Mapping of psoriasis to 17q terminus

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Psoriasis is a chronic, inflammatory, hyperproliferative disease of the skin, scalp, nails, and joints, with a prevalence of up to 2% in Caucasians^{1,2} but well under 1% in the Mongoloid races of the Far East.³ The disease varies in severity. Some patients display mild disease with isolated scaling erythematous plaques on the elbows or knees, whereas for others most of their cutaneous surface can be affected. At the cellular level, psoriasis is characterised by markedly increased epidermal proliferation and incomplete differentiation, elongation, dilation, and leakiness of the superficial plexus of dermal capillaries, and by a mixed inflammatory and immune cell infiltrate of the epidermis and papillary dermis.^{1,2} Dermal infiltrates comprised of T cells and macrophages typically appear in early lesions before epidermal changes.⁴ The therapeutic effect of immunosuppressive agents suggests psoriasis has a primary immune pathogenic basis.⁵

Susceptibility to the development of psoriasis is likely to have a significant genetic component. Accumulating evidence is consistent with the idea that psoriasis is a multifactorial disorder caused by the concerted action of multiple disease genes in a single individual and triggered by environmental factors.⁶ Some of these genes control the severity of a variety of diseases, via their regulation of the inflammatory and immune processes (severity genes), whereas others are unique to psoriasis (specific genes).

A number of genetic studies have sought to identify the psoriasis susceptibility loci. Associations between psoriasis and human lymphocyte antigen alleles were first described in 1990.⁷ Subsequently, genome-wide linkage scans have mapped psoriasis to several chromosomal regions including PSORS1 at 6p21,^{8,9} PSORS2 at 17q,^{8–10} PSORS3 at 4q,¹¹ PSORS4 at 1q,¹² PSORS5 at 3q,¹³ PSORS6 at 19p,¹⁴ and PSORS7 at 1p.¹⁵ Recently, the International Psoriasis Genetics Consortium reassessed these candidate loci using a cohort of 942 affected sib pairs. This reassessment confirmed the significant linkage on chromosomes 6p21, 16q, and 10q22–q23.¹⁶ PSORS2 on chromosome 17q24–25 did not exceed a maximum LOD score of 0.9 in that study, even though this region has been implicated in psoriasis in several family studies.^{8–10} However, a RUNX1 binding site variant located in the 17q25 region between SLC9A3R1 and NAT9 has recently been identified as the first putative susceptibility gene for psoriasis.¹⁷

The present study was prompted by the debatable importance of 17q25 in psoriasis. We present information obtained from a large, five generation kindred with psoriasis. Using a genome-wide scan and single nucleotide polymorphism (SNP) fine mapping, the psoriasis locus was mapped to the chromosome 17q terminus, a region close to the 17q PSOR2 locus.

METHODS

Family ascertainment

A five generation psoriasis kindred with apparent autosomal dominant inheritance (fig 1) was identified at the National

Key points

- Psoriasis is a common inflammatory skin disorder affecting up to 2% of the general population. To investigate a five generation kindred with psoriatic lesions of variable severity, genome-wide scans, single nucleotide polymorphism (SNP) fine mapping, and association analysis were performed.
- Genome-wide scans showed a highly significant linkage with a maximum two point LOD score of 7.164 ($\theta=0.01$) and a multipoint LOD score of 4.58 at D17S928, the most telomeric microsatellite marker at 17q.
- Fine mapping with SNP markers demonstrated two plateaus of significant LOD score (>3.3) with the highest LOD score of 7.67 surrounding D17S784, and a LOD score of 3.404 near the 17q terminus.
- Modified PDT (Pedigree Disequilibrium Test Analysis Program version 4.1) analysis revealed a strong association ($p=0.0008$) between psoriasis and the SNP #206 marker (rs3744165) located within 400 kb of the 17q terminus.
- Our study maps the psoriasis susceptibility locus to the distal end of chromosome 17q, which is distal to and distinct from a recently reported putative susceptibility gene SLC9A3R1 located in 17q25 for the PSORS2 locus.

