, is provided as three supplemental appendices at <http://www.jmedgenet.com/supplemental>.

Subject recruitment

Psoriasis was defined as previously described,¹⁷ and ascertainment was for age at onset of ≤ 40 years in the proband.¹⁸ 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 south eastern Michigan. Enrolment 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

Genomic DNA amplification was performed using the same primers employed by Helms *et al.*¹⁰ 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, Foster City, CA). Microsatellites were typed utilising ³²P-labelled oligonucleotide primers as described.¹⁹

Linkage analysis

The sample consisted of 274 families, including 115 families that were used in a prior genome-wide linkage analysis.⁷ 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}

Non-parametric linkage analysis was performed using Merlin version 0.10.6.²⁰ Logarithm of the odds (LOD) scores and p values were computed using the Kong and Cox linear model,²¹ with both the NPL-all and NPL-pairs scoring functions.²² 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.²³

Haplotype reconstruction

Maximum likelihood haplotypes for analysis by the transmission/disequilibrium test (TDT) and the pedigree disequilibrium test (PDT) were created using the "best" option of Merlin (version 0.10.2). Phase ambiguities in the most likely Merlin haplotypes were resolved whenever possible using PHASE version 2.1.1.^{24,25} Although PHASE is designed for constructing haplotypes of independent individuals, when families are available current methods of haplotype reconstruction in pedigrees (for example, 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.²⁴ 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 for the family based association test (FBAT) 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.²⁶

Family based association analysis

Pedigrees were analysed for the putative disease associated alleles and haplotypes with three different FBATs: the TDT,²⁷ the PDT,^{28,29} and the FBAT.^{26,30} 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. For the PDT, we utilised the PDT-avg test which gives equal weighting to all families. All trios and discordant sibpairs in a family contributed to the test. We also computed \bar{D} , a standardised measure of LD between the disease and marker loci as assessed by the PDT.³¹ \bar{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 software³² was used with the empirical variance and an offset of 0.

Power tests

All power computations used a type I error rate of 0.05, a range of values for GRR for test allele homozygotes (GRR2) broad enough to encompass 60–99% power, and four genetic models (dominant, additive, multiplicative, and recessive). The marker locus was assumed to be in complete LD (r^2 measure of disequilibrium = 1.0) with the true disease locus. Power for the TDT was determined analytically by the first approximation method of Knapp,³³ using the observed number of fully typed and independent trios in our family sample and the observed frequency of the test 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 simulated pedigrees identical in structure, founder allele frequencies, and disease phenotypes to the observed sample were generated using a gene drop algorithm with rejection sampling. One thousand pedigree samples were simulated for each combination of locus, GRR2, and genetic model of inheritance and subjected to a Monte Carlo test of power.

RESULTS

Linkage analysis

As shown in fig 1, non-parametric multipoint linkage analysis of the chromosome 17 markers yielded suggestive evidence for linkage by genome-wide criteria³⁴ for both allele sharing statistics (maximum LOD = 2.73, $p = 2.0 \times 10^{-4}$ with S_{all} and maximum LOD = 2.26, $p = 6.3 \times 10^{-4}$ with S_{pairs}). Evidence for linkage in both cases peaked at 127.9 Haldane cM (marker D17S785) in chromosome region 17q25.1, only 1.7 cM distal to the RUNX1 SNPs.

Both the older⁷ and newer families independently showed evidence for linkage. The original 115 families yielded maximum LOD = 1.32, $p = 0.0068$ with S_{all} and maximum LOD = 0.93, $p = 0.019$ with S_{pairs} , and the subsequent 159 families yielded maximum LOD = 1.63, $p = 0.0031$ with S_{all} and maximum LOD = 1.53, $p = 0.0040$ with S_{pairs} . LOD scores peaked between D17S674 and D17S1847 (134.5–135.5 Haldane cM) for the original sample and at D17S785 (127.9 Haldane cM) for the subsequent sample. The similar linkage peak locations and positive LOD scores obtained in two independent samples increase our confidence that our linkage results for the pooled sample are not a false positive.

