Abstract  Chromosome 17q25 harbors a susceptibility locus for psoriasis (PSORS2). This locus may overlap with loci for atopic dermatitis and rheumatoid arthritis. To further refine the location of PSORS2, we genotyped 242 primarily nuclear families for 15 polymorphic microsatellites mapping to chromosome 17q23-q25. Non-parametric linkage analysis revealed a linkage peak lying close to a novel cluster of genes from the immunoglobulin (Ig) superfamily. This cluster spans >250 kb and harbors five CMRF35-like genes and a sixth inhibitory receptor (CMRF35H) with three ITIM motifs that is transcribed in the opposite direction from the rest. The Ig domains encoded by these genes are most similar to those of the TREM (triggering receptor expressed selectively in myeloid cells) molecules, NKp44 and the polymeric immunoglobulin receptor. CMRF35-like genes are only expressed in sub-populations of cells of the myeloid lineage. In order to investigate the association of this region with psoriasis, we genotyped the families for 13 novel microsatellites and 19 SNPs from the region of linkage. A maximum NPL of 1.6 (P=0.05) was obtained within the interval. Two SNP-based haplotypes revealed some evidence for association with psoriasis. One spanned CMRF35H and includes a non-synonymous polymorphism within CMRF35H (R111Q) (TDT P=0.03). The second was a three-locus haplotype lying within the first intron of CMRF35A2 (TREM5) (TDT P=0.04). The novel markers described here will facilitate additional linkage and association studies between the CMRF35 family and disease.

Introduction  Psoriasis is a debilitating, chronic inflammatory skin disorder that affects 2–3% of the U.S. population. It is characterized by scaly skin patches caused by infiltration of inflammatory cells into the dermis and epidermis, with secondary epidermal cell (keratinocyte) hyperproliferation (Menter and Barker 1991). Psoriasis is a complex disease and a combination of genetic and environmental factors are likely to be causative. Psoriasis vulgaris, or chronic plaque psoriasis is the most common form of the disease. Streptococcal infection may exacerbate chronic plaque psoriasis and is strongly associated with guttate psoriasis, possibly through the release of superantigenic toxins (Davison et al. 2001). The frequency of psoriasis is also elevated in patients with HIV infection (Duvic 1990; Mallon and Bunker 2000). Approximately 10% of patients also have psoriatic arthritis of five different subtypes, one of which may be identical to rheumatoid arthritis (Gladman and Brockbank 2000).

The exact pathogenesis of psoriasis as a T-cell mediated disease is still unclear. However, epidemiological and family studies (Bhalerao and Bowcock 1998) have demonstrated that a genetic component exists in this disorder. Psoriasis was initially shown to be associated with HLA antigens, particularly those encoded by class I genes: HLA-Cw6 and HLA-B57 (Gottlieb and Krueger 1990; Tiilikainen et al. 1980). The role of a susceptibility locus in the HLA region at chromosome 6p21, now known as PSORS1, has been confirmed by multiple linkage and association studies (Enlund et al. 1999a; Nair et al. 1997; Trembath et al. 1997), although identification of the exact genetic lesion(s) within this region has proved difficult (Allen et al. 1999; Asumalahti et al. 2000; Veal et al. 2002; Saccone et al., manuscript submitted).

Our group localized a non-HLA psoriasis susceptibility locus (PSORS2) to 17q24-q25 following a genome-
wide scan of eight multiply affected families (Tomfordre et al. 1994). Further linkage studies on independent family sets from the U.S., Germany, Ireland and Sweden supported linkage to this region (Enlund et al. 1999a; Matthews et al. 1996; Nair et al. 1997). Genetic studies on independent sets of multiply affected families have localized additional susceptibility loci to 1q21-q23 (PSORS4) (Bhalerao and Bowcock 1998; Capon et al. 1999), 4q (PSORS3) (Matthews et al. 1996), 3q21 (PSORS5) (Enlund et al. 1999b) and 19p13.3 (PSORS6) (Lee et al. 2000a). Genome-wide scans, primarily on sib pairs or nuclear families have also provided some evidence for loci on chromosomes 2p, 16q, 20p and 1p (Nair et al. 1997; Trembath et al. 1997; Veal et al. 2001). Some of these localizations overlap with loci for other inflammatory diseases such as rheumatoid arthritis (3q21 and 17q24-q25) and atopic dermatitis (1q21, 3q21, 17q24-q25) (Cookson et al. 2001; Cornelis et al. 1998; Jawaeer et al. 2001; Lee et al. 2000b).

