(Invitrogen) to remove contaminated genomic DNA and precipitated with ethanol. The quality of total RNA was assessed by agarose gel electrophoresis (visual absence of significant 28S and 18S band degradation) and by spectrophotometry. Double-stranded cDNA was synthesized from 5 g of total RNA by using a SuperScript Choice system (Life Technologies, Rockville, MD, USA) and a T7-(dT)24 primer (GENSET). The cDNA was subjected to in vitro transcription in the presence of biotinylated nucleoside triphosphates with the use of a BioArray High-Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY, USA). The biotinylated cRNA was hybridized with the GeneChip arrays for 16 h at 45°C. After washing, the hybridized biotinylated cRNA was stained with streptavidin–phycoerythrin (Molecular Probes, Eugene, OR, USA) and scanned with the HP GeneArray Scanner.

Data analysis

The raw Affymetrix data (fluorescence intensity of each probe) was quantified and further analysed using the 'Affymetrix MicroArray Suite version 5.01' (MAS) [15, 16], with the unfiltered, generated data being exported to text files for post processing. The Affymetrix recommended default parameters were used for the MAS analysis.

The data sets consisted of 11 Affymetrix GeneChip data files. Three data files were of three normal subjects (no psoriasis), labelled as N02, N05 and N09. The other eight data files (S21N, S21P, S23N, S23P, S24N, S24P, S29N and S29P) were of four psoriatic subjects (S21, S23, S24 and S29), with four data files of skin samples showing evidence of active psoriasis (P or affected skin) and four data files of samples from a symptomless skin area (N or unaffected skin) close to an active area but showing no signs of activity.

The algorithm used by the Affymetrix MicroArray Suite was designed to detect the presence of valid gene expression by testing the probes of a gene for the reliable detection of signals and by 'calling' one of three states, 'absent', 'marginal' or 'present', compared with the reference. A gene may also show an increase, a decrease or no change in expression. This information was used to increase the stringency of our analysis by eliminating the genes that showed an ambiguous call across the samples. Therefore, up- or down-regulated genes were further analysed only if the Affymetrix algorithm recorded a consistent present state.

Postprocessing was used to further refine the analysis of the Affymetrix data using a combination of Microsoft Excel, Gnumeric and R to cluster and filter the data. Clustering was based on generating a *t* statistic for related samples based on the Affymetrix MAS-generated signal level for each gene. The resulting *t* statistics were ranked according to value, and a significance threshold of 5% was used to eliminate genes that were unlikely to contribute to further analysis. The remaining genes were annotated using the Affymetrix NetFX facility at http://www.affymetrix.com/analysis/index.affx [17]. These data were subjected to manual analysis to identify biological trends in the data. In compiling the gene lists, tables were constructed to include the official gene names and symbols, NCBI gene identifier (ID), Affymetrix sequence identifier, NCBI GenBank accession number, OMIM identifier, chromosomal location, *t* statistic and reference to previous Affymetrix studies on psoriasis (AFFYREF) [7, 8, 10] and to indicate whether the genes were known to respond to IFN, viral antigens or infections. The manual and automated analysis included using the Affymetrix sequence identifiers to obtain the GeneID, OMIM, official gene name and symbol and chromosomal locations from NCBI (http://www.ncbi.nlm.nih.gov/). The NCBI data files for the GeneID, OMIM and PubMed references assisted with manual compiling of the functional categories of the genes. The full psoriasis gene expression data can be accessed from NCBI GEO as accession number GSE2737.

Results

Global comparison of gene expression in psoriatic and normal skin

The global comparison of gene expression using the HUG95AV2 oligoarray revealed the presence of an average of 2,209 expressed genes in normal skin, 2,711 expressed genes in unaffected psoriatic skin and 3,403 expressed genes in affected psoriatic skin. Thus, 22.6 and 54.1% more genes were expressed in unaffected and affected psoriatic skin, respectively, than in normal skin. This difference in the number of genes expressed in psoriatic skin when compared with normal skin probably reflects the complex and mixed cellular changes that can occur in the psoriatic epidermis, such as hyperplasia and atypical differentiation of keratinocytes, neurogenesis, angiogenesis and the increased number of cellular infiltrates such as antigen-presenting cells, leukocytes, monocytes and lymphocytes into the psoriatic skin (Fig. 1). To determine the differences in gene expression between normal and psoriatic skin, the average frequency gene expression values were calculated, and a statistical *t* test was performed between normal and affected psoriatic skin, between normal and unaffected psoriatic skin, and between affected and unaffected psoriatic skin for four psoriatic patients and three normal controls.

