Combined Analysis of Polymorphisms of the Tumor Necrosis Factor-α and Interleukin-10 Promoter Regions and Polymorphic Xenobiotic Metabolizing Enzymes in Psoriasis

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Environmental and genetic factors are thought to interact in the manifestation of psoriasis, but knowledge about the involved genes and antigens is incomplete. This study has focused on the association between psoriasis and inherited variations in xenobiotic metabolism and cytokine production as two components that may influence cutaneous immune responses to foreign substances. Polymorphisms of *N*-acetyltransferase 2, glutathione *S*-transferases T1 and M1, and promoter polymorphisms of the genes encoding for tumor necrosis factor-α and interleukin-10 were investigated in 151 Caucasian patients with psoriasis (100 with type I and 51 with type II psoriasis) and in 123 healthy controls. Polymorphisms were detected by polymerase chain reaction-based methods, restriction enzyme analysis, and direct sequencing. There were no significant differences in the distribution of enzyme polymorphisms or point mutations at position –1082 of the interleukin-10 promoter between the psoriasis groups and the control group. The G→A polymorphism at position –238 of the tumor necrosis factor-α promoter (*TNFα-238*A* allele) was more common in type I psoriasis (27%) than in the controls [9.8%; odds ratio 3.4 (95% confidence interval 1.6–7.2); $p = 0.0012$; $p_{corr} = 0.018$]. Surprisingly, this overrepresentation of the *tumor necrosis factorα – 238*A* allele was observed in male patients [4.1 (1.5–11.0); $p = 0.0046$; $p_{corr} = 0.064$] but not in female patients [1.8 (0.5–6.5); $p = 0.5$]. The G→A polymorphism at position –308 of the tumor necrosis factor-α promoter was less frequent in type I psoriasis (23%) compared with controls (35.7%), although the negative association was weak [0.54 (0.3–0.97); $p = 0.041$; p_{corr} = not significant]. The distribution of the *TNFα-238*A* and *TNFα-238*A* alleles was similar in type II patients and controls. Our results suggest that male carriers of the G→A polymorphism at position –238 of the tumor necrosis factor-α promoter are at an increased risk to develop early-onset psoriasis. *Key words: cytokines/heritable factors/immune reaction/xenobiotic metabolism. J Invest Dermatol 113:214–220, 1999*

Psoriasis is a chronic inflammatory skin disorder affecting about 2% of Caucasian populations (Lomholt, 1963). Population and twin studies provide clear evidence of a heritable component to the etiology (Elder *et al*, 1994), and a strong association has been established between the disease and genes of the major histocompatibility complex on human chromosome 6. The highest relative risk (nine to 15 times normal) has been found for HLA-Cw6 (Tiilikainen *et al*, 1980), and the HLA association of psoriasis was shown to include the B and C loci (HLA-B17, HLA-B37, HLA-B57) and extend to the class II genes DRB1*0701, HLA-DQA1*0201, and HLA-DQB1*0303 (Schmitt-Egenolf *et al*, 1993). A classification of disease subtypes has been proposed on the basis of differences in the age of onset and frequency of HLA (Henseler and Christophers, 1985). According to this classification Caucasian patients with type I psoriasis (onset before the age of 40 y) are characterized by a high proportion of carriers of Cw6, B57, and DR7, whereas in type II psoriasis (onset beyond 40 y), HLA-Cw2 is increased and the association with Cw6 is weaker (Henseler, 1997). The concept of disease subtypes that differ in their genetic background was supported by the demonstration of a strong association between the extended risk haplotype Cw6-B57-DRB1*0701-DQA1*0201-DQB1*0303 and German patients with familial early-onset but not sporadic late-onset psoriasis (Schmitt-Egenolf *et al*, 1996). Whereas these data suggest that at least one predisposing gene resides in the HLA region, a more complex genetic basis is indicated by genome scans in affected families that have yielded additional susceptibility loci on chromosomes 17q (Tomfohrde *et al*, 1994) and 4q (Matthews *et al*, 1996).

Recent studies into the genetic factors associated with allergic asthma, rheumatoid arthritis, and lupus erythematosus took advantage of the increasing knowledge about disease-specific immunologic abnormalities and the genetic control of immunoregulatory mechanisms such as the production of protective or harmful cytokines (Eskdale *et al*, 1998a; Lim *et al*, 1998; Mok *et al*, 1998). Such an approach may also be considered when investigating the genetics of psoriasis, the psoriatic inflammatory process being characterized by a disturbance of the local cytokine network

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Abbreviations: GST M1, glutathione *S*-transferase class mu type 1; GST T1, glutathione S-transferase theta type 1; NAT2, N-acetyltransferase 2.

with overexpression of proinflammatory mediators, such as tumor necrosis factor (TNF)-α (Uyemura *et al*, 1993; Schlaak *et al*, 1994), and a relative deficiency of anti-inflammatory factors such as interleukin (IL)-10 (Nickoloff *et al*, 1994; Asadullah *et al*, 1998). Importantly, several findings suggest that both cytokines play a critical part in the disease pathogenesis. TNF-α together with other mediators induces the production of IL-8 in fibroblasts and keratinocytes (Larsen *et al*, 1989; Barker *et al*, 1990) that acts as a chemotactic factor for T cells and neutrophils and is strongly upregulated in psoriatic epidermis (Kulke *et al*, 1996). Immune cell traffic into psoriatic lesions may also be enhanced by TNF-α via induction of adhesion molecules on endothelial cells (Uyemura *et al*, 1993). Finally, TNF-α and IFN-γ stimulate the production of transforming growth factor-α by epidermal cells that may together with IL-8 be involved in autocrine stimulation of keratinocyte proliferation (Barker *et al*, 1991). On the other hand, IL-10 is a potent antagonist of cutaneous T cell-mediated immune responses (Ferguson *et al*, 1994), suppresses epidermal cell growth *in vitro* (Michel *et al*, 1997), and has strong anti-psoriatic effects when administered to affected patients (Asadullah *et al*, 1998; Reich *et al*, 1998).