To further refine the location of PSORS2, we genotyped 242 (primarily nuclear) families for polymorphic microsatellites mapping to chromosome 17q23-q25. Non-parametric genetic linkage analysis revealed a linkage peak lying close to a novel cluster of genes from the immunoglobulin (Ig) superfamily. We next identified and validated a set of single nucleotide polymorphisms (SNPs) peak lying close to a novel cluster of genes from the im-

Materials and methods

Clinical material

Psoriasis families were ascertained via letters to collaborating dermatologists or through the National Psoriasis Foundation. All participating relatives were from the U.S. and all patients had chronic plaque psoriasis. Presence or absence of psoriasis was determined from thorough medical history and clinical evaluation. Informed consent was obtained from all family members available for venipuncture. Lymphoblastoid cell lines were established by Epstein-Barr virus transformation of peripheral blood lymphocytes, and genomic DNA was isolated from these cells or from whole blood by routine methods.

A total of 242 nuclear families were ascertained via these means. Most had at least two children with psoriasis and all except for eight were Caucasian (three were Asian, three Hispanic, and two African-American). There were 199 families with two affected offspring, 29 families with one affected child, ten with three, two with four, and one family each with six and eight affected offspring. The total number of diagnosed affected individuals was 572; 224 were of unknown diagnosis, and there were 209 unaffected individuals. The average age at onset of psoriasis was 23 years. Eighty individuals had been diagnosed with psoriatic arthritis. Thirty-nine additional individuals had joint involvement considered likely to be psoriatic arthritis, so that a total of ~20% of patients in this cohort may have had psoriatic arthritis.

Semi-automated genotyping

Genotyping of polymorphic microsatellites was performed with fluorescently tagged oligonucleotide primers (Research Genetics or Integrated DNA Technologies) and semi-automated genotyping as described elsewhere (Bennett et al. 2000). Polymorphic microsatellites were identified by querying the Marshfield database (for markers within the 17q24-q25 region, or were developed with Benson’s Tandem Repeats Finder (Benson 1999), in which case primers were designed with the aid of “Primer 3” (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3 www.cgi). Following amplification of known or predicted exons and regulatory sequences from the region, SNPs were identified by sequencing pooled DNA samples of 10–30 unrelated affected and unaffected individuals as described elsewhere (Taillon-Miller et al. 2000). DNA pools consisted of samples from ~20 unrelated psoriasis cases, unrelated cases from nine large families demonstrating linkage to this region (unpublished results; Tomfordre et al. 1994) and ~20 unrelated Caucasian controls from CEPH. The psoriasis cases harboring haplotypes associated with psoriasis from this region or from families providing some evidence for linkage to this region were selected to maximize the chance of identifying psoriasis-associated variants/mutations.

SNP genotyping was performed with fluorescently labeled allele-specific primer extension assayed by fluorescence polarization template-directed dye incorporation (FP-TDI) (Chen and Kwok 1997). ExoSAP-IT and AcyloPrime kits were obtained from USB (Cleveland, Ohio) and PerkinElmer (Boston, Mass.), respectively. Unlabeled dideoxynucleotides (Boehringer Mannheim) were used for the liquid chromatography assay. Genotyping methods were verified by sequencing a subset of the samples. Further information on polymorphic microsatellites and SNPs are provided in detail on our web site at http://hg.genome.wi.mit.edu/bowcock/papers/lg2002data/index.html.

Linkage analyses

Following gel runs, the Genotyper 2.0 software was used to size alleles. PEDMANAGER (Whitehead Institute for Biomedical Research, http://www.genome.wi.mit.edu) was used to check for non-Mendelian inheritance of alleles and to estimate allele frequencies. Parametric and non-parametric analyses and multipoint TDT analyses were performed with GENEHUNTER v2.0 (Kruglyak et al. 1996) with pairs set to 1 (the first pair of affected sibs being included in the analysis only). Association studies were performed with the haplotype relative risk (HRR) test (Falk and Rubinstein 1987) as implemented in the Analyze program (Terwilliger 1995) using both a likelihood ratio and chi-square test. The HRR test has a higher expected power for detecting association than does the TDT test (Schaid and Sommer 1994; Terwilliger 1995), but can give false positive results in the presence of dominance and/or sample heterogeneity. However, such false positives would not be expected to be clustered. Initial marker orders were obtained from the Marshfield database and refined with physical mapping.

Due to the difficulty in determining a reasonable correction for multiple testing, and the loss of the interpretability of P values if they were corrected, we have chosen to present raw P values throughout.

Physical mapping and gene characterization

Bacterial artificial chromosomes (BACs) from the 17q24-q25 region were identified by hybridizing pools of oligonucleotide primers for the polymorphic microsatellites D17S617, D17S1352, D17S1864, D17S1829, D17S1602, D17S929, D17S1807 and D17S114 with the RPCI-11 BAC filters and by querying the TIGR BAC End Database with sequences from the region. Genomic sequences containing these loci were identified by querying the NCBI (http://www.ncbi.nlm.nih.gov) and Celera databases (Venter et al. 2000) with BLAST.