Up-regulated genes in psoriatic skin

In determining the difference in gene expression between psoriatic and normal skin, we found that the number of significantly ($p < 0.05$) up-regulated genes was 102 in unaffected psoriatic skin and 263 in affected genes (Supplementary Tables S1 and S2). Of the 266 up-regulated genes in psoriatic patients, 99 were up-regulated in both the affected and unaffected psoriatic skin and 164 were up-regulated in affected but not in unaffected psoriatic skin. The three genes up-regulated in unaffected but not in

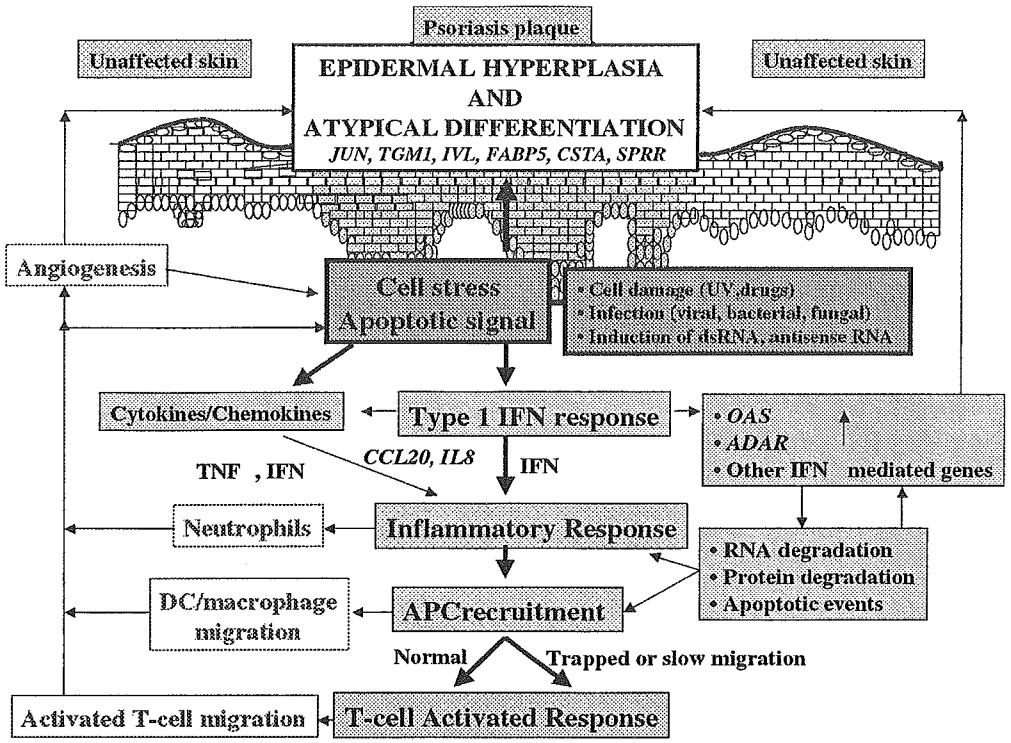

Fig. 1 Immunopathogenesis of psoriasis. This simplified model was adopted from [1–6] and is described briefly in "Discussion". *Arrows* between the labelled boxes indicate the interaction between the different steps or temporal changes involved in psoriasis. Neurogenesis is not included in the figure, but probably contributes to sensory sensitivity and itchiness of psoriatic skin. Redness of psoriatic skin is due to the increase in capillary blood vessels trafficking in red cells, neutrophils and T cells to sites of psoriatic plaques and blisters. The psoriatic pathogenic cycle is hypothesized to stem from unidentified stress, antiviral or apoptotic signals. The expression of the TNF , IFN and IFN genes had not changed significantly in our study, and their suspected key roles in psoriasis are taken from [1–6, 20]. Some of the overexpressed genes observed in the present study are shown in *italics*. A fuller complement of changes in gene expression in psoriatic affected and unaffected skin is presented in Tables 1 and 2 and Supplementary Tables S1, S2, S3, S4, S5

affected skin were degenerative spermatocyte homolog, lipid desaturase (DEG5, 8560), metallothionein IV (MT4, 84560) and perilipin (PLIN, 5346). The top seven up-regulated genes based on their *t*-test ranking were *ALOX12B*, *EIF5*, *CTSC*, *CDC42EP1*, *HDGF*, *MX1* and *ITM2B* in unaffected psoriatic skin and *SYNCRIP*, *MYO5A*, *ALOX12B*, *UBE2L6*, *NAPA*, *TGM1* and *SPRR1A* in affected psoriatic skin. In the list of 263 genes induced in affected psoriatic skin, at least 104 were identified in previous Affymetrix experiments on psoriasis [7, 8, 10].