Polymorphisms have now been detected in the promoter regions of the *TNF*α and *IL10* gene that affect cytokine production. The G→A polymorphism at position –308 of the *TNF*α promoter (*TNF*α*-308*A* allele) is associated with a 6–7-fold increased transcriptional activity (Wilson *et al*, 1997). Monocytes of carriers of another G→A polymorphism at position –238 (*TNF*α*-238*A* allele) show a higher TNF-α production after stimulation than monocytes isolated from homozygous carriers of the *TNF*α*-238*G* allele, although in the original study this finding was not statistically significant (Pociot *et al*, 1995). In contrast, the *IL10–1082*A* allele (G→A transition at position –1082 of the *IL10* promoter) is associated with decreased inducible levels of IL-10 in peripheral blood mononuclear cells (Turner *et al*, 1997).

Given the observed constellation of cytokine expression in psoriatic skin it is natural to ask whether these promoter polymorphisms are associated with the disease. In the case of *TNF*α promoter polymorphisms, research has also been stimulated by the fact that the gene for TNF-α is located within the class III region of the major histocompatibility complex between HLA-B and HLA-DR (Campbell and Trowsdale, 1993), and two recent studies have presented evidence for an association between the *TNF*α*-238*A* allele and early-onset psoriasis (Arias *et al*, 1997; Höhler *et al*, 1997).

In this study a case-control design was used to reinvestigate the distribution of the *TNF*α*-238*A* and *TNF*α*-308*A* alleles in a larger series of patients with early- and late-onset psoriasis compared with healthy individuals, and to search for associations between the disease and IL-10 promoter polymorphism. We extended our study to functionally relevant polymorphisms of *N*-acetyltransferase 2 (NAT2), glutathione *S*-transferases (GST) M1 and T1, because these have lately been recognized as heritable risk factors for the development of cutaneous inflammatory reactions in response to exogenous stimuli such as ultraviolet irradiation (Kerb *et al*, 1997) and contact allergens (Schnuch *et al*, 1998).

MATERIALS AND METHODS

Patients We included only German Caucasians in this study. One-hundred patients with early-onset psoriasis (onset not later than at the age of 40 y and a positive family history) and 51 patients with type II psoriasis (onset after the age of 40 y, negative family history) were recruited at the Department of Dermatology, University Clinic of Göttingen. All patients had psoriasis vulgaris and none suffered from other pathologic skin conditions. The median age of the type I group was 41 y (range 11–92) and included 33 female (33%) and 67 male subjects (67%). The median age at onset was 25 y (range 3–39). The type II group had a median age of 61 y (range 42–86), including 22 female (43.1%) and 29 male patients (56.9%). The median age at onset was 55 y (range 41–85). The control population consisted of 123 locally recruited, unrelated, healthy volunteers (56 females, 67 males). Health status was determined by skin examination and a record of the personal and familial medical history. Individuals with a history suspicious of atopy or contact allergy were excluded from the control group.

Determination of the NAT2 phenotype Systemic *N*-acetyltransferase activity was determined using the urinary caffeine metabolite ratio of 5-acetylamino-6-formylamino-3-methyluracil to 1-methylxanthine as outlined by Grant *et al* (1984). Participants were asked to ingest a beverage containing caffeine. Urine was collected at least 4 h later, acidified, and stored at –80°C. Analysis of caffeine metabolites was done within 3 weeks, because the quotient of 5-acetylamino-6-formylamino-3-methyluracil/1-methylxanthine is stable for this period under the given storing conditions. Extraction and separation of caffeine metabolites were performed according to Grant *et al* (1984) with the exception that formic acid instead of acetic acid was used to improve the stability of the compounds during chromatography. Thus, 0.025% formic acid with methanol (99% vol/vol) served as mobile phase on a Kontron high-performance liquid chromatography (Kontron M 800 with diode array detector; Neufahrn, Germany) and a Knauer 100 C18 column (Knauer, Berlin, Germany).

Genotyping for NAT2, GST T1, and M1 Blood was collected in ethylenediamine tetraacetic acid tubes. Extraction of DNA from buffy coats was performed by a salting out procedure. Briefly, lymphocytes were collected at 400 g and 4°C, and lysed in 50 mM trisect pH 8.0, 20 mM ethylenediamine tetraacetic acid pH 8.0 and 2% sodium dodecyl sulfate. Protein was digested with Proteinase K (6 h at 56°C) and pelleted with 6 M NaCl following centrifugation at 5000 × *g* for 20 min. DNA was precipitated with 1.5 × Vol. 100% ethanol (pa). DNA yields were between 0.1 and 1 μg per μl.

The genotype of NAT2 was characterized by polymerase chain reaction and restriction enzyme analysis (PCR–RFLP) at nucleotide positions 191, 282, 341, 481, 590, 803, and 857 as described by Cascorbi *et al* (1995) with the published modifications (Cascorbi *et al*, 1996). From the mutation pattern, seven different alleles were determined, including the wild-type allele *NAT2*4* and the six mutant alleles *NAT2*5a*, *NAT2*5b*, *NAT2*5c*, *NAT2*6a*, *NAT2*7b*, and *NAT2*12a*.