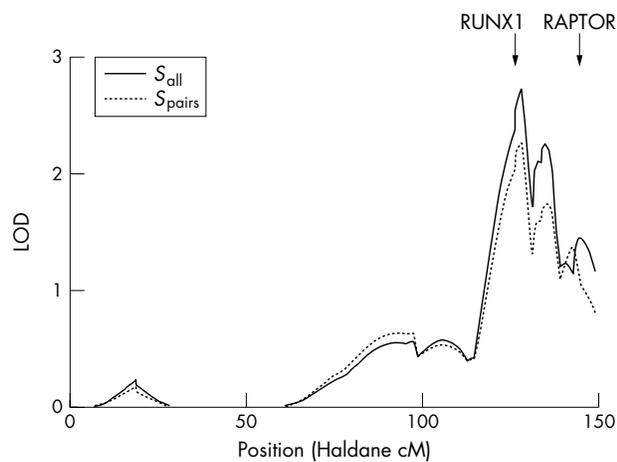


Figure 1 Non-parametric linkage results for chromosome 17. Solid and dotted lines are LOD score plots using the linear Kong and Cox model with the S_{all} or S_{pairs} allele sharing statistic, respectively. The locations of the RUNX1 binding site and RAPTOR gene are shown.

Haplotype reconstruction

The two approaches used for reconstructing haplotypes (Merlin combined with PHASE or FBAT), although very different, gave nearly identical results. For the RUNX1 interval, the combined frequency of the two most common haplotypes was estimated as 99.2% by both methods; for the RAPTOR interval it was estimated as 99.8% using Merlin and PHASE and 99.9% using FBAT. The preponderance of only two of the eight possible haplotypes indicates nearly complete LD ($r^2 \approx 1$) among the three biallelic loci of each interval.

Family based association tests

Using the full pedigree sample, no significant association of psoriasis was found with any of the previously reported

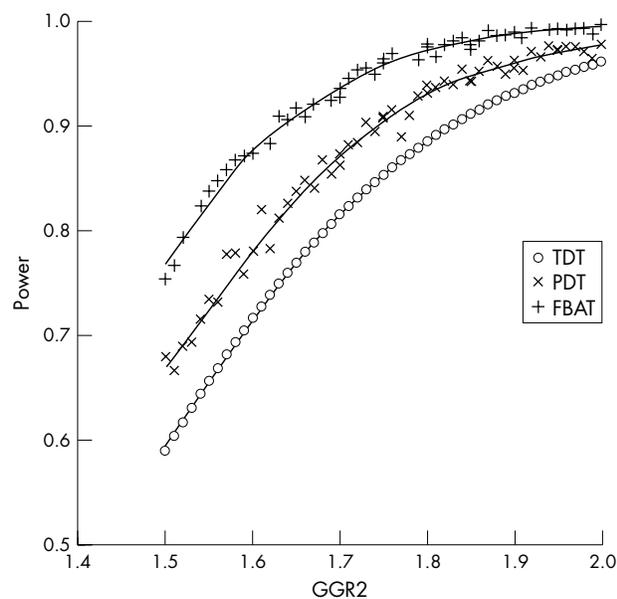


Figure 2 Comparison of power curves for the RUNX1 binding site locus under the additive model, type I error rate = 0.05. The TDT power curve was analytically derived using the method of Knapp,³³ whereas the power calculations for the PDT and FBAT were carried out as described in Methods. For purposes of comparison, GRR2 for the MHC linked PSORS1 locus is approximately 10 under the additive model.³¹

disease associated alleles for either the RUNX1 or RAPTOR intervals (table 1A). As expected given the nearly complete LD among SNPs in each interval, results for the three-locus haplotypes were essentially identical to those for individual SNPs. A weak positive but clearly non-significant (p value ≥ 0.40) association with psoriasis was measured by all three tests with both the putative RUNX1 disease alleles (51.5–52.0 percent transmission (%T), 0.006 to 0.010 \bar{D} , 0.07 to 0.17 FBAT statistic Z) and the putative RAPTOR disease alleles (50.6–51.2 %T, 0.030 to 0.041 \bar{D} , 0.17 to 0.35 Z). The strength of association originally reported for the RUNX1 SNPs and haplotypes, 55–58 %T by the TDT or PDT, was much higher.¹⁰ Neither of the reports of significant association with RAPTOR^{10 15} provided measures of the strength of association.