Although 164 genes were found to be up-regulated in affected but not in unaffected psoriatic skin of four patients in comparison with normal skin of three patients by using an unpaired *t*-test analysis, we undertook a more stringent comparison of gene expression between the affected and unaffected skin of the four patients by using a paired *t*-test analysis. Table 1 shows the top 51 up-regulated genes in psoriatic affected tissue relative to unaffected tissue of the four Japanese patients based on the paired *t*-test statistic. This table shows the gene names and symbols, NCBI gene identifier, Affymetrix sequence probe identifier, NCBI GenBank accession number, OMIM identifier, chromosomal location, function, *t* statistic, reference to previous Affymetrix studies on psoriasis, and which genes respond to IFN, viral antigens or infections. There were 26 significantly ($p<0.05$) up-regulated genes in psoriatic affected skin compared with unaffected skin. The top four significantly ($p<0.01$) up-regulated genes based on the paired *t*-test ranking were *JUNB*, *YWHAB*, *LAMP3* and *SEC61G* in affected psoriatic skin compared with unaffected psoriatic skin.

To clarify the relative functions of the up-regulated genes for affected and unaffected psoriatic skin, we grouped the genes into 31 categories (Supplementary Table S3) representing molecular or biological functions based on the available gene information provided by NCBI or found in the published literature (PubMed). The top 10 functional categories, based on 10 or more induced genes within a functional category, were genes coding for protein degradation (37 genes), IFN responses (23 genes), phosphatases and kinases (22 genes), binding proteins and transporters (18 genes), mitogen-activated responses (16 genes), epidermal differentiation (16 genes), RNA binding, regulation, and degradation (15 genes), cytoskeleton organization, myosin and actin-related protein 2/3 complex (13 genes), apoptosis (11 genes) and cell adhesion and interaction (10 genes). The protein degradation category contained four main subcategories of genes encoding proteinases (12 genes), proteosome components (10 genes), ubiquitination factors (8 genes) and proteinase inhibitors (7

Table 1 Top 51 up-regulated genes in the affected compared with the unaffected skin of four psoriatic patients based on a paired *t*-test ranking

Gene symbol	OMIM	Affymetrix ID	Accession No.	GeneID	IFN/viral response	Reference	Chromosome	Function	*t* Statistic	Gene name
JUNB	165161	32786_at	X51345	3726			19p13.2	mRNA regulation	6.93	JunB proto-oncogene
YWHAB	601289	32324_at	X57346	7529	Viral target		20q13.1	Cell cycle	6.75	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide
LAMP3	605883	37168_at	AB013924	27074		B3/7	3q26.3–q27	DS	6.49	Lysosome-associated membrane protein 3
SEC61G		39169_at	AF054184	23480			7p11.2	PT	6.12	Sec61 gamma, protein translocation complex
KIAA0101		38116_at	D14657	9768		O	15q22.31	Cell cycle	5.82	KIAA0101 gene product
CSTA	184600	39581_at	AA570193	1475		B3/7	3q21	PD	5.55	Cystatin A (stefin A)
OAS1	164350	38389_at	X04371	4938	IFN, viral target		12q24.1	IFN response	5.38	2 ,5 -Oligoadenylate synthetase 1 (40–46 kDa)
CCL20	601960	40385_at	U64197	6364	Viral target	N	2q33–q37	DS	5.02	Small inducible cytokine subfamily A (Cys-Cys), member 20
TGM1	190195	35947_at	M98447	7051		B26/29, O, N	14q11.2	KR	4.91	Transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine gamma-glutamyltransferase)
SEC61B		32564_at	AA083129	10952			9q22.32–q31.3	PD	4.83	Protein translocation complex beta, SEC61 beta
GBA	606463	32632_g_at	J03060	2629		B26/29	1q21	KR	4.6	Glucosidase, beta; acid
H2AFY		36576_at	AF054174	9555	viral target		5q31.3–q32	Cell cycle	4.45	H2A histone family, member Y
UBE2L6	184600	40505_at	AA883502	9246		B26/29	11q12	PD	4.35	Ubiquitin-conjugating enzyme E2L 6
GM2A	272750	35820_at	X62078	2760		B11, N	5q31.3–q32	PD	3.85	GM2 ganglioside activator protein
SULT2B1	604125	41034_s_at	U92315	6820		O	19q13.3	KR	3.79	Sulfotransferase family, cytosolic, 2B, member 1
P4HB	176790	691_g_at	J02783	5034			17q25	Apoptosis	3.68	Procollagen-proline, 2-oxoglutarate 4-dioxygenase, beta polypeptide (thyroid hormone binding protein p55)
RER1		41551_at	AW044624	11079			1pter-q24	PD	3.67	Similar to *Saccharomyces cerevisiae* RER1
PSMB6	600307	941_at	D29012	5694	IFN, viral target	B11 (PSMA6)	17p13	PD	3.61	Proteasome (prosome, macropain) subunit, beta type, 6
NMI	603525	36472_at	U32849	9111	IFN, viral target	B26/29	22p24.3–q21.3	IFN response	3.55	N-myc (and STAT) interactor
IVL	147360	36355_at	M13903	3713		B11	1q21	KR	3.53	Involucrin, part of insoluble cornified cell envelope of stratified squamous epithelium
STAT1	600555	33338_at	M97936	6772	IFN, viral target	B11, O	2q32.2	mRNA regulation	3.5	Signal transducer and activator of transcription 1, 91 kDa, STAT91
YWHAQ		409_at	X56468	10971			2p25.1	Cell cycle	3.48	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide
CYCS	123970	35818_at	D00265	54205		B11	7p21.2	Apoptosis	3.44	Cytochrome *c*, somatic