Homozygous deletions of *GSTM1* and *GSTT1* were characterized simultaneously with a multiplex PCR with β-globin as internal standard. Primers for GST M1 were according to Bell *et al* (1992). Primers for GST T1 were according to Pemble *et al* (1994). Each PCR contained ≈200 ng of genomic DNA, 11.25 pmol dNTPs (Amresco, Solon, OH), 20 pmol of each primer (Eurogentec, Seraing, Belgium), 1 U *Taq* polymerase (Appligene Oncor, Heidelberg, Germany), and Taq-buffer according to the supplier in a final volume of 30 μl. The samples were denaturated for 4 min at 94°C, and subsequently amplified with 31 cycles of 94°C, 66°C, and 72°C for 1 min each, without a final extension time. PCR products were separated on a 2% agarose gel (molecular biology grade; Eurogentec).

Enzyme expression in skin biopsies, dendritic cells, and keratinocytes To study the expression of polymorphic enzymes in the skin, 3 mm punch biopsies were taken from active plaque lesions from three psoriatic patients participating in the study that had not received any topical treatment for at least 2 wk and any systemic treatment including phototherapy for at least 4 wk. Skin specimens were also collected from three healthy volunteers. Written informed consent was obtained in all cases. Biopsies were immediately snap frozen in liquid nitrogen and stored at –70°C. Total mRNA was extracted from 30 consecutive 20 μm sections using Trizol (Gibco, Eggenstein, Germany) according to the instructions supplied by the manufacturer. RNA was also obtained from peripheral blood mononuclear cells of the same donors. The absence of gDNA contamination was confirmed by running a β-actin PCR. Reverse transcription was then performed with Superscript II (Gibco) according to the manufacturer's protocol.

Expression of polymorphic enzymes was also analyzed in peripheral blood derived dendritic cells and freshly isolated Langerhans cells. The former were generated according to a modified published protocol (Pickl *et al*, 1996). Briefly, $CD14^+$ cells were positively selected from peripheral blood mononuclear cells of healthy volunteers (n = 3) by high gradient magnetic sorting using the *VARIO*MACS technique (Miltenyi Biotec, Bergisch Gladbach, Germany). Isolated $CD14^+$ cells (purity > 85%) were cultured for 5 d in RPMI 1640/5% fetal bovine serum, containing 50 ng recombinant human (rh) IL-4 (Pharma Biotechnologie, Hanover, Germany) per ml and 50 ng rhGM-CSF (kindly provided by the Schering-Plough Research Institute, Kenilworth, NJ) per ml. After this time, part of the cells were subjected directly to mRNA extraction (referred to as immature dendritic cells). The remaining cells were incubated for 3 additional days with 100 ng TNF-α (Pharma Biotechnologie) per ml and 50% vol/vol monocyte conditioned medium (Romani *et al*, 1996). Sixty to 90% of the

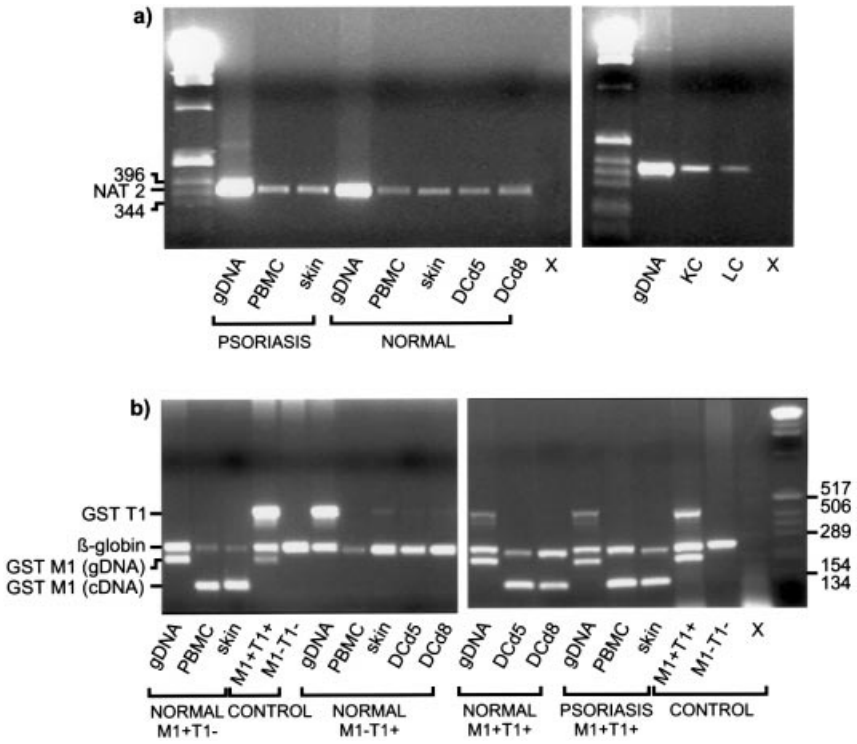

Figure 1. Expression of NAT2 and GST in PBMC, skin, dendritic cells, and keratinocytes. (*a*) *Left:* example for the constitutive expression of NAT2 in healthy skin, lesional psoriatic skin, and peripheral blood mononuclear cells (PBMC) from the same donors as determined by reverse transcription–PCR. Expression of NAT2 was also detected in immature (DCd5) and mature (DCd8) peripheral monocyte-derived dendritic cells (see *Materials and Methods*). *Right*: constitutive expression of NAT2 in cultured keratinocytes (KC) and freshly isolated epidermal Langerhans cells (LC). PCR of gDNA samples prepared from lymphocytes of the corresponding individuals served as positive control. (*b*) Multiplex PCR with β-globin as internal standard demonstrating constitutive expression of GST M1 but not GST T1 in PBMC, healthy and lesional psoriatic skin, and immature (DCd5) and mature dendritic cells (DCd8). For each individual the mutation pattern (M1–, homozygous deletion of GST M1; T1–, homozygous deletion of GST T1) as determined by gDNA analysis is shown compared with control gDNA samples with confirmed combined homozygous deletion of both enzymes (M1–T1–) or absence of gene deletions (M1+T1+). X, negative control reaction without genomic or cDNA.

resulting cell population displayed a dendritic morphology in cytospin preparations ("veils"), and expressed the phenotype of mature dendritic cells as measured by fluorescence-activated cell sorter analysis ($CD83^+$, $CD86^+$, $HLA\text{-}DR^{high}$, $CD40^+$, $CD1a^+$, $CD14^-$). The cells produced IL-12p40, IL-1β, and TNF-α but only minor quantities of IL-10 and were potent stimulators of proliferatory responses in resting T cells (referred to as mature dendritic cells; data not shown).