Taiwan University Hospital. All individuals were examined by at least two dermatologists. This family comprised a total of 93 members, including 16 with classical skin manifestations of psoriasis, 10 with mild skin lesions, and 43 who were unaffected (fig 2). Because of age dependent variation in the expression of psoriasis, 24 family members aged less than 30 who presented with a normal appearance received the “unknown disease” classification. The study was approved by the institutional review board at the National Taiwan University Hospital. Informed consent was obtained from each participating person.

Genotyping

Genomic DNA was extracted using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). Genotyping was performed for 382 highly polymorphic microsatellite markers from the ABI PRISM Linkage Mapping Set v 2.5 HD5 (Applied Biosystems, Foster City, CA, USA). The average heterozygosity of markers was 0.72% with estimated 10 cM spacing. Allele sizing was calculated

Abbreviations: SNP, single nucleotide polymorphism; TBCD, tubulin specific chaperone d

Figure 1 A five generation psoriasis kindred with large intrafamilial variation. Most spouses are not depicted. Those numbered are included in genotyping.

using the GeneMapper software program (Applied Biosystems). Allele calling and binning were performed using the SAS program. All genotyping was performed with the inclusion of four CEPH control individuals (1331-01, 1331-02, 134702, H20) for quality control purposes. In order to improve the mapping on 17q25, six additional markers between D17S785 and D17S784 were selected from Génethon (<http://www.cephb.fr/ceph-genethon-map.html>), the Cooperative Human Linkage Center (<http://gai.nci.nih.gov/CHLC/>), and the Marshfield Clinic Research Foundation (Marshfield, WI, USA; <http://www.marshfieldclinic.org>) for genotyping: D17S801 (75.1 Mb), D17S722 (75.59 Mb), D17S939 (76.05 Mb), D17S802 (76.83 Mb), D17S1806 (78.04 Mb), and D17S1822 (78.48 Mb).

Single nucleotide polymorphism selection and typing

High density SNPs with minor allele frequency between 0.1 and 0.5 was selected from the SNP Consortium (<http://snp.cshl.org/>) and the NCBI website (<http://www.ncbi.nlm.nih.gov/>) (for sequence names and allele frequencies of the SNP markers see the supplemental table available at <http://jmg.bmjournals.com/supplemental>). Primers and probes that flanked the SNPs were designed in multiplex format using SpectroDESIGNER software (Sequenom, San Diego, CA, USA). Multiplex PCR was performed. Unincorporated dNTPs were dephosphorylated using 0.3 U of shrimp alkaline phosphatase (Hoffman-LaRoche, Basel, Switzerland) followed by primer extension. A cation exchange resin (SpectroCLEAN, Sequenom) was used to remove residual

Figure 2 Skin lesions of the patients. (A) Plaque psoriasis on the upper arm of the proband (IV-5). (B) Clearly demarcated skin lesions on the legs of V-8 at the age of 3. (C) Mild psoriatic lesion on the elbow of III-1. (D) Atrophic skin of III-2 at age 71. (E) Marked hyperkeratosis with parakeratosis, loss of granular layer, epidermal acanthosis, and elongation of rete ridges, thin suprapapillary plates, vascular dilatation, inflammatory cell infiltration, and a suspicious Munro's microabscess (arrow) are apparent in V-4. (F) Less severe changes with alternating orthokeratosis and parakeratosis are evident in V-8. Reproduced with permission.

Figure 3 Genome-wide linkage analysis of the extended psoriasis kindred. This analysis includes 43 family members. The y axis represents the LOD score calculated using the MLINK program. The x axis represents the distance from the chromosomal p terminus expressed in Haldane cM.

Figure 4 Multipoint analysis using the LINKMAP program with six additional microsatellite markers in the 17q25 region. The y axis represents the LOD score. The x axis represents the distance in Mb. The LOD scores (in parenthesis) and positions for markers D17S785, D17S784, and D17S928 are also depicted.

salt from the reactions, and 15 nl of the purified primer extension reaction was spotted onto a 384 element silicon chip (SpectroCHIP, Sequenom). SpectroCHIPS were analysed using a Bruker Biflex III MALDI-TOF SpectroREADER mass spectrometer (Sequenom) and spectra processed using SpectroTYPER (Sequenom).