Table 1 (continued)

Gene symbol	OMIM	Affymetrix ID	Accession No.	GeneID	IFN/viral response	Reference	Chromosome	Function	*t* Statistic	Gene name
SFRS9	601943	32573_at	AL021546	8683			12q24.31	RNA degradation	3.29	Splicing factor, arginine/serine-rich 9
VDAC1	604492	40198_at	L06132	7416	Viral target		5q31	Apoptosis	3.29	Voltage-dependent anion channel 1
CASP4	602664	195_s_at	U28014	837	IFN	O	11q22.2–q22.3	Apoptosis	3.26	Caspase 4, apoptosis-related cysteine protease
UBE2N	603679	1660_at	D83004	7334	IFN		12q22	PD	3.18	Ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
EPHA2	176946	1379_at	M59371	1969		B26/29	1p36	Signaling	3.14	EphA2, tyrosine protein kinase receptor, ECK
TUBB	191130	297_g_at	X79535	7280	Viral target	O	6p25	CytoR	3.14	Tubulin, beta polypeptide
ATP6V0D1	607028	38686_at	X71490	9114			16q22	ATPase	3.14	ATPase, H^+ transporting, lysosomal (vacuolar proton pump), subunit D, isoform 1
ALOX12B	603741	33029_at	AF038461	242		B11, N	17p13.1	KR	3.13	Arachidonate 12-lipoxygenase, 12R type
LILRB1	604811	35926_s_at	AF004230	10859	IFN	B11	19q13.4	Immunity	3.11	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
PSMB10	176847	384_at	X71874	5699	IFN, viral target	O	16q22.1	PD	3.08	Proteasome (prosome, macropain) subunit, beta type, 10
IFI30	604664	39728_at	J03909	10437	IFN	B26/29	19p13.1	IFN response	3.08	Interferon, gamma-inducible protein 30
SYNCRIP		40122_at	AF037448	10492	IFN	B26/29	6q14–q15	RNA regulation	3.06	Synaptotagmin binding, cytoplasmic RNA-interacting protein
IER2		36097_at	M62831	9592		O	19p13.13	Signaling	3.01	Immediate early response 2
ITGB4BP	603741	35262_at	AF022229	3692			20q12	KR	3.01	Integrin beta 4 binding protein, euk translation initiation factor, eIF3a
OTUB1	608337	38710_at	AL096714	55611			11q13.1	PD	3	OTUB1-OUT domain, ubiquitin aldehyde binding
YWHAE	605066	1011_s_at	U54778	7531	Viral target		17p13.3	Cell cycle	3	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide
CKMT1	123290	31887_at	J04469	1159			15q15	Kinase	2.97	Creatine kinase, mitochondrial 1 (ubiquitous)
CBX3	604477	38085_at	AI740522	11335		B26/29	7p15.2	RNA degradation	2.97	Chromobox homolog 3 (HP1 gamma homolog, *Drosophila*)
CIG5	607810	38549_at	AF026941	91543	IFN		2p25.2	IFN response	2.96	Viperin
FABP5	605168	39799_at	M94856	2171		B3/7, O	8q21.13	KR	2.95	Fatty acid binding protein 5 (psoriasis-associated)
ADAR1	601059	38014_at	X79448	103	IFN, viral target		1q21.1–q21.2	IFN response	2.85	Adenosine deaminase, RNA-specific
PAIRBP1		40441_g_at	AL080119	26135			1p31–p22	RNA regulation	2.84	PAI-1 mRNA-binding protein
ARPC1B	604223	39043_at	AF006084	10095	IFN		7q22.1	CytoR	2.83	Actin related protein 2/3 complex, subunit 1A (41 kDa)