Langerhans cells (>95% $CD1a^+$ by fluorescence-activated cell sorter analysis) were isolated from the foreskin of individuals undergoing circumcision as described (Simon *et al*, 1995). Keratinocytes were prepared from individuals undergoing plastic surgery according to Boyce and Ham (1983). Reverse transcription–PCR was performed with keratinocytes derived from the second passage of culture (>95% cytokeratin positive, <1% CD1a positive). Extraction of total mRNA from suspensions of these cells and cDNA synthesis were performed as above.

Primers for the detection of NAT2 cDNA were 5′-GAC-ATT-GAA-GCA-TAT-TTT-GAA-AG-3′ (sense; Woolhouse *et al*, 1997) and 5′-ACC-CAG-CAT-CGA-CAA-TGT-AAT-TCC-TGC-CCT-CA-3′ (antisense; Cascorbi *et al*, 1996), which amplify a 369 bp fragment. PCR conditions were as follows: denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, 60°C for 1 min, and 74°C for 4 min, and a final extension time of 10 min.

Primers and PCR conditions for GST T1 and M1 were the same as for the gDNA analysis. The sequence amplified from GST M1 gDNA comprises 219 bp including a 86 bp intron (Zhong *et al*, 1993). Therefore, amplification of cDNA should yield a 133 bp fragment (**Fig 1*b***).

Analysis of *TNFα* promoter polymorphisms Polymorphisms of the *TNFα* promoter at position –238 were analyzed by PCR–RFLP according to Day *et al* (1998). Allelic variations at position –308 were detected by allele-specific PCR as described by Couchane *et al* (1997).

Analysis of the polymorphism at position –1082 of the *IL10* promoter Base pair substitutions at position –1082 of the *IL10* gene (Turner *et al*, 1997) were detected by cycle sequencing of PCR products generated with the following primers: IL-10 sense: 5′-ATCCAAGAC-AACACTACTAA-3′ (Turner *et al*, 1997), and anti-sense: 5′-GTGGAA-GAAGTTGAAATAAC-3′ (Mok *et al*, 1998). PCR conditions were 35 cycles of 94°C, 51°C, and 72°C for 1 min each with a final extension time of 10 min at 72°C. The anti-sense primer was used for cycle sequencing on an ABI Prism 310 Genetic Analyzer (Perkin Elmer ABI, Foster City, CA) equipped with the Data Collection 1.02 and Sequencing Analysis 3.0 software. PCR fragments were purified by ultrafiltration via Microcon 100 filters (Millipore, Bedford, MA). The purified fragments were amplified and labeled with Big-Dye-Terminator-Ready-Reaction-Mix (Perkin Elmer ABI) as follows: 4 μl of mix, 110 ng DNA and 3.2 pmol primer in a final reaction volume of 20 μl. Following 25 cycles of 95°C for 30 s, 50°C for 15 s, and 60°C for 4 min, labeled DNA was separated by Na-acetate/ethanol-precipitation.

Statistical analysis The frequencies of cytokine promoter and enzyme alleles were compared between patients with type I psoriasis, type II psoriasis, and controls by the two-sided Fisher's exact test (Kleinbaum *et al*, 1982). The Bonferroni method was used to adjust for multiple comparison. The frequencies of seven variables *NAT2*4*, *GSTT1*0*, *GSTM1*0*, *GSTT1*0/M1*0*, *TNFα-238*A*, *TNFα-308*A*, and *IL10–1082*A* were compared separately between the early-onset and the control groups and the late-onset and the control groups, and the initial p-values were multiplied by 14 (Rothmann and Greenland, 1998). Odds ratios and 95% confidence intervals were calculated for each variable. In each group the distribution of TNF-α and IL-10 promoter genotypes was tested for deviations from Hardy–Weinberg equilibrium by an exact test (Guo and Thompson, 1992). Linkage disequilibrium between the polymorphisms at positions –238 and –308 of the TNFα promoter was also tested by an exact test (Zaykin *et al*, 1995). The prism 2.01 software (GraphPad, San Diego, CA) was employed for calculation of the Fisher's exact test and the 95% confidence interval.

RESULTS

NAT2, GST M1, and T1 are expressed in normal and psoriatic skin, keratinocytes and dendritic cells By reverse transcription–PCR we detected NAT2 expression in freshly isolated peripheral blood mononuclear cells and skin biopsies from healthy donors and psoriasis patients (**Fig 1*a***). As epidermal dendritic cells and keratinocytes represent the first line of cells that may handle transepidermally absorbed antigens, we also investigated the expression of NAT2 in freshly isolated Langerhans cells and keratinocytes, and expression of NAT2 mRNA was confirmed in both cell types (**Fig 1*a***). Furthermore, NAT2 expression was detected in immature dendritic cells cultured from peripheral blood in the presence of granulocyte-macrophage colony-stimulating factor and IL-4. Expression of NAT2 was maintained after induction of maturation by incubation with TNF-α (**Fig 1*a***).

