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SHORT COMMUNICATION

Strong associations of psoriasis with antigen processing LMP and transport genes TAP differ by gender and phenotype

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Psoriasis, a skin disease with autoimmune features, can be triggered and exacerbated by genetic and environmental factors. Chemicals can break tolerance to self-antigens by interfering with antigen processing and presentation; therefore, proteins involved in antigen processing may affect susceptibility. We test here whether variants of immunoproteasome subunits LMP2 and LMP7, or antigen peptide transport proteins TAP1 (transporters associated with antigen presentation) and TAP2 are associated with psoriasis. We analyzed 7 single-nucleotide polymorphisms in 321 Caucasian (German) psoriasis patients and 235 unrelated controls by time-of-flight mass spectrometry, using the Sequenom platform. We found strong associations of psoriasis with variant alleles of LMP and TAP (OR_{TAP_687}: 3.3, 95% CI: 1.9–5.7). Genotype effects were generally stronger for males and LMP effects were mainly seen for psoriasis arthropathica. Our results will help define behavioral or drug treatment suggestions to patients and contribute to a better understanding of the role of low molecular weight chemicals in genetic susceptibility to autoimmune diseases.
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Psoriasis is a disease with autoimmune features, affecting about 2% of Caucasians, with major impact on patient's quality of life. Manifestations include red, heavily scaled pruritic plaques, and can affect joints as well. Several distinct but overlapping clinical phenotypes can be distinguished including psoriasis vulgaris and psoriasis arthropathica. Strong evidence suggests a multilocus model of inheritance in association with environmental factors.[1] The etiology of the disease is still unclear. In several cases, however, low molecular weight chemicals (LMWC), such as drugs, were identified as etiologic agents, and variants of genes responsible for metabolizing LMWC are risk factors.[2] A proven concept of immunotoxicology holds that LMWC can break tolerance by interfering with antigen processing and presentation, either exposing cryptic neo-antigens, or by haptenating and thus immunoconverting presented self-antigens.

Self-antigen processing and presentation is an essential part of the autoimmune response. Consequently, presenting human leukocyte antigen (HLA) genes, notably HLA-Cw*0602, belong to the identified susceptibility genes of psoriasis.[3] Antigen presentation is essentially dependent on the proteasome, an intracellular protease complex catalyzing important steps in the breakdown of proteins to peptides to be presented as antigens. The proteasome is a multicatalytic proteinase complex with a highly ordered ring-shaped 20S core structure. The core structure is composed of four rings of 28 non-identical subunits; two rings are composed of seven α-subunits and two rings are composed of seven β-subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave cellular as well as foreign peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of peptides destined for antigen presentation. The γ-interferon-controlled expression of the proteasomal subunits LMP2 and LMP7 enables the immunoproteasome to generate such peptides with a high efficiency. These peptides are transported into the lumen of the endoplasmic reticulum via the 'transporters associated with antigen presentation' (TAP), where they bind the major histocompatibility complex (MHC) class I proteins.[4] Proteasome inhibitors have been found to be effective in the treatment of psoriasis in an animal model.[5]

The LMP2/TAP1/LMP7/TAP2 gene cluster is located in the HLA class II region on chromosome.[6] It spans a region of 38 kbp, starting at position 32 929 916 upstream. Two recombination hot spots at the downstream end of TAP2 have been identified.[6] TAP genes have already been investigated in some autoimmune diseases. In psoriasis vulgaris, the G-allele of TAP2_665 has been

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found to be significantly more frequent than in controls in two of three studies,[7–9] and for TAP1_637, the A-allele was found more often in three of four studies.[8–11] LMP polymorphisms have only been investigated in two studies[8,10] with no significant results. Two small studies, one in HLA-B27-negative ankylosing spondylitis[12] and another in rheumatoid arthritis,[13] indicated that the G-allele in LMP_60 was more frequent in cases than in controls. We therefore investigated psoriasis in the subgroups P. arthropathica and P. vulgaris.