Statistical analyses

The Mendelian inheritance for the 382 microsatellite markers of this pedigree was verified by using the PedCheck program (<http://watson.hgen.pitt.edu/register/docs/pedcheck.html>). The allele frequencies were estimated by using 90 individuals randomly chosen from our previously collected control population. Two point analyses for these markers were first calculated using the MLINK program of the LINKAGE package. For the most significant results obtained in the region covering D17S785, D17S784, and D17S928, multipoint analysis using the LINKMAP program was carried out. Given that the Asian prevalence of psoriasis is under 1% and many candidate genes have been identified, the prevalence of a single gene that might segregate in this particular family should be low and we therefore assumed the disease gene frequency to be 0.00062 with 0.12% prevalence. Although segregation analyses for the dominant and polygenic models were not conclusive, given that a more mildly affected father (IV-7) transmitted a severe disease (V-8) to his daughter, a dominant model with reduced penetrance (80%) and 0.1% phenocopy rate was assumed for the calculations. Two point analyses were also performed for the SNP markers in this region. Multipoint analysis was performed using the SimWalk2 program. A modified version of the PDT (Pedigree Disequilibrium Test Analysis Program version 4.1),¹⁸ which utilises a

permutation test to calculate the empirical p value, was used to test the allelic association between SNP markers and psoriasis. In calculating the empirical p value, 10 000 permutations were carried out for each SNP.

RESULTS

The age of onset of psoriasis and the severity of the disease varied in family members. The proband (individual IV-5, indicated by an arrow in fig 1), a 41 year old woman, showed plaque-like scaling skin lesions with cracking and fissuring over most of her skin surface including her palms and scalp since the age of 10 (fig 2A). However, her son (V-4) exhibited erythrodermic psoriasis with generalised, inflamed erythematous lesions, and fever. In V-8, well demarcated, palm sized, pink-to-red coloured skin lesions had been noted since the age of 3 (fig 2B), which extended from her extremities to her trunk within 6 months of follow up. However, her father (IV-7) and some other family members exhibited only ichthyosis-like skin lesions over their legs, or lesions over the extensor side of their knuckles, elbows, and knees (III-1, fig 2C). Diffuse skin atrophy was seen in older patients (III-2, fig 2D). No active arthritis was seen in this kindred, but some patients had deformed finger joints and had experienced arthralgia during disease flare up. There was no sex difference in the number affected, disease severity, or transmission rate. Skin biopsy specimens from V-4 revealed marked hyperkeratosis and parakeratosis with follicular plugging, loss of granular layer, epidermal acanthosis, and elongation of rete ridges, thin suprapapillary plates, vascular dilatation, and inflammatory cell infiltration in the dermis and epidermis (fig 2E). Histological changes in V-8 were similar but much less severe. Of particular interest was the presence of alternating orthokeratosis and parakeratosis, a histopathologic finding reminiscent of pityriasis rubra pilaris (fig 2F).

We performed a genome wide scan with polymorphic microsatellite markers for 43 DNA samples (those numbered in fig 1) from 13 individuals with classical skin manifestations of psoriasis, 10 with mild skin lesions, seven with unknown disease status, and 13 family members who were unaffected. One region covering D17S785, D17S784, and D17S928 on chromosome 17q showed highly significant linkage with the disease, with a maximum two point LOD score of 7.164 at D17S928 ($\theta = 0.01$) (fig 3). The LOD scores for markers over other regions of chromosome 17 and other chromosomes were not significant, including the PSORS1 locus (flanking markers D6S257 with LOD = 0.0028, and D6S460 with LOD = -2.313). To further validate our results and narrow down the candidate region, six additional microsatellite markers between D17S785 and D17S784 were added, and multipoint analysis demonstrated a LOD score of 1.21 for D17S785, 2.57 for D17S784, and 4.58 for D17S928 (fig 4). These data are consistent with a gene location close or distal to D17S928, hitherto the most distal known polymorphic microsatellite marker on 17q. The distance between D17S928 and the end of 17q is around 800 kb.