Of the polymorphic GST investigated, only GST M1 was reproducibly detected in peripheral blood mononuclear cells, normal and psoriatic skin, and immature and mature dendritic cells,

Table I. Frequencies of enzyme polymorphisms in the investigated groups

Enzyme	Control group n/total (%)	Psoriasis group: Early onset, n/total (%)	Psoriasis group: Late onset, n/total (%)
NAT2rapid[a]	37/123 (30.1%)	40/100 (40%)	19/51 (37.3%)
GST T1-[b]	18/123 (14.6%)	8/100 (8%)	10/51 (19.6%)
GST M1-[b]	62/123 (50.4%)	57/100 (57%)	28/51 (54.9%)
GST M1-T1-[c]	7/123 (5.7%)	2/100 (2%)	7/51 (13.7%)[d]

[a]NAT2 wild-type allele homozygous or heterozygous.
[b]Homozygous deletion of GST T1 or M1, respectively.
[c]Combined homozygous deletion of GST T1 and M1.
[d]p=0.0073, p_{corr}=0.1; late onset compared with early onset psoriasis.

Table II. Frequencies of rapid acetylator allelic combinations

Genotype	Control group (37/123)[a] n	(%)	Psoriasis group: Early onset (40/100) n	(%)	Psoriasis group: Late onset (19/51) n	(%)
*NAT2*4/2*4*	5	(4.1)	5	(5)	2	(3.9)
*NAT2*4/2*5b*	13	(10.6)	15	(15)	9	(17.6)
*NAT2*4/2*6a*	18	(14.6)	16	(16)	8	(15.7)
*NAT2*4/2*5c*	0		3	(3)	0	
*NAT2*4/2*7b*	0		1	(1)	0	
*NAT2*4/2*12a*	1	(0.8)	0		0	

[a]Genotypically rapid acetylators/total.

whereas the cutaneous expression of GST T1 was weak or absent (**Fig 1*b***).

Lack of association between psoriasis and polymorphisms of xenobiotic metabolizing enzymes at the genotypic level There were no significant differences in the distribution of genotypically rapid acetylators (presence of at least one *NAT2*4* wild-type allele) or rapid acetylator allelic combinations between the psoriasis groups and the control group (**Tables I** and **II**). Material for a phenotypic analysis (see *Materials and Methods*) was available from the first 91 patients with psoriasis (55 type I; 36 type II) and 84 controls consecutively enrolled into the study. The proportions of genotypically rapid acetylators in psoriasis patients and controls in this subgroup were similar to those observed in the entire study population (controls: 29.8%, type I: 43.6%, type II: 37.8%), and differences were not significant. When the NAT2 phenotype was determined, however, a rapid acetylator phenotype was significantly more common among patients with early-onset psoriasis (45.5%) than among healthy controls [23.8%; 2.7 (1.3–5.5); p = 0.0096; **Fig 2**].

Frequencies of homozygous gene deletions of GST T1 (*GSTT1*0*) or GST M1 (*GSTM1*0*) that lead to abolition of enzymatic function were similar among psoriasis patients and controls (**Table I**). Combined homozygous deletions of both enzymes were more common in type II (13.7%) than in type I patients (2%), but the difference was not significant after correction for multiple comparisons [7.8 (1.6–39.1); p = 0.0073; p_{corr} = 0.1]. There was no association of the *GSTM1*0/GSTT1*0* genotype with late-onset psoriasis compared with controls [2.6 (0.9–8); p = 0.12]. Frequencies of NAT2, GST T1 and M1 polymorphisms were similar among male and female patients.

The *TNFα-238*A* allele is increased in male but not in female patients with early-onset psoriasis The allele frequencies of the TNFα-238 and TNFα-308 polymorphism in the control group were similar to those reported in other studies for west European Caucasians (D'Alfonso and Richiardi, 1994; Pociot *et al*, 1995;