Table 1 (continued)

Gene symbol	OMIM	Affymetrix ID	Accession No.	GeneID	IFN/viral response	Reference	Chromosome	Function	*t* Statistic	Gene name
PSME2	602161	41171_at	D45248	5721	IFN	B11, O	14q11.2	PD	2.83	Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)
AIM1		32113_at	U83115	202			6q21	CytoR	2.75	Absent in melanoma 1
HLA-G	142871	40369_f_at	AL022723	3135	IFN, viral target	[40]	6p21.3	Immunity	2.75	HLA-G histocompatibility antigen class I, G
IFI35	600735	464_s_at	U72882	3430	IFN, viral target	[27]	17q21	IFN response	2.75	Interferon-induced protein 35
IFI16	147586	1456_s_at	M63838	3428	IFN, viral target	B26/29	1q22	Cell cycle	2.71	Interferon, gamma-inducible protein 16

In the Reference column, B refers to Bowcock et al. [13] and the numbers 3/7, 11 and 26/29 refer to the genes in their up-regulated gene clusters. O refers to Oestreicher et al. [14]. N refers to Nomura et al. [16]. Of the top 51 listed genes, 27 (53%) were previously determined to be significantly up-regulated by B, O or N
CytoR Cytoskeleton regulation, *DS* dendritic cell regulation, *KR* keratinocyte regulation, *PD* protein digestion, *PT* protein transporter
t Statistic > 3.182=$p < 0.05$, *t* statistic > 5.841=$p < 0.01$

genes). Other categories of interest include antioxidant/antistress factors, heat responses/chaperones, signal transduction/intracellular signalling and immune- and inflammatory-related factors. The functions for 4 genes were unknown.

Down-regulated genes in psoriatic skin

The number of significantly ($p<0.05$) down-regulated genes was 23 in unaffected psoriatic skin and 58 in affected psoriatic genes compared with normal skin (Supplementary Tables S4 and S5). In total, there were 61 down-regulated genes detected in psoriatic skin, 38 were down-regulated only in affected skin, 2 (*OTUB* and *SMOX*) were down-regulated only in unaffected skin, and 21 were down-regulated both in the affected and unaffected skin, compared with normal skin. A more stringent comparison of gene expression between the affected and unaffected skin of the four patients by a paired *t*-test analysis revealed that 9 genes were significantly ($p<0.05$) down-regulated in affected psoriatic skin compared with unaffected psoriatic skin (Table 2). All 9 of these down-regulated genes were also listed in either Supplementary Table S4 or S5. The functions of the down-regulated genes in affected psoriatic skin compared with unaffected psoriatic skin are varied and with at least one of the gene products (*TXNIP*) involved in epidermal regulation.