To improve the mapping, we further genotyped 202 SNP markers located between D17S785 and the 17q terminus

Figure 5 Distribution of 202 SNPs. The SNPs are depicted according to their relative position to the microsatellite markers D17S785, D17S784, and D17S928.

Figure 6 Multipoint linkage analysis with 38 SNP markers surrounding D17S784 using the SimWalk2 program. The y axis represents the LOD score. The x axis represents the distance in kb. SNPs #154 and 155 have the highest LOD score of 7.67.

(fig 5). Since computation for a multipoint analysis with 202 SNP markers in such a complicated pedigree structure was not feasible, two point analyses were carried out first in order to narrow down the region. Results from two point linkage

analysis peaked at regions surrounding D17S784 and near 17q terminus, and these two regions were studied by multi-point analyses using 38 and 45 SNPs, respectively. These analyses further disclosed two plateaus of significant

Figure 7 Multipoint linkage analysis with 45 SNP markers near the 17q terminus. The y axis represents the LOD score. The x axis represents the distance in kb. SNP #211 has the highest LOD score of 3.404.

Table 1 Allelic association between nine SNP markers and psoriasis

SNP number	p Value
203	0.156
204	0.215
205	0.135
206	0.0008
207	0.11
208	0.136
209	0.181
210	0.115
211	0.198

The empirical p value was calculated from 10 000 permutations with the PDT program using PDT average statistics.

LOD score (>3.3) with the highest LOD of 7.67 at SNPs #154 and #155 (fig 6), and a LOD of 3.404 at SNP #211 (fig 7).

We calculated the allelic associations of 29 and nine SNP markers in the two regions (#130 to #158 surrounding D17S784, and #203 to #211 near the 17q terminus) using a modified version of the PDT program. The variance for the PDT in the original program is obtained from more than one family. Since only a single family is involved in our analysis, a permutation test was used to derive the empirical p value. For the association test in the region from #130 to #158, no significance was found. The results were possibly due to low heterozygosity especially for #154 ($H=0$) and #155 ($H=0.16$). The results in the region from #203 to #211 revealed a significant association between SNP #206 and psoriasis ($p=0.0008$) as shown in table 1. This SNP marker (rs3744165) is located at an intronic region of the tubulin specific chaperone d (TBCD) gene (MIM 604649), approximately 400 kb from the 17q terminus.

DISCUSSION

We have mapped the psoriasis susceptibility locus to the distal end of chromosome 17q. No significant linkage to other chromosomes, including 6p21, 10q, 16q, and other loci previously reported for psoriasis, was evident in our analyses.

PSORS2 at 17q25 was one of the earliest identified psoriasis loci.¹⁰ In that study of eight Caucasian families, family 1 (PS1, 19 affected and 12 unaffected members) revealed strong linkage to D17S784 with a maximal two point LOD score of 5.33. The investigators proposed a psoriasis susceptibility gene between D17S784 and D17S928.¹⁰ Two subsequent studies confirmed the psoriasis susceptibility locus at 17q, employing either nuclear or medium size families, or sib pairs, but the linkage was not strong.^{7,9} Recently, in the absence of significant linkage to micro-satellite markers in the genotyping data from 242 nuclear families, non-parametric analysis performed with Gene-Hunter provided evidence for linkage to D17S1301.¹⁹ Their follow up study demonstrated a putative RUNX1 binding site variant 8 Mb proximal to the 17q terminus, which maybe associated with susceptibility to psoriasis.¹⁷

The above studies represent the sole sources of published fine mapping data on PSORS2. The region near the 17q terminus that we have identified in our familial population appears to be distal to the regions previously reported. The discrepancy in the precise localisation of the psoriasis susceptibility locus on chromosome 17q25 could be due to our use of a different study design. We used a single, large, extended family, while previous studies examined multiple, smaller families. Alternatively, the discrepancy could be simply due to genetic heterogeneity. As a third possibility, it is conceivable that the family studied was somehow unique in their presentation of psoriasis. With respect to the latter

possibility, it may be germane that the clinical manifestations included not only both plaque psoriasis and erythrodermic psoriasis, but also very mild skin lesions and histopathologic findings reminiscent of pityriasis rubra pilaris.