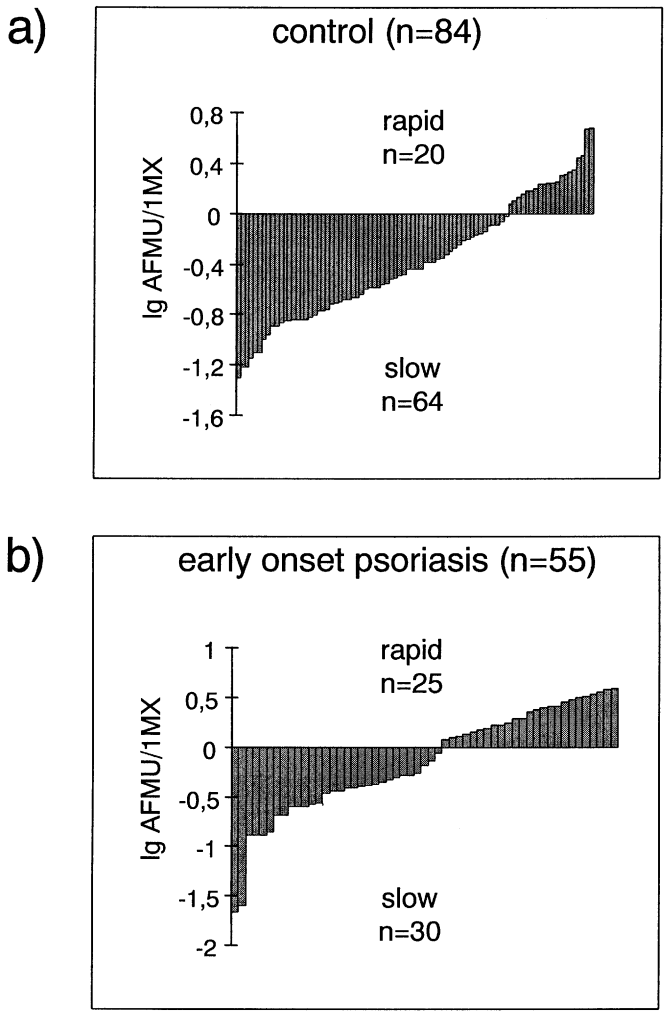

Figure 2. Association of a rapid acetylator phenotype with early-onset psoriasis. Systemic NAT2 activity expressed as the ratio of caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil and 1-methylxanthine in (*a*) 84 noncontact allergic, nonatopic healthy controls and (*b*) 55 patients with early-onset psoriasis. The values were logarithmically transformed; a log ratio < 0 corresponds to a slow, a log ratio > 0 to a rapid acetylator phenotype. The difference between study and control group was significant (two-sided Fisher's exact test: p = 0.0096).

Arias *et al*, 1997). We found a significantly increased frequency of the *TNFα-238*A* allele in patients with early-onset psoriasis (27%) compared with controls [3.4 (1.6–7.2); p = 0.0012; p_{corr} = 0.017], whereas the frequency in late-onset psoriasis (19.6%) compared with controls was not increased [1.7 (0.7–4.5); p = 0.3] (**Table III**). Homozygosity for the *TNFα-238*A* allele was present in one patient of the type I group, and none of the type II patients or controls, which is probably due to chance as homozygosity according to the Hardy–Weinberg law can be expected in 2, 0.3, and 0.3 individuals of the type I, type II, and control groups, respectively. When the distribution of the *TNFα-238*A* allele was investigated separately in male and female patients, an increased frequency was only detected in male but not in female patients with early-onset psoriasis (**Table IV**).

The frequency of the *TNFα-308*A* allele in type I patients (23%) was lower than in the control group (35.8%); however, this finding was only of borderline significance [0.5 (0.3–0.97); p = 0.041; p_{corr} = 0.6]. As the negative association between early-onset patients and *TNFα-308*A* could result from a linkage disequilibrium between *TNFα-238*A* and *TNFα-308*G* alleles, it is noteworthy that in accordance with the literature (Höhler *et al*, 1997) variations at the –238 locus were independent from

those at the –308 locus. Independence was also suggested by the observation that the *TNFα-308*A* allele was similarly distributed among male and female type I patients. The association between male patients with type I psoriasis and the *TNFα-238*A/-308*G* haplotype [4.8 (1.5–15.1); p = 0.0047; p_{corr} = 0.066] was not stronger than the association found for *TNFα-238*A* alone.

The *IL10–1082*A* allele was in Hardy–Weinberg equilibrium and no significant associations were found with early- or late-onset psoriasis (**Table III**). Frequencies of the *IL10–1082*A* allele were comparable in males and females in all groups.

Interactions between enzyme and promoter polymorphisms
To address the issue of possible interactions between cytokine promoter and enzyme polymorphisms we assessed the frequencies of combined polymorphisms in the psoriasis groups and controls. The *NAT2*4/GSTM1*0*, *NAT2*4/IL10–1082*A*, and *GSTM1*0/IL10–1082*A* combined polymorphisms were similarly distributed among type I patients, type II patients, and controls.

There was evidence for interactions between the TNFα-238 polymorphism and enzyme polymorphisms. **Table V** shows the frequencies of enzyme and promoter polymorphisms and of combined polymorphisms in male patients with early-onset psoriasis compared with male controls. Compared with the association between disease and the *TNFα-238*A* allele independent from other polymorphisms [4.1 (1.5–11); p = 0.0046] the association was weaker for the *TNFα-238*A/GSTM1*0* [2.8 (0.9–9.4); not significant], of comparable strength for the *TNFα-238*A/IL10–1082*A* [3.5 (1.2–10.1); p = 0.02] and appeared stronger for the *TNFα-238*A/NAT2*4* combined polymorphisms [8.5 (1.1–69.6); p = 0.021]; however, the numbers of individuals with combined polymorphisms were small and corrected p-values were not significant (**Table V**). The frequencies of combined TNFα-238 and enzyme or IL10–1082 promoter polymorphisms were similar in female type I patients and controls, and type II patients and controls

Table III. Frequencies of cytokine promoter polymorphisms in the investigated groups

		Psoriasis group	
	Control group n/total (%)	Early onset, n/total (%)	Late onset, n/total (%)
TNFα-238			
G/G	111/123 (90.2%)	73/100 (73%)	43/51 (84.3%)
G/A	12/123 (9.8%)	26/100 (26%)[a]	8/51 (19.6%)
A/A	0	1/100 (1%)	0
TNFα-308			
G/G	79/123 (64.2%)	77/100 (77%)	36/51 (70.6%)
G/A	42/123 (34.1%)	23/100 (23%)[b]	15/51 (29.4%)
A/A	2/123 (1.6%)	0	0
IL10-1082			
G/G	27/123 (22%)	26/100 (26%)	12/51 (23.5%)
G/A	67/123 (54.5%)	48/100 (48%)	22/51 (43.1%)
A/A	29/123 (23.6%)	26/100 (26%)	17/51 (33.3%)

[a]p=0.0012, p_{corr}=0.017; G/A and A/A *versus* G/G in early onset psoriasis compared with controls.
[b]p=0.041, p_{corr}=0.6; G/A and A/A *versus* G/G in early onset psoriasis compared with controls.

