Network: Diseases Due to Environmental Factors

Project: Disease Gene on Chromosome 6p

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Introduction

Psoriasis vulgaris [MIM 177900] is a chronic inflammatory dermatosis with a prevalence of 0.5-5%, depending on race and countriy. Psoriasis is a multi-factorial disease involving the interaction of several genes as well as environmental factors like streptococcal infections, physical trauma, drug usage, and stress.

Genome-wide scans have revealed linkage to several regions across the genome. Linkage has been shown on chromosomes 6p21, 1q21, 1p and 16q. Suggestive linkage includes regions at chromosomes 3p, 8q, 15p and 20p. Other studies have also shown chromosomes 17q and 4q to be of interest but these regions have not been replicated in any genome-wide scans (reviewed in 1).

All the genomewide studies have revealed highly significant linkage to a region on the major histocompatability complex (MHC) at 6p21.3, designated “psoriasis susceptibility region 1” (PSORS1). Within that region, HLA-Cw6 was the first allele to show a strong association with familial psoriasis; and HLA-Cw6 is still the only marker allele present in all investigated ethnic groups .Our own group refined PSORS1 to a region of approximately 170 kb telemoric of HLA-C (2). A case control study in a Japanese population using microsatellite markers distributed over a 1060 kb region surrounding the HLA-C locus located PSORS1 to an interval of 111 kb telomeric of the HLA-C gene (3). Interestingly these two studies, although based on different ethnic groups, showed an overlapping DNA interval of approximately 110 kb suggesting that PSORS1 is likely to be located here. Seven genes have been identified within this candidate DNA interval including POU5F1 or octamer transcription factor 3 gene (OTF3), the cell growth regulated gene TCF19, HCR gene encoding α-helix coiled coil rod homologue protein, SPR1 encoding small proline rich protein, S gene or corneodesmosin (CDSN), SEEK1 and STG. TCF19 is far less polymorphic than the other genes in the region and showed no association with psoriasis, suggesting that the true gene(s) for psoriasis could be telomeric to TCF19.

However, several other studies also investigating the relation of psoriasis with loci centromeric of HLA-Cw, i.e. MICA, TNFα, and HLA-DRB1 and -DQB1.These studies demonstrated that the prevalence of the risk alleles of these loci seems to be propably lower than that of PSORS1 or Cw6, but that the presence of alleles of these loci results in higher risk for psoriasis HLA-Cw.

In order to further clarify the psoriasis risk region in the MHC, we examined a 1,923 kb DNA interval located from 910 kb telomeric to 1013 kb centomeric of HLA-C for psoriasis mutation(s).

Patients and Methods

Patients and controls: 530 patients from 56 families with multiple occurance of chronic plaque type psoriasis (age at onset < 40) and 696 healthy controls recruited in the Kiel area were included into the study. For confirmatory purposes, an independent cohort (480 patients, 1468 unaffected) derived from Ann Arbor, MI, was genotyped for a subset of the SNPs (n=40). **SNP- and HLA-Genotyping:** A total of 99 SNPs was selected encompassing 1,923 kb on 6p21.3 from DDR1 to BTNL2 (table 1). SNP genotyping was done by TaqMan^R technology. SNPs were selected either from the literature or from the Kiel NGFN SNP program. HLA genotypes (HLA-B, Cw, DRB1, and –DQB1) were estimated using the DYNAL Allset Plus^R genotyping kits or by PCR-based HLA genotyping-tests as published (4). **Statistical analysis:** For case-control and transmission disequilibrium analysis, odds ratios and p-values were calculated for single markers and for marker haplotypes. All calculations were done using the program HAPLOVIEW (5). **Haplotype construction:** For construction of SNP haplotypes, either HAPLOVIEW or a self-written program (http://www.hlainformatik.de/) were used. Shortly, this program uses the segregation information of chromosome 6 as provided by HLA data and is able to define SNP haplotypes as far as siblings and parents are not heterozygous at a given position. **Stratification for PSORS1:** Patients and probands were designated to be positive for PSORS1 if they were positive for HLA-Cw6.