Given the pedigree of multiple affected members in our family and the presence of a milder affected father (IV-7) who transmitted a severe disease to his daughter (V-8), a dominant model with variable expression seemed likely. We propose that a major, specific psoriasis gene segregates in a dominant way in this kindred with reduced penetrance, while other less severe or modifying genes together determine the phenotypes. The lack of linkage to PSORS1 in the human lymphocyte antigen region (the only consistently replicated psoriasis locus) in the family is also consistent with a monogenic inheritance pattern. Because of this, we chose parametric linkage analysis over a non-parametric approach for this large pedigree to allow adequate power (power = 83% with LOD of 3). In addition, in order to perform non-parametric analysis, this large extended family would need to be broken down into smaller families to fit into any available calculation algorithm (for example, GeneHunter or SimWalk2). Obviously, these smaller families are not independent and hence the results would be unreliable. In our parametric linkage analyses, various values of prevalence ranging from 0.12 to 1% were tested and LOD scores with small fluctuations were observed, demonstrating the robustness of the results.

The lack of association for SNP markers surrounding D17S784 was possibly due to low heterozygosity especially for #154 and #155 (low heterozygosity was also the reason for the negative LOD scores for SNP markers immediately surrounding D17S928). Further study with new and informative SNPs in this region is ongoing.

In this study, SNP #206 (lying 0.38 Mb distal to D17S928) revealed significant evidence from both linkage and association analyses. SNP #206 is located at an intronic region of the TBCD gene (MIM 604649). TBCD is one of the tubulin specific chaperone proteins responsible for microtubule assembly and regulation,²⁰ and is expressed in skin. One of its partners, tubulin specific chaperone cofactor C, may functionally overlap with the retinitis pigmentosa 2 protein. It is possible that a mutation or polymorphism in TBCD could alter the integrity of keratinocytes and so prelude the inflammatory change that is a hallmark of psoriasis.

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ELECTRONIC-DATABASE INFORMATION

The URLs for this paper are as follows: Cooperative Human Linkage Center, <http://gai.nci.nih.gov/CHLC/>; Génethon, <http://www.cephb.fr/ceph-genethon-map.html>; Marshfield Clinic Research Foundation, <http://www.marshfieldclinic.org/NCBI>, <http://www.ncbi.nlm.nih.gov/>; SNP Consortium, <http://snp.cshl.org/>; the PedCheck program is available at <http://watson.hgen.pitt.edu/register/docs/pedcheck.html>; and the supplemental table is available at <http://bmjournals.com/supplemental>.

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ECHO

Polymorphisms in neprilysin gene affect the risk of Alzheimer's disease in Finnish patients

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Please visit the *Journal of Medical Genetics* website [www.jmedgenet.com] for a link to the full text of this article.

Objectives: Neprilysin (NEP) is an amyloid β -peptide ($A\beta$) degrading enzyme expressed in the brain, and accumulation of $A\beta$ is the neuropathological hallmark in Alzheimer's disease (AD). In this study we investigated whether polymorphisms in the NEP gene have an effect on the risk for AD.

Methods: The frequencies of seven single nucleotide polymorphisms (SNPs) and apolipoprotein E (APOE) were assessed in 390 AD patients and 468 cognitively healthy controls. Genotypes of the study groups were compared using binary logistic regression analysis. Haplotype frequencies of the SNPs were estimated from genotype data.

Results: Two SNPs, rs989692 and rs3736187, had significantly different allelic and genotypic frequencies (uncorrected $p = 0.01$) between the AD and the control subjects and haplotype analysis showed significant association between AD and NEP polymorphisms.

Conclusion: Taken together, these findings suggest that polymorphisms in the NEP gene increase risk for AD and support a potential role for NEP in AD.

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