Table IV. Gender-specific distribution of TNFα-238 genotypes

			Psoriasis group			
	Control group		Early onset		Late onset	
TNFα-238	female (n=67) n (%)	male (n=56) n (%)	female (n=33) n (%)	male (n=67) n (%)	female (n=22) n (%)	male (n=29) n (%)
G/G	46 (91)	50 (89)	28 (85)	45 (67)	18 (82)	25 (86)
G/A	6 (9)	6 (11)	5 (15)[a]	21 (31)[b]	4 (18)	4 (14)
A/A	0	0	0	1 (2)	0	0

[a]OR=1.8 [95% CI 0.5–6.5]; p=0.5; G/A and A/A *versus* G/G in females with type I psoriasis compared with female controls.
[b]OR=4.1 [95% CI 1.5–11]; p=0.0046, p_{corr}=0.064; G/A and A/A *versus* G/G in males with type I psoriasis compared with male controls.

DISCUSSION

In this case-control study a significant association was observed between familial early-onset but not sporadic late-onset psoriasis and the G→A polymorphism at position –238 of the TNF-α promoter. The study confirms in a larger population the finding of two previous studies reporting the same association. Together these findings strongly suggest that the *TNFα-238*A* allele is a risk factor for susceptibility to psoriasis.

In this study the frequency of the *TNFα-238*A* allele in type I patients (27 of 100) was lower than the frequencies observed by Arias *et al* (1997; 29 of 64) and Höhler *et al* (1997; 23 of 60) in Caucasian patients. In all three studies patients were recruited locally, and ethnicity among Caucasians could account for the difference, but the similar *TNFα-238*A* allele frequency in the locally recruited controls (this study: 9.8%; Arias: 8.9%; Höhler: 7%) argues against this explanation. With our striking new finding of an overrepresentation of the *TNFα-238*A* allele in male type I patients only, the difference could alternatively be due to differences in the proportions of males and females enrolled into the studies. The paper by Arias *et al* (1997) did not include information about the gender of recruited patients. In the study by Höhler *et al* (1997) the proportions of males and females in the control group and in the early-onset group were similar to the proportions in our study, but *TNF238*A* allele frequencies were reported to be similar among male and female patients. Gender influences the inheritance of psoriasis as documented by a paternal effect on disease susceptibility in affected families (Traupe *et al*, 1992; Burden *et al*, 1998); however, this finding is not related to our observation. Clearly, the different distribution of the TNFα-238 polymorphism in male and female type I patients needs to be controlled in future studies. It will be interesting to see whether there are gender-specific variations in the role of this polymorphism as a risk factor in psoriasis. All other polymorphisms investigated in this study were evenly distributed among the sexes.

As the TNFα-238 promoter polymorphism is in linkage disequi-

Table V. Frequencies of combined polymorphisms in males

Genotype	Control (males=56) n/males (%)	Early onset (males=67) n/males (%)	OR (95% CI)	p
*NAT2*4*	16/56 (28.6)	26/67 (38.8)	1.6 (0.7–3.4)	p=NS
*GSTM1*0*	29/56 (51.8)	39/67 (58.2)	1.3 (0.6–2.7)	p=NS
*IL10-1082*A*	45/56 (80.4)	49/67 (73.1)	0.7 (0.3–1.6)	p=NS
*TNFα-238*A*	6/56 (10.7)	22/67 (32.8)	4.1 (1.5–11.0)	p=0.0046; p_{corr}=0.064
*TNFα-238*A/NAT2*4*	1/56 (1.8)	9/67 (13.4)	8.5 (1.1–69.6)	p=0.021; p_{corr}=NS
*TNFα-238*A/GSTM1*0*	4/56 (7.1)	12/67 (17.9)	2.8 (0.9–9.4)	p=NS
*TNFα-238*A/IL10-1082*A*	5/56 (8.9)	17/67 (25.4)	3.5 (1.2–10.1)	p=0.02; p_{corr}=NS

librium with HLA-B and HLA-DR loci (Wilson *et al*, 1993; D'Alfonso and Richiardi, 1994), it is possible that the observed association at least partly reflects the known linkage between these HLA loci and the disease. Noteworthy, Höhler *et al* (1997) showed that the *TNFα-238*A* allele is associated with a higher relative risk to develop psoriasis than HLA class I antigens. In addition, there is no proof yet of a direct functional involvement of HLA as antigen-presenting molecules in psoriasis, whereas an increased production of TNF-α has been detected in lesional skin, peripheral blood mononuclear cells and sera of psoriasis patients (Bonifati *et al*, 1994; Uyemura *et al*, 1993; Ettehadi *et al*, 1994; Asadullah *et al*, 1998), and is likely to play an important part in various phases of psoriatic skin inflammation (Barker *et al*, 1991). As the TNF-238 polymorphism lies in a putative regulatory box of the TNF-α promoter region (D'Alfonso and Richiardi, 1994), a genetically determined alteration of the cytokine production constitutes a mechanism for the involvement of the *TNFα-238*A* allele in the disease pathogenesis; however, several findings point to a more complex role of TNF-α promoter polymorphisms in psoriasis. *In vitro* studies have shown a significantly increased production of TNF-α in association with the *TNFα-308*A* allele (Wilson *et al*, 1997), but not in association with the *TNFα-238*A* allele (Pociot *et al*, 1995). It appears therefore paradoxical that in our study as well in the two previous studies (Arias *et al*, 1997; Höhler *et al*, 1997) the frequency of the *TNFα-308*A* allele was lower in type I patients than in controls, although the negative association was only of borderline significance. It is possible that the –238 or –308 polymorphisms do not themselves regulate the production of TNF-α, but are in linkage disequilibrium with certain microsatellite alleles of the *TNFα* gene that have been shown to affect TNF-α levels (Turner *et al*, 1995).