MHC I	#	*pos*	*MHC III*	#	*Pos*
Unknown	3	-910	BAT1	3	174
DDR1	1	-458	IkBL	2	192
VARS2L	1	-438	LTA	2	216
UNQ541	1	-420	TNF Alpha	3	219
Unknown	2	-280	unknown	1	224
C6orf115a	1	-245	BAT2	2	579
C6orf115b	1	-241	DDAH2	1	374
CDSN	2	-238	C6orf29	1	514
PSORS1C1	6	-225	unknown	1	608
HCR	8	-212	unknown	1	706
C6ORF18	1	-199	GKIV2L Intron	1	719
TCF19	6	-194	CREBL1	1	732
Pou5F1	5	-189	PBX2	1	796
Unknown	3	-181	NOTCH4	2	828
NOB4	1	-118	unknown	1	837
HCGII-2	4	-102	C6orf10	1	905
HLA-Cw	6	0	BTNL2	1	1014
KIAA0055	6	9			
MICA	4	54			
Unknown	1	84			
P5-1	4	106			
Unknown	1	116			
MICB	6	150			
	74			25	

Tab 1: *SNP probes used in the study. Positions of loci are given relative to HLA-Cw in kb/1000. SNPs labeled als “unknown” indicate localization within uncoding regions. Square: PSORS1 as defined in the text*

Results:

Association analysis: Several regions in the investigated area yielded highly significant association with psoriasis (CDSN, HCR, POU5F1, HCG2-II, KIA0055hom) all p<0.00001; fig 1). All of these loci are already known to demonstrate strong association with psoriasis. However, in both cohorts the calculation of odds ratios and family based transmission disequilibrium ratios yielded the highest values for loci located centromeric of HLA-Cw, close to or within the central MHC (fig. 1). The highest odds ratios were observed in both cohorts for P5-1, MICB, and the TNFα promoter polymorphism at position –238. **Haplotype construction:** Using 530 individuals from 56 families of the primary cohort, we were able to deduce the SNP sequence of a total of 307 chromosomes. Among those, the phase of 83% of the SNPs could unambigously be identified. Analysis of SNP blocks both by HAPLOVIEW and sequence comparison revealed an extremely well conserved block of 30 kb in the region telomeric of HLA-C which comprises 20 SNPs and the genes HCR, TCF19, and POU5F1. This block (block I in fig. 2) is present in 101 of the 307 chromosomes, and 37 of the 46 affected founders are positive for this haplotype. Of the 56 chromosomes positive for Cw6 (18%), 52 (93%) are positive for Block I, and the SNPs which

yielded the strongest association (figure 1) are all present in this block.

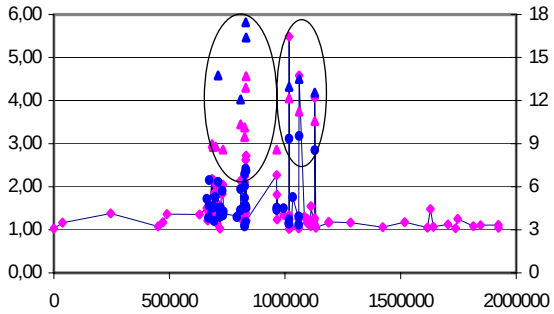

Fig 1: *Association and odds ratios for the investigated markers. Connected squares indicate the observed odds ratios for each marker (left), triangles indicate the respective – log(p) values (right). Magenta: Kiel-, blue: Ann Arbor-cohort. The left circle indicates markers belonging to block I, the right circle markers belonging to block III (see below.)*

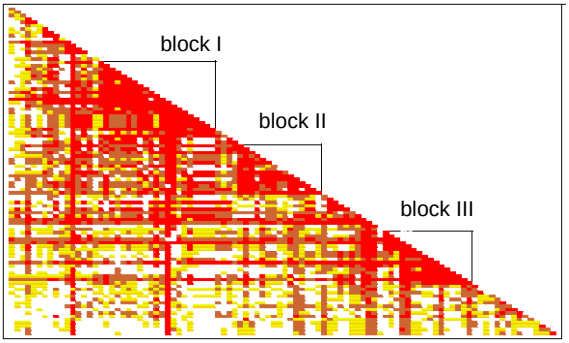

Fig 2: *Two point LD as calculated out of 307 deduced haplotypes: Red squares: LD > 95%; orange: LD > 75%, yellow: LD > 50%; white: LD < 50%. The triangles indicate conserved blocks. I: HCR, TCF19,POU5F1, II: NOB4, HCG II, HLAC, KIA0055hom; III: MICB, IkBL, LTA, TNFa*

A further block (block III, Fig. 2) contains the three SNPs which yielded the highest odds ratios. Again, these three SNPS are in almost perfect linkage disequilibrium (LD) and form a well conserved haplotype which in turn is in very close linkage with block I (31 out of 33 chromosomes; 94%). However, block III is less frequent than block I (33 vs 101 chromosomes).

			psoriasis		
		block III	Yes	No	P
Cw6 pos:	Kiel	-	82	62	0,000031
		+	87	20	
	AA	-	27	37	0,003014
		+	44	21	
	S	-	109	99	0,000001
		+	131	41	
Cw6 neg:	S	+	6	7	n.s.