Discussion

The concordance between our Affymetrix results and those of others was at best only moderate. Of the 51 genes listed in Table 1, 27 (53%) were reported previously to be up-regulated as part of either the collection of the 177 psoriatic genes identified by Bowcock et al. [7] or the 159 psoriatic genes identified by Oestreicher et al. [8] in their Affymetrix studies of psoriatic lesions. Surprisingly, only 5 genes (*PSME2*, *FABP5*, *STAT1*, *TGM1* and *IFI27*) were found to be up-regulated in all three of the previous studies [7, 8, 10]. The concordance was highest between the genes in our study (Table 1) and those of Bowcock et al. [7], with at least 21 genes identified in both studies. The genes ranked in the top 10 of the 51 up-regulated genes in all four of our psoriatic patients and which were not part of the list of the three previous Affymetrix studies [7, 8, 10] are *JUNB*, *YWHAB*, *SEC61G*, *OAS1*, *CCL20*, *SEC61B*, *H2AFY*, *P4HB*, *RER1* and *YWAQ*. Moreover, the up-regulated genes *YWHAB*, *YWHAG* and *YWHAQ* (Table 1) have not been previously associated with psoriasis. These genes are members of a family of genes encoding for tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation enzymes that mediate signal transduction and participate in apoptosis, cell cycle, and cellular spreading and migration [18]. The up-regulated genes reported by Bowcock et al. [7] and Oestreicher et al. [8] that are not in our list of top 51 genes (Table 1) include some previously well described psoriatic marker genes, such as *S100A7*, *SKALP*, *CRAB2*,

Table 2 Top 32 down-regulated genes in the affected compared with the unaffected skin of four psoriatic patients based on a paired *t*-test ranking

Gene symbol	OMIM	Affymetrix ID	GeneID	Chromosome	Function	*t* Statistic	Gene name
CSPG4	601172	38004_at	1464	15q23	Epidermal stem cell clustering	−11.425	Chondroitin sulfate proteoglycan 4 (melanoma-associated)
ANP32A	600832	37034_at	8125	15q22.3–q23	Histone acetylation, apoptosis	−10.250	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A
SPTAN1	182810	33833_at	6709	9q33–q34	Cell adhesion	−7.750	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
C11orf11		35511_at	747	11q12.2	Not known	−7.539	Chromosome 11 open reading frame 11
SSA2	600063	35294_at	6738	1q31	RNA-binding protein, autoantigen	−6.750	Sjogren syndrome antigen A2 (60 kDa, ribonucleoprotein autoantigen SS-A/Ro)
LAMA5	601033	41610_at	3911	20q13.2–q13.3	Extracellular glycoprotein	−5.750	Laminin, alpha 5
ICA1	147625	32634_s_at	3382	7p22	Diabetes antigen	−5.662	Islet cell autoantigen 1, 69 kDa
MECP2	105830	34355_at	4204	Xq28	Bind methylated DNA	−5.662	Methyl CpG binding protein 2 (Rett syndrome)
ALDH3A2	270200	40409_at	224	17p11.2	Detoxification of aldehydes	−5.629	Aldehyde dehydrogenase 3 family, member A2, Sjogren–Larsson syndrome
NAB1	600800	38692_at	4664	2q32.3–q33	Transcriptional repressor	−5.511	NGFI-A binding protein 1 (EGR1 binding protein 1)
DDHD2		35177_at	23259	8p12	Metal ion binding	−5.196	DDHD domain containing 2
ANXA8	602396	37954_at	244	10q11.2	Phospholipid-binding protein	−4.899	Annexin A8
ZNF384		38648_at	171017	–	Transcription factor	−4.750	Zinc finger protein 384
OSBPL1A	606730	36689_at	114876	18q11.1	Intracellular lipid receptor	−4.470	Oxysterol binding protein-like 1A
ZBTB16	176797	39681_at	7704	11q23.1	Transcription factor, cell cycle	−4.396	Zinc finger and BTB domain containing 16
ATXN3	109150	36820_r_at	4287	14q24.3–q32.2	Polyubiquitin-binding protein	−4.327	Ataxin 3
DDR1	600408	36643_at	780	6p21.3	Regulatory protein	−4.308	Discoidin domain receptor family, member 1
GPM6B	300051	37251_s_at	2824	Xp22.2	Neurogenesis	−4.047	Glycoprotein M6B
–		39156_at	–	–	Not known	−3.834	–
KCNC1	176258	32322_at	3746	11p15	K channel membrane protein	−3.755	Potassium voltage-gated channel, Shaw-related subfamily, member 1
PCDH21		37857_at	92211	10q22.1–q22.3	Cell–cell adhesion	−3.670	Protocadherin 21
CMAH	603209	39317_at	8418	6p21.32	Neu5Gc biosynthesis	−3.618	Cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase
DTX2		37977_at	113878	7q11.23	Ubiquitin-protein ligase activity	−3.525	Deltex homolog 2 (*Drosophila*)
TXNIP	606599	31508_at	10628	1q21.1	Epidermal regulation	−3.483	Thioredoxin-interacting protein
PLCL4		35454_at	9651	1p36.32	Intracellular signalling cascade	−3.474	Phospholipase C-like 4
MEIS4		37486_f_at	4213	17p12	Retrotransposed pseudogene	−3.464	Meis1, myeloid ecotropic viral integration site 1 homolog 4 (mouse)
KIAA0556		36279_at	23247	16p12.1–p11.2	Not known	−3.294	KIAA0556 protein
–		32393_s_at	–	–	Not known	−3.278	–