We analyzed genetic variations in the LMP/TAP cluster in 321 Caucasian psoriasis patients and 235 unrelated controls.[2] We chose seven coding single-nucleotide polymorphisms (SNPs), already investigated in some other studies (LMP2_60/rs17587, TAP1_637/rs1800453, LMP7_49/rs2071543, TAP2_565, TAP2_651/rs4148876, TAP2_665/rs241447, TAP2_687/rs241448), to investigate the association between genetic variations in cytosolic antigen processing and transport genes, and psoriasis. All SNPs lead to amino-acid exchanges in the encoded protein. We especially focused on effect homogeneity between males and females, and the subphenotypes P. vulgaris and P. arthropathica.

The study population was already described.[2] In short, 50% of the psoriasis cases were female, mean age was 48.5 years with a range of 11–84 years. Controls were slightly younger (45.7 years). A total of 87% had psoriasis vulgaris, 2.2% psoriasis pustulosa and 26% psoriasis arthropathica. This relation was not different between males and females; an onset before the age of 20 years was reported by 44% of the females and 37% of the males. Albeit the age range of patients was high, there was no effect modification, except for SNP LMP7_49 (OR: 8.5, 95% CI: 1.8–39.6, in the age group 20–39 years).

A descriptive overview of genotype frequencies in cases and controls is given in Table 1. The order of the SNPs in Table 1 corresponds to their location on chromosome 6. The TAP and LMP loci are embedded in the MHC region. None of the SNPs showed a deviation from Hardy–Weinberg equilibrium, neither in cases nor in controls. No deviation from the assumption of additive allele effects could be detected either. For the neighboring SNPs LMP2-60, TAP1_637 and LMP7_49, a haplotype analysis was performed. This analysis revealed that the A-A-C genotype was present in 19% of cases and 28% of controls. This difference was significant ($P = 0.0007$, $\chi^2 = 11.37$). This was only slightly higher than the differences for the single SNPs. For four SNPs, one in the LMP2 gene and three in TAP genes, we found a strong association with disease even after Bonferroni post-test: the G- allele was significantly more often in psoriasis cases for LMP2_60, TAP1_637, TAP2_665 and TAP2_687. Linkage disequilibrium between SNPs was investigated in the controls. TAP2_665 and TAP2_687 were in complete linkage disequilibrium (with only one exception); therefore, the results are the same and only those for TAP2_687 are given in Table 2. LMP2_60, TAP1_637 and LMP7_49 showed linkage disequilibrium, which was highly significant (LD > 15), but was independent of TAP2 SNPs. This pattern was expected because of the known recombination hot spot between LMP7 and TAP2. To test the independent effect of each SNP on the phenotype, all six SNPs were included simultaneously in the logistic regression model.

Logistic regression revealed that the genotype effects on psoriasis and especially on psoriasis arthropathica were stronger for males than for females. For TAP2_665/TAP2_687, the effect was 4.1 times stronger in males than in females (95% CI: 1.4–12.5) and for LMP2_60, it was 3.1 times stronger (95% CI: 0.94–10.1; $P = 0.06$). Therefore, the final logistic regression was performed separately for P. vulgaris and P. arthropathica, and for males and females (Table 2). LMP2_60 is especially relevant for males with P. arthropathica. With the exception of females with P. arthropathica, the association of the TAP2_665/TAP2_687 polymorphisms with the disease was highly significant. Haplotype analysis did not reveal a different picture or stronger associations.

Psoriasis is a complex multifactorial disease, triggered or exacerbated by genetic and environmental factors. The analysis of allelic variation has been a powerful tool to identify susceptibility genes, also helping to distinguish environmental factors. In common with most other autoimmune diseases, psoriasis is associated with several HLA antigens. However, the presence of HLA genes such as Cw6 is not sufficient for developing the disease; other loci and unknown environmental factors, such as exposure to LMWC, are assumed to be involved as well. LMWC can bind to reactive groups in cysteines (-SH), lysines (-NH_2) or tyrosines (-OH), and might conceivably change the enzymatic capacity or the conformational structure of a protein.[14] We therefore analyzed genes of components of the antigen presentation pathway for associations with psoriasis in a large group of patients. We confirmed a strong association between the SNPs TAP_665/TAP2_687 with psoriasis. TAP1 and TAP2 contribute to efficient peptide transport, and single point mutations in TAP2 are able to alter the peptide transport specificity, and presumably affect epitope selection.[15] The associations we found were especially strong in men. According to our data, the chance to develop psoriasis was more than five times higher for those carrying the GC-allele of TAP2_665/TAP2_687. The gender differences were even stronger in P. arthropathica. Here, the chance of men with the allele was more than 18 times higher than in those without the allele, whereas no association was visible in women.