Results were not improved by restricting the analysis to those pedigrees with a documented family history of psoriasis, which we defined as two or more affected individuals in a family larger than a trio (table 1B). p values were generally higher (≥ 0.6), and the strength of association was diminished for RUNX1 (49.7–50.5 %T, -0.037 to -0.026 \bar{D} , -0.53 to -0.36 Z) and essentially unchanged for RAPTOR (50.9–51.2 %T, 0.021 to 0.037 \bar{D} , 0.11 to 0.23 Z).

Power tests

Power of the full pedigree sample is very good for detecting association with the markers or haplotypes in the vicinity of the RUNX1 binding site under all genetic models (table 2). Power is good for all three tests of association but consistently best for the FBAT (table 2 and fig 2). When carrying one copy of the test allele (two copies for the recessive model), 80% power with the FBAT is attained with GRRs of 1.23 to 1.53 (depending on genetic model; details in table 2) and 95% power with GRRs of 1.31 to 1.75. Power is also very good for detecting association with the RAPTOR markers or haplotypes under all models except the dominant (table 2); 80% power with the FBAT is attainable with GRRs of 1.31 to 1.41 and 95% power is achieved with GRRs of 1.42 to 1.64. The putative disease allele and haplotypes for the RAPTOR locus are very common (frequency 0.77) and carried by 94.7% of individuals in our sample. Therefore, power to detect associations with RAPTOR under a dominant model is poor, being only 24% and 52% for GRR2 of 2 and 5, respectively. As expected, for either interval, association tests of the haplotype had power essentially identical to tests of single SNPs (data not shown).

DISCUSSION

The search for genes conferring risk for common diseases with a genetic component continues to be a major challenge.³⁵ Independent replication of findings is a critical component of this process. In this study, we have analysed 517 pedigrees of various structures for association to three SNPs within a 2.5 kb interval including the RUNX1 binding site, and three SNPs within a 26.5 kb interval in the RAPTOR gene, that were previously described by Helms *et al.*¹⁰ However, we failed to demonstrate any significant associations.

Given these disappointing findings, we considered it essential to carefully evaluate the power of our full sample to detect significant association. Our sample contains a diverse mix of families, including many extended pedigrees. The TDT is restricted to a single trio per family when implemented as a test of association, but the PDT and FBAT can more fully use multiplex and multigenerational families, including those with missing parents. Analytical power methods are available for the TDT³⁶ but not for the PDT and FBAT when applied to samples like ours. We therefore developed a gene drop algorithm with rejection sampling to

Table 1 Results of family based tests of the association of psoriasis with RAPTOR and RUNX1 SNPs and three-locus haplotypes

Locus	Associated allele*		TDT			PDT				FBAT				
	Base	Freq	No. fam†	No. inf fam‡	T:NT§	p value¶	No. fam†	No. inf fam‡	\bar{D}^{**}	p value¶	No. fam†	No. inf fam‡	Z††	p value¶
A: All pedigrees														
RUNX1														
rs745318	T	0.442	467	351	233:218	0.51	503	372	0.009	0.53	515	307	0.13	0.89
rs734232	A	0.440	466	350	235:219	0.48	502	371	0.006	0.51	514	306	0.17	0.87
rs895691	A	0.444	466	350	233:219	0.54	502	371	0.010	0.55	514	307	0.07	0.95
Haplotype	TAA	0.435	466	350	236:218	0.43	502	371	0.009	0.48	514	364	0.17	0.87
RAPTOR														
rs1564864	T	0.772	468	286	163:159	0.87	504	304	0.030	0.96	516	271	0.17	0.86
rs2019154	C	0.772	467	284	168:160	0.73	503	302	0.037	0.83	515	270	0.31	0.76
rs869190	G	0.773	468	284	165:159	0.78	505	303	0.039	0.86	517	269	0.31	0.76
Haplotype	TCG	0.771	467	285	164:157	0.76	503	301	0.041	0.84	515	315	0.35	0.79
B: Non-trio pedigrees														
RUNX1														
rs745318	T	0.452	181	143	90:89	1.00	217	164	-0.031	0.87	229	129	-0.40	0.69
rs734232	A	0.448	180	142	91:90	1.00	216	163	-0.033	0.90	228	129	-0.36	0.72
rs895691	A	0.452	181	143	88:89	1.00	217	164	-0.037	0.74	229	130	-0.53	0.59
Haplotype	TAA	0.442	181	143	92:90	0.94	217	164	-0.026	0.98	229	187	-0.36	0.72
RAPTOR														
rs1564864	T	0.767	182	112	65:62	0.86	218	130	0.032	0.88	230	110	0.11	0.91
rs2019154	C	0.766	182	112	59:57	0.93	218	130	0.031	0.86	230	111	0.11	0.91
rs869190	G	0.768	181	110	59:57	0.93	218	129	0.037	0.88	230	108	0.17	0.87
Haplotype	TCG	0.765	182	112	62:59	0.86	218	130	0.036	0.91	230	149	0.23	0.82