A cutaneous overproduction of TNF-α may also result from a defective production of IL-10, an anti-inflammatory cytokine that inhibits the synthesis of proinflammatory cytokines, chemokines, and inflammatory enzymes in activated macrophages, T cells, and neutrophils (Moore *et al*, 1993). In normal skin, upregulation of endogenous IL-10 production is a key mechanism in the suppression of T cell driven immune responses (Ferguson *et al*, 1994; Li *et al*, 1994); however, expression of IL-10 is weak or absent in psoriatic skin compared with other inflammatory dermatoses (Nickoloff *et al*, 1994; Asadullah *et al*, 1998). The 5′ flanking region of the *IL10* gene, which controls transcription, is polymorphic. In addition to two microsatellite loci located 1.2 kb and 4.0 kb upstream of the coding region, there are three single base-pair substitutions at positions –1082, –819, and –592 that produce three different haplotypes, GCC, ACC, and ATA (Turner *et al*, 1997). Peripheral blood mononuclear cells from individuals carrying an A at position –1082 (*IL10–1082*A*) showed significantly lower inducible IL-10 levels compared with individuals negative for an A at this position, and this correlation was independent of the polymorphisms at positions –819 and –592 (Turner *et al*, 1997). In this study we therefore focused on polymorphisms at position –1082. We were unable to detect associations between early- or late-onset psoriasis and the *IL10–1082*A* allele, and there was no evidence for significant interactions between the TNFα-238 and IL10–1082 polymorphisms. These data do, of course, not exclude the possibility that the distribution of the ATA and ACC haplotypes or of particular combinations of microsatellite alleles varies between psoriasis patients and healthy controls, the latter being also associated with differential IL-10 secretion (Eskdale *et al*, 1998b).

The second focus of this study was the analysis of polymorphisms of xenobiotic metabolizing enzymes. *N*-acetyltransferase 2 catalyzes the acetylation of primary amino and hydroxylamino groups and plays a central part in the metabolic pathways that produce or prevent toxicity following exposure to homocyclic and heterocyclic arylamine and hydralazine xenobiotics. Combinations of any two of 15 mutant alleles identified until now define a slow acetylator genotype, while rapid acetylators have one or two wild-type alleles (Grant *et al*, 1997). GST are a complex multigene family of enzymes that conjugate electrophiles with reduced glutathione. An important biologic function is the protection against inflammation, mutagenicity, and genotoxicity as a result of oxidative stress. Homozygous gene deletions with an abolition of enzymatic function have been elucidated for GST M1 (Seidegård *et al*, 1988) and T1 (Pemble *et al*, 1994). In addition to the well established role of NAT2 and GST polymorphisms as risk factors in human cancer, enzyme polymorphisms have also been implicated in the susceptibility to inflammatory reactions of the intestine (Duncan *et al*, 1995) and, more recently, the skin (Kerb *et al*, 1997; Schnuch *et al*, 1998). We found no significant associations between psoriasis and a rapid acetylator genotype or homozygous deletion of GST. As there is a biologic variability in NAT2 phenotype expression of certain NAT2 genotypes (Cascorbi *et al*, 1995), however, this finding does not automatically exclude an association between the disease and the acetylator status. To gain more information about the acetylator status in psoriasis we analyzed simultaneously the NAT2 genotype and phenotype in a subgroup of study participants (84 controls, 55 type I, 36 type II). Interestingly, there was a significant association between early-onset but not late-onset psoriasis and a rapid acetylator phenotype [OR 2.7 (1.3–5.5); $p = 0.0096$] that was not present at the genotypic level [1.8 (0.9–3.7); $p = 0.11$]. Moreover, we found NAT2 to be constitutively expressed in normal and psoriatic skin suggesting a direct effect of NAT2 polymorphisms on the local generation of metabolites from arylamines in food, cigarette smoking, or environmental amines of various types. As NAT2 was detected in keratinocytes and Langerhans cells, the enzyme may participate in cutaneous antigen processing, a critical step in the induction of inflammatory T cell responses (Anderson *et al*, 1995). At a functional level complex interactions between polymorphic enzymes affecting the "preimmunologic phase" and cytokine promoter polymorphisms affecting the "immunologic phase" of cutaneous immune responses may occur. It may be speculated that these underlie our finding that males carrying the *GSTM1*0* and *TNFα-238*A* alleles were not at an increased risk to develop psoriasis [OR 2.8 (0.9–9.4); $p = 0.11$] whereas males carrying the *NAT2*4* and *TNFα-238*A* alleles were over eight times more likely to develop the disease [8.5 (1.1–69.6); $p = 0.021$; **Table V**]. Corrected p-values were not significant, however, and interactions between cytokine promoter and enzyme polymorphisms will have to be addressed in larger studies in the future.

According to current knowledge psoriasis has a complex genetic basis, involving one or more major determinants and a variety of associated genetic factors that may contribute to the disease manifestation or control certain aspects of the disease but are no major predisposing genes themselves. The results of our study in concert with the findings of two previous reports (Arias *et al*, 1997; Höhler *et al*, 1997) clearly suggest that the *TNFα-238*A* allele is a risk factor for susceptibility to early-onset psoriasis; however, as neither HLA nor TNFα loci have been shown to be linked to susceptibility loci identified by replicated linkage analyses in affected families, both loci do not appear to represent major genes. A genetically determined overproduction of proinflammatory cytokines could lower the threshold for the manifestation of cutaneous inflammatory reactions in response to physical, chemical, or antigenic stimuli. Clinical observations such as the well-established Koebner's phenomenon (i.e., the development of psoriatic lesions after nonspecific irritation of normal appearing skin) and the high rate of contact sensitizations (Heule *et al*, 1998) support the presence of an inflammatory predisposition in psoriasis.

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