Psoriasis does not exhibit sexual dimorphism as many other autoimmune diseases, where women are primarily affected. In autoimmune-like illnesses caused by recognized environmental agents, sex discrepancy is usually explained by differences in exposure.[16] Our data suggest that in the case of psoriasis, either the influence of the environment compensates the male-specific genetic effect, that is, men are more resistant to such effects, or women are affected more strongly by environmental factors such as LMWC. As a result, the frequency of the disease is not gender-biased. Which factors might determine the higher susceptibility or resistance to environmental factors is unclear. Both exposure and/or metabolic differences might be responsible. It is interesting to note that for one SNP, LMP7_49, a risk could be found in the younger patients; however, the risk effect disappeared in the older age groups. The majority of all patients suffered from early onset of psoriasis.[2] This finding might indicate again that gene effects can be assimilated by the environment or simply by age. Owing to a recombination hot spot, a second independent

Table 1 Genotype frequencies in cases and controls of psoriasis patients[2] ordered by frequency

Locus/effect	*Genotype*	*Genotype frequencies*		*Odds ratio (95% confidence limits)*	χ^2	P-*value*	P-*value after Bonferroni correction (six independent tests)*
		Cases	*Control*				
LMP2_60/Arg60His	**G/G**	0.64	0.52	2.50 (1.41–4.45)	9.73	0.0018	0.0108
	A/**G**	0.33	0.41				
	A/A	0.04	0.07				
TAP1_637/Asp637Gly	A/A	0.71	0.79	2.50 (1.25–5.02)	6.64	0.0100	0.0600
	A/**G**	0.26	0.19				
	G/G	0.03	0.02				
LMP7_49/Lys49Gln	C/C	0.66	0.74	1.93 (0.98–3.80)	3.66	0.0556	0.3336
	A/C	0.31	0.24				
	A/A	0.03	0.02				
TAP2_565/Thr565Ala	**C/C**	0.88	0.86	1.25 (0.52–2.99)	0.24	0.6215	—
	C/T	0.12	0.13				
	T/T	0.01	0.01				
TAP2_651/Arg651Cys	**C/C**	0.89	0.87	1.40 (0.50–3.90)	0.40	0.5621	—
	C/T	0.11	0.13				
	T/T	0.00	0.00				
TAP2_665/Thr665Ala	A/A	0.37	0.53	3.18 (1.84–5.48)	17.23	0.00002	0.00012
	A/**G**	0.49	0.41				
	G/G	0.14	0.06				
TAP2_687/−687Gln	T/T	0.37	0.53	3.27 (1.90–5.63)	18–27	0.00002	0.00012
	C/T	0.49	0.41				
	C/C	0.14	0.06				

Genotyping analyses were carried out by using the MassARRAY system (Sequenom, San Diego, CA, USA). A 5 ng portion of genomic DNA was amplified by PCR using HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). PCR primers were used at 167 nM final concentrations for a PCR volume of 6 μl. The PCR condition was 95°C for 15 min for hot start, followed by denaturing at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for 45 cycles and finally incubation at 72°C for 10 min. PCR products were first treated with shrimp alkaline phosphatase (SAP, Amersham, Freiburg, Germany) for 20 min at 37°C to remove excess dNTPs and afterwards for 10 min at 85°C to inactivate SAP. ThermoSequenase (Amersham) was used for the base extension reactions. Extension primers were used at a final concentration of 5.4 μM in 10 μl reactions. The base extension reaction condition was 94°C for 2 min, followed by 94°C for 5 s, 52°C for 5 s and 72°C for 5 s for 40 cycles. All reactions (PCR amplification, base extension) were carried out in a Tetrad PCR thermal cycler (MJ Research). The final base extension products were treated with SpectroCLEAN resin (Sequenom) to remove salts in the reaction buffer. This step was carried out with a Multimek 96-channel autopipette (Beckman Coulter), and 16 μl of resin–water suspension was added into each base extension reaction, making the total volume 26 μl. After a quick centrifugation (2000 r.p.m., 3 min) in an Eppendorf Centrifuge 5810, 10 nl of reaction solution was dispensed onto a 384 format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid (3-HPA) by using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Biflex matrix-assisted laser desorption ionization-time-of-flight mass spectrometer (Sequenom) was used for data acquisitions from the SpectroCHIP. Genotyping calls were made in real time with MASSARRAY RT software (Sequenom).
The index allele is given in bold. Loci are ordered as on the chromosome 5′ to 3′.