Non-trio pedigrees: those families larger than a trio with at least two affected members.

*The alleles and haplotypes tested are those reported to be associated with psoriasis (Helms *et al*⁹ for RUNX1; Anne Bowcock, personal communication for RAPTOR). Allele frequency is based on all founders in the pedigrees.

†The number of families shown for each association test counts only those families with at least one typed and phenotypically informative unit. For the TDT this unit is a trio (an affected child and both parents), for the PDT it is a trio or a discordant sibpair (an affected and unaffected sib), and for the FBAT with the settings used here it is a trio, discordant sibpair, or a sibship with three or more affected sibs.

‡The number of informative families is the subset of the typed and phenotypically informative families that are genotypically informative for the allele being tested.

§T:NT is the ratio of transmissions to non-transmissions of the test allele from heterozygous parents to affected children in the TDT.

¶All p values are uncorrected for multiple testing.

** \bar{D} is a standardised measure of disequilibrium for the PDT (see Methods).

††Large sample test statistic for association, distributed as a standard normal.

determine power of the PDT and FBAT by simulating family samples with founder allele frequencies, pedigree structures, and disease phenotypes matching our observed sample. As illustrated in fig 2 for an additive model and the RUNX1 locus, the power of our families to detect association is substantially greater with the PDT and FBAT than with the TDT. The PDT and FBAT not only utilise a larger variety of pedigrees (compare number of families in table 1) but also a larger portion of the information contained in each family. Greatest power was achieved with the FBAT, which uses the greatest number of phenotypically informative families and which does the best job of utilising families with missing parental information.

Our findings for the RUNX1 binding site constitute the third negative report for this locus.^{15 16} The power of the recent study by Hüffmeier *et al*¹⁶ is good but substantially less than ours. In order to directly compare the power of the TDT in these two samples according to the method of Knapp,³³ we reset our type I error rate to 0.01, the genotype relative risk of test allele heterozygotes (GRR1) to 1.6, and the GRR2 to 2.8, as specified by Hüffmeier *et al*.¹⁶ With these parameters, the power of our sample was 0.999, as compared to 0.93 for the family sample of Hüffmeier *et al*. Power calculations were not reported by Capon *et al*,¹⁵ but given their sample size, the power of their study should be lower than that of Hüffmeier *et al* or of the present study (233 v 300 v 467 trios,

Table 2 Genotype relative risks needed to achieve power of 0.80 and 0.95 in family based tests of the association of psoriasis with RAPTOR and RUNX1 SNPs*