Tab 2: *Distribution of the P5-1/MICB/TNF-238 risk haplotype (block III) in Cw6 positive and Cw6 negative individuals. Cw6 indicates presence or absence of PSORS1.*

Haplotype association: Individuals positive for PSORS1 and Block III were selected and the strenght of association was calculated in dependence of the presence or absence of the respective haplotypes. In both cohorts, the frequency of psoriasis was significantly increased when block III was present in individuals positive for block I when compared with individuals positive only for block I but negative for block III. Block III occurred only very rarely in the absence of PSORS1 (or HLA-Cw6, respectively). Table 2 shows the results of this analysis.

Discussion:

Psoriasis is a common, immunologically-mediated, hyperproliferative skin disease that is influenced by multiple genes, including a major gene in the major histocompatibility complex. Recently discussed candidate genes within the MHC are CDSN, HCR, and HLA-Cw6. However, because the penetrance of the disease allele at this locus is only about 10%, and based on recurrence risk(6) and linkage(1) analysis, it is apparent that additional loci also influence susceptibility to psoriasis. Such additional susceptibility regions migth be PSORS2-PSORS6 which have been shown to be in significant linkage with the disorder (1).

We have analysed association and odds ratios of a total of 99 SNP marker located within the MHC I and MHC III. Not surprisingly, markers located within or close to the PSORS1 region as described in the literature yielded the highest significance. The respective marker alleles are very frequent among patients (> 80%, data not shown) but do not carry high relative risks. In contrast, we observed three markers centromeric of PSORS1, which yield much higher odds ratios (fig. 1) but are less frequent in patients. In order to clarify this discrepancy, we deduced the SNP frequencies of the MHC chromosomes present in the investigated families. These investigations demonstrated that the PSORS1 haplotype is in very close linkage with a second haplotype located centromeric of HLA-Cw. The presence of this haplotype significantly increases the risk to develop psoriasis, but only if the PSORS1 haplotype is also present. Without PSORS1, this haplotype does not confer any risk for psoriasis, indicating that there is an interaction of at least two different genes. Genes located in the centromeric region significantly associated with psoriasis are P5.1, TNFα and MICB. Due to their immunological functions, at least TNFα and MICB are well suited psoriasis susceptibility candidates. Taken together, these data indicate that in fact genes additional to the one present in PSORS1 are necessary to develop psoriasis. One of such a gene might also be present in the human MHC, located centromeric of PSORS1 close to the central MHC. Since the psoriasis gene located in PSORS1 is probably involved in the epidermal differentiation dysregulation characteristic for psorisis, this second gene might be reponsible for the immunological dysregulation of psoriasis.

Project Status

The SNP based gene scan of the MHC is now finished. Due to the observed extreme LD within the investigated areas, genotyping probably won't provide futher information. Functional analyses now have to show the role of the interacting genes located in that region.

Outlook

To further clarify the role of a second psoriasis gene in the MHC, (i) functional studies will be performed to investigate the role of the identified candidates in the pathogenetics of psoriasis. (ii) The chip based genome wide scan will be used to identify further additional genes in the human genome which interact with PSORS1.

Lit.: ***1.*** *Elder JT. Psoriasis clinical registries, genetics, and genomics. Ann Rheum Dis. 2005 Mar;64 Suppl 2:ii106-7.* ***2.*** *Nair RP et al. Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C. Am J Hum Genet. 2000 Jun;66(6):1833-44.* ***3.*** *Oka A et al. Association analysis using refined microsatellite markers localizes a susceptibility locus for psoriasis vulgaris within a 111 kb segment telomeric to the HLA-C gene. Hum Mol Genet. 1999 Nov;8(12):2165-70.* ***4.*** *Welsh K et al. Molecular typing for the MHC with PCR-SSP . Rev Immunogenet. 1999;1(2):157-76.* ***5.*** *Barrett JC et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005 Jan 15;21(2):263-5.* ***6:*** *Elder JT et al. The genetics of psoriasis. Arch Dermatol 1994;130(2):216-24.*