Table 2 Association between genotype and different types of psoriasis in males and females

	Psoriasis vulgaris						*Psoriasis arthropathica*					
	All, OR, 95% CI	P	*Males, OR, 95% CI*	P	*Females, OR, 95% CI*	P	*All, OR, 95% CI*	P	*Males, OR, 95%CI*	P	*Females, OR 95%CI*	P
LMP2_60	1.35, 0.65–2.79	0.42	1.48, 0.56–3.96	0.43	1.02, 0.34–0.06	0.97	2.92, 0.96–8.91	0.06	**6.42, 1.16–35.54**	0.03	1.35, 0.29–6.33	0.70
TAP1_637	2.16, 0.85–5.48	0.11	2.74, 0.72–10.42	0.14	1.50, 0.39–5.74	0.56	**3.55, 1.04–12.10**	0.04	**7.16, 1.16–44.16**	0.03	2.78, 0.41–18.88	0.29
LMP7_49	1.93, 0.84–4.41	0.12	**3.33, 1.06–10.42**	0.04	1.04, 0.29–3.73	0.96	0.77, 0.22–2.61	0.68	0.61, 0.10–3.75	0.59	1.02, 0.17–6.21	0.98
TAP2_565	0.74, 0.25–2.17	0.58	0.60, 0.15–2.46	0.48	0.82, 0.15–4.55	0.82	4.21, 0.58–30.6	0.16	1.04, 0.09–12.36	0.98	—	—
TAP2_651	1.37, 0.37–5.06	0.74	3.93, 0.60–25.52	0.15	0.37, 0.05–2.63	0.32	1.33, 0.23–7.82	0.75	7.49, 0.46–122.40	0.16	0.28, 0.02–3.51	0.33
TAP2_687	**4.35, 2.21–8.57**	0.00002	**5.28, 1.92–14.51**	0.0013	**3.57, 1.39–9.21**	0.0084	**4.20, 1.62–10.91**	0.003	**18.36, 3.77–89.380**	0.0003	1.26, 0.33–4.85	0.74

Psoriasis vulgaris ($N = 175$) or psoriasis arthropathica ($N = 65$) comparison with controls ($N = 235$), mutually adjusted odds ratios with 95% confidence limits.

Logistic regression was used for analysis. Significant results are indicated in bold. The allele more frequent in cases was coded as one, the less frequent with zero. Genotype differences between cases and controls were tested assuming additive allele effects. The validity of this assumption was tested. Homogeneity of the genotype/psoriasis associations between men and women was tested by including second-order interaction terms into the logistic model (SAS Stat 9.1). The analysis was repeated using cases with the diagnosis of psoriasis vulgaris alone and those with the diagnosis of psoriasis arthropathica alone. Hardy–Weinberg equilibrium and linkage between single-nucleotide polymorphisms were tested and haplotypes were compared using SAS/Genetics.

contribution to the manifestation of psoriasis from the TAP/LMP cluster could be observed: the risk to contract P. arthropathica was higher for men if LMP2_60 and TAP1_637 showed the G-allele. Interestingly, in rheumatoid arthritis, the same association has been observed. An association of these gene polymorphisms with psoriasis has not been observed before. P. arthropathica is epidemiologically and clinically distinct, and demonstrates greater heritability among first-degree relatives than P. vulgaris, which is congruent with our data here.[17] We could conclude that the presence of these alleles might predispose specifically to P. arthropathica. A study with similar size but without gender stratification found no association.[7] Currently, knowledge of the genetic factors necessary to understand the reasons for variable age at onset and predisposition towards P. arthropathica is incomplete. The effect of environmental triggers may also be understood once the altered pathways are elucidated.