Locus†	Genetic model	GRR2/GRR1 for power of 0.80			GRR2/GRR1 for power of 0.95		
		TDT	PDT	FBAT	TDT	PDT	FBAT
RUNX1	Dominant	1.69/1.69	1.61/1.61	1.52/1.52	2.00/2.00	1.92/1.92	1.75/1.75
	Additive	1.69/1.35	1.62/1.31	1.53/1.27	1.96/1.48	1.87/1.44	1.73/1.37
	Multiplicative	1.69/1.30	1.62/1.27	1.52/1.23	1.96/1.40	1.86/1.36	1.72/1.31
	Recessive	1.69/-	1.63/-	1.53/-	1.93/-	1.84/-	1.70/-
RAPTOR	Dominant	‡	‡	‡	‡	‡	‡
	Additive	2.09/1.54	1.99/1.50	1.81/1.41	2.76/1.88	2.59/1.80	2.28/1.64
	Multiplicative	1.92/1.38	1.84/1.36	1.71/1.31	2.33/1.53	2.23/1.49	2.01/1.42
	Recessive	1.50/-	1.47/-	1.40/-	1.69/-	1.65/-	1.55/-

*GRRs needed to achieve power of 0.80 and 0.95 when testing for association of the three-locus RUNX1 and RAPTOR haplotypes are essentially identical to the GRRs of their constituent SNPs.

†For each locus, power was tested for the allele listed in table 1.

‡Power of 0.80 or 0.95 is not attainable. For both single SNPs and the three-locus haplotype, power at a GRR2 (=GRR1) of 2 and 5 is 0.18 and 0.42 for the TDT, 0.22 and 0.47 for the PDT, and 0.24 and 0.52 for the FBAT.

GRR1, genotype relative risk for test allele heterozygotes; GRR2, genotype relative risk for test allele homozygotes

respectively). Furthermore, the only positive report of RUNX1 binding site association¹⁰ found no significant association of psoriasis with any of the three RUNX1 SNPs we tested or their haplotype when a single affected individual was drawn from each family and tested against an independent control sample. This is puzzling, as the TDT and case control tests are known to have nearly identical power for a given genetic model when the numbers of trios and case control pairs are equivalent.³⁷ The difference is not likely to be due to population admixture, as the allele frequencies of the associated SNPs in the independent control sample did not differ from those of non-transmitted alleles and haplotypes identified by the family based tests.

Our findings stand in contrast to two previous positive reports of association between psoriasis and RAPTOR.^{10, 15} However, the Capon *et al* study found only weak evidence of association to only one of the RAPTOR SNPs ($p = 0.027$ for rs2019154) unless analysis was restricted to those trios with a documented family history of psoriasis.¹⁵ A similar stratification in the present study failed to increase the significance or strength of association for any of the RAPTOR SNPs or their haplotype (table 1B).

Association analysis is generally more powerful than linkage analysis.³⁸ Capon *et al*¹⁵ cite this fact to explain why they found significant association with the RAPTOR loci when two previous studies^{39, 40} using many of the same families failed to find significant linkage. By the same reasoning, one would expect association analysis of our sample to readily reveal a bona fide PSORS2 locus, given that our pedigree sample provides evidence for linkage to the PSORS2 region of chromosome 17q. However, it did not. Indeed, our current linkage results attain a much higher level of significance than those previously reported by Speckman *et al*⁴¹ for the cohort used to identify associations to the RUNX1 binding site and the RAPTOR gene¹⁰ ($p = 0.00020$ or 0.00063 for this study v $p = 0.05$ for Speckman *et al*).

In the original report,¹⁰ only one marker each in the RUNX1 binding site and RAPTOR peaks of association remained significant at the $p = 0.05$ level after correcting for multiple testing with the false discovery rate method under the assumption of correlated data,⁴² raising the possibility of a false positive result. The appearance of three independent negative attempts to confirm this result for the RUNX1 binding site (Capon *et al*¹⁵, Huffmeier *et al*,¹⁶ and this study) heightens this concern. Our failure to find any evidence for association to the RAPTOR locus could be due to the low power of our sample to detect a dominant gene. However, if this were the case, it would be very surprising that two prior studies,^{10, 16} both of which reported allele frequencies similar to our sample but were based on fewer families than this one, would be able to detect it. Taken together, the available information suggests that the PSORS2 locus or loci exist, but the corresponding gene(s) remains to be identified. Additional studies, and meta-analysis of available studies,⁴³ will be necessary to provide a definitive answer to this important question.

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ELECTRONIC-DATABASE INFORMATION



Three supplemental appendices describing the methods used are available at <http://www.jmedgenet.com/supplemental>.

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