Evaluation of the IRF-2 Gene as a Candidate for PSORS3

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Type 1 interferon can trigger flares of psoriasis. Hypersensitivity to type 1 interferon signaling causes a psoriasis-like skin disease in mice deficient for the transcription factor interferon regulatory factor 2 (IRF2). The human IRF2 gene is located at a previously identified candidate psoriasis susceptibility locus on chromosome 4q (PSORS3 at D4S1535). Therefore, we tested association of psoriasis with IRF2. We generated a sample consisting of 157 families with a total of 521 individuals. Five novel microsatellite markers were developed and typed, and complemented with three known markers to yield a set of eight markers spaced within 600 kb around the IRF2 gene, three of which are located in the gene. We detected association of IRF2 with type 1 psoriasis at two markers in the IRF2 gene. Haplotype sharing analysis confirmed association of IRF2 with type 1 psoriasis (p = 0.0017; pcorr = 0.03). The 921G/A SNP in exon 9 was found to obliterate a predicted exon splice enhancer in an allele-specific manner. There was a suggestive increase of homozygosity for the splicing-deficient allele in type 1 psoriasis patients. Our data identify IRF2 as a potential susceptibility gene for psoriasis.

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Psoriasis is a multifactorial skin disease with a strong genetic component (reviewed in Elder et al., 2001). Clinically, psoriasis has been classified into type 1 and type 2 subgroups (Henseler and Christophers, 1985; Christophers and Henseler, 1989). The type 1 form is characterized by an early age at onset (≤ 40 years) and a positive family history. Mice deficient for the transcription factor interferon regulatory factor 2 (IRF2) sustain a skin disease with striking parallels to psoriasis (Hida et al., 2000), which is caused by a hyperresponsiveness to type 1 interferon signaling. This is in line with the role of IRF2 as a transcriptional repressor of α- and β- (i.e., type 1) interferon target genes (Taniguchi and Takaoka 2001). Intriguingly, this hyperresponsiveness is limited specifically to the skin, despite the ubiquitous expression pattern of IRF2. Administration of type 1 interferon can also trigger flares of psoriasis in humans (Downs and Dunnill 2000; Kowalzick, 1997). Moreover, the human IRF2 gene is located only 50 kb from the PSORS3 locus (marker D4S1535 on chromosome 4q25 (Matthews, 1996). We analyzed 198 families with a total of 521 individuals and 201 psoriasis patients (41% type 1 psoriasis). All patients underwent in-patient treatment at the time of sampling. Figure 1 shows the results of single locus alleleic (Fig 1a), genotypic (Fig 1b), and two-locus haplotype (Fig 1c) association analysis. Although a suggestive peak was observed in the unstratified patient sample at marker PS4005 for allelic association, none of the p values reached statistical significance. Nevertheless, when limiting the patient sample to the type 1 subgroup, suggestive results were detected at marker PS4005 both for alleleic (p = 0.005, when corrected for testing on eight markers in two subgroups; pcorr = 0.07) and genotypic (p = 0.007; pcorr = 0.11) association, as well as a suggestive peak in the two-locus haplotype (p = 0.04; pcorr = 0.5) association analysis (Fig 1a–c, gray symbols). These data suggest that an association exists between IRF2 and type 1 psoriasis. Notably, we observed low or absent linkage disequilibrium between the markers (data not shown) suggesting that association of IRF2 with psoriasis would have gone unnoticed in previous genomewide scans employing marker D4S1535 (Nair et al., 1997; Enlund et al., 1999; Zhang et al., 2002).