Although the functional relevance of these SNPs is not known, the strong associations point to either direct or near by gene loci to influence psoriasis. Our results will help define behavioral or drug treatment suggestions to patients and contribute to a better understanding of the role of LMWC in genetic susceptibility to autoimmune diseases.

References

1 Liu Y, Krueger JG, Bowcock AM. Psoriasis: genetic associations and immune system changes. *Genes Immun* 2007; **8**: 1–12.
2 Richter-Hintz D, Thier R, Steinwachs S, Kronenberg S, Fritsche E, Sachs B *et al*. Allelic variants of drug metabolizing enzymes as risk factors in psoriasis. *J Invest Dermatol* 2003; **120**: 765–770.
3 Jenisch S, Henseler T, Nair RP, Guo SW, Westphal E, Stuart P *et al*. Linkage analysis of human leukocyte antigen (HLA) markers in familial psoriasis: strong disequilibrium effects provide evidence for a major determinant in the HLA-B/-C region. *Am J Hum Genet* 1998; **63**: 191–199.
4 Kloetzel PM. Antigen processing by the proteasome. *Nat Rev Mol Cell Biol* 2001; **2**: 179–187.
5 Zollner TM, Podda M, Pien C, Elliott PJ, Kaufmann R, Boehncke WH. Proteasome inhibition reduces superantigen-mediated T cell activation and the severity of psoriasis in a SCID-hu model. *J Clin Invest* 2002; **109**: 671–679.
6 Overview Namespace Document Object Tree Project Catalog Help. 2007. http://www.hapmap.org.
7 Hohler T, Weinmann A, Schneider PM, Rittner C, Schopf RE, Knop J *et al*. TAP-polymorphisms in juvenile onset psoriasis and psoriatic arthritis. *Hum Immunol* 1996; **51**: 49–54.
8 Pyo CW, Hur SS, Kim YK, Kim TY, Kim TG. Association of TAP and HLA-DM genes with psoriasis in Koreans. *J Invest Dermatol* 2003; **120**: 616–622.
9 Saeki H, Kuwata S, Nakagawa H, Asahina A, Tamaki K, Shibata Y *et al*. Analysis of HLA class II and TAP alleles in Japanese patients with psoriasis vulgaris. *Hum Immunol* 1998; **59**: 503–511.
10 Hohler T, Schneider PM, Rittner C, Hasenclever P, Meyer zum Buschenfelde KH, Marker-Hermann E. LMP polymorphisms do not influence disease expression in psoriatic arthritis. *Clin Exp Rheumatol* 1996; **14**: 661–664.
11 Witkowska-Tobola AM, Szczerkowska-Dobosz A, Nedoszytko B, Roszkiewicz J. Polymorphism of the TAP1 gene in Polish patients with psoriasis vulgaris. *J Appl Genet* 2004; **45**: 391–393.
12 Maksymowych WP, Tao S, Vaile J, Suarez-Almazor M, Ramos-Remus C, Russell AS. LMP2 polymorphism is associated with extraspinal disease in HLA-B27 negative Caucasian and Mexican Mestizo patients with ankylosing spondylitis. *J Rheumatol* 2000; **27**: 183–189.
13 Vinasco J, Fraile A, Nieto A, Beraun Y, Pareja E, Mataran L *et al*. Analysis of LMP and TAP polymorphisms by

polymerase chain reaction-restriction fragment length polymorphism in rheumatoid arthritis. *Ann Rheum Dis* 1998; **57**: 33–37.
14 Esser C. Immunotoxicology. In: Taylor J, Triggle DJ (eds). *Comprehensive Medicinal Chemistry II*. Elsevier: Oxford, 2007, pp 215–229.
15 Armandola EA, Momburg F, Nijenhuis M, Bulbuc N, Fruh K, Hammerling GJ. A point mutation in the human transporter associated with antigen processing (TAP2) alters the peptide transport specificity. *Eur J Immunol* 1996; **26**: 1748–1755.
16 Lockshin MD. Sex differences in autoimmune disease. *Orthop Clin N Am* 2006; **37**: 629–633.
17 Rahman P, Elder JT. Genetic epidemiology of psoriasis and psoriatic arthritis. *Ann Rheum Dis* 2005; **64** (Suppl 2): ii37–ii39.