Association study All clinical investigation in this study were conducted according to Declaration of Helsinki principles and were subject to prior institutional review board approval. We identified five novel microsatellite markers suitable for genotyping, three of which are located within the IRF2 gene (primer sequences and genotyping conditions are available upon request). To these, we added three known markers (D4S408, D4S3047, D4S1535). Marker D4S1535 has been used previously as the PSORS3 marker (Matthews, 1996). We analyzed 198 families with a total of 521 individuals and 201 psoriasis patients (41% type 1 psoriasis). We previously described the Haplotype sharing statistic (HSS), which detects an excess in the mean length of haplotypes shared among patients to those shared among controls (Te Meerman et al., 1995;
This method does not compare allele frequencies and has been shown to be independent of association analysis (Nolte, 2002a). It can thus be used to complement the latter to increase overall statistical power. HSS also allows incorporation of multiallelic and biallelic genotyping data. Therefore, SNP 921G/A genotyping results (see below) were included in addition to the markers mentioned above in the HSS analysis of the IRF2 locus. The results are shown in Fig 2. A clear peak is visible at marker PS4005 for the entire patient sample (black line), with a maximum –log₁₀ p value of 2.1 (corresponding to a p value of 0.008 and pcorr = 0.13 after Bonferroni correction for 18 tests), and the type 1 psoriasis subgroup (gray line) with a maximum –log₁₀ p value of 2.76 (p = 0.0017; pcorr = 0.03). Thus, HSS analysis also suggest an association of IRF2 with type 1 psoriasis.

**SNP 921G/A** We next screened all nine IRF2 exons (omitting the noncoding part of exon 9) including exon–intron boundaries, as well as the proximal promoter region.
(300 nt upstream of the major transcription initiation site) for sequence variations associated with psoriasis risk haplotype status by WAVE analysis. This analysis revealed only the already established 921G/A variant in exon 9 (data not shown). We then genotyped the entire patient sample for this SNP using the assay described by (Nishio et al., 2001). The results are summarized in Table I. There was a notable but not statistically significant increase in frequency of individuals homozygous for the G allele among type 1 psoriasis patients compared to controls (41.1% vs. 30.5%). The age at onset also differed between the A/A and G/G groups in the type 1 psoriasis subgroup.

**Putative function of the 921G allele**

The 921G/A SNP represents a translationally silent variation. Nevertheless, it is located at the +3 position of exon 9. The sequence of intron 8 preceding the 3′-splice site of exon 9 contains a notably weak polypyrimidine tract (Roscigno et al., 1993). 3′-Splice sites with a weak polypyrimidine tract exhibit low binding affinity to the U2AF auxiliary factor requiring an exon splicing enhancer (ESE) for optimal splicing (Hastings and Krainer 2001). When subjecting the IRF2 sequence to an ESE prediction program (http://exon.cshl.org/ESE; threshold 3.0; predictions of ASF/SF2-binding sites), we detected a strong predictive score for an ESE permitting binding of the SR family member ASF/SF2 in the IRF2 921A allele. This binding site was completely abolished in the 921G allele. Independently of the 921G/A site, two other equally strong ASF/SF2 ESE predictions were detected downstream (not shown).

The suggestive p values observed for an association between IRF2 and type 1 psoriasis suggest that IRF2 likely represents a susceptibility or disease-modifying gene only in a subgroup of psoriasis patients. We used the type 1 subgroup, but it is evident that this group is still heterogeneous. Recently, IRF2 was reported to be aberrantly expressed in suprabasal psoriatic lesional epidermis (van der Fils et al., 2003). Although this study offers no support for a loss of function of IRF2 in psoriasis, it does not exclude it, because the low number of patients studied (six) does not address the problem of genetic heterogeneity.

We find suggestive evidence for an increased frequency of the 921G/G allele among type 1 psoriasis patients and for a decreased age at onset in the type 1 921G/G subgroup. We also present indirect evidence that the 921G/A SNP may differentially affect splicing. Therefore, individuals with the IRF2 921G/G genotype may harbor increased sensitivity to type 1 interferon owing to deficient IRF2 expression or functioning. The magnitude of this effect may only be moderate, because two other ESE sites close to the 921G/A site are intact in both haplotypes. More importantly, the frequency differences at this SNP between type 1 psoriatic patients and controls are small. Thus, our results may be exerted by the combined action of multiple SNPs. Several intronic SNPs have been mapped in the IRF2 gene (Carstens et al., 2000). The fact that intronic SNPs may affect the binding of transcription factor to intronic enhancers has recently been shown for systemic lupus erythematosus (Prokunina et al., 2002).

In conclusion, we present evidence for a putative association between the transcription factor IRF2 and type 1 psoriasis. Future studies must reveal whether the 921G/A SNP itself acts as a disease-modifying variant in a subgroup of psoriatics or is merely in linkage disequilibrium with another causal variation.

**References**


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