Table 2 (continued)

Gene symbol	OMIM	Affymetrix ID	GeneID	Chromosome	Function	*t* Statistic	Gene name
GBAS	603004	39793_at	2631	7p12	Vesicular transport	−3.264	Glioblastoma amplified sequence
PPP1R3C	602999	39366_at	5507	10q23–q24	Regulates diverse functions	−3.264	Protein phosphatase 1, regulatory (inhibitor) subunit 3C
DBN1	126660	37981_at	1627	5q35.3	Cytoplasmic actin-binding protein	−3.240	Drebrin 1
ALS2CR3	607334	40064_at	66008	2q33	Not known	−3.213	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 3

t Statistic $< -3.182 = p < 0.05$, *t* statistic $< -5.841 = p < 0.01$

KRT17, *KRT18*, *DEFB2*, *SPRR2A* and *SPRRB1B*. Furthermore, although we and Oestreicher et al. [8] found that the *SULT2B1* gene was significantly ($p<0.05$) overexpressed in psoriatic lesions, another study reported that the SULT2B1 protein product was absent from a psoriatic lesion when it was compared with a normal tissue by immunohistochemistry [19]. Therefore, the differences between our study and the other four published Affymetrix studies [7–10] on psoriasis highlight the variability in gene expression occurring between individual patients, probably on the basis of their age, ethnicity, sex, genetics, skin types and environmental influences. In addition, laboratory and statistical errors and biases are likely to contribute to some of the differences between the results of different studies.

Unaffected psoriatic skin in close proximity to psoriatic lesions is known to be morphologically symptomless and histologically normal. However, gene expression profiling in this and previous studies have found that gene expression activity is abnormally high in both psoriatic unaffected and affected skin when compared with normal skin [7–9]. For example, in our study, there were 102 up-regulated genes and 26 down-regulated genes in unaffected psoriatic skin in comparison with normal skin. Thus, the results of gene expression profiling and genetic studies strongly suggest that people with psoriasis have a skin type that is genetically different to normal skin and that the psoriatic unaffected skin type is prone to the development of psoriatic lesions by as yet unidentified signals or triggers. This underlying genetic difference between symptomless psoriatic skin and normal skin was also indicated in animal model studies when transplanted human symptomless prepsoriatic skin, but not normal skin, was converted spontaneously into a psoriatic skin lesion with clear morphological evidence of elevation, erythema and scaling and histological evidence of epidermal hyperplasia, parakeratosis, papillomatosis and prominent vessels of the papillary dermis [20].

At least six distinct but interrelated immunopathogenic events have been identified in the development and persistence of psoriasis (Fig. 1). These are an apoptotic or immunity stress signal, inflammatory response and recruitment of neutrophils, recruitment and activation of professional antigen-presenting cells (especially DCs), recruitment and activation of T cells, angiogenesis (endothelial recruitment and vascular change), and lymphocyte–keratinocyte interactions resulting in abnormal keratinocyte proliferation and differentiation [1–6]. The prepsoriatic skin is believed to undergo a transition from symptomless skin to form psoriatic plaques and lesions when pathogenic T cells infiltrate the epidermis in response to an apoptotic/stress signal and provoke epidermal hyperplasia in the basal cell layer [11, 20]. This proliferative burst within the epidermis was postulated by Nickoloff [21] to be of sufficient intensity and duration to trigger keratinocytes in the suprabasal cell layers to undergo premature senescence and form the chronic, stable psoriatic plaques. The senescent keratinocytes within the epidermal compartment of

psoriatic plaques may then become the source of the apoptotic/stress signals that continue to trigger an inflammatory and pathological T-cell response (Fig. 1). We found that this hyperplastic and plaque-forming phase (psoriatic affected skin) of psoriasis was marked by a further increase in the number of up- and down-regulated genes when compared with either psoriatic unaffected skin or normal skin. In addition, the number of overexpressed genes was at least three times greater than the number of suppressed genes.

Although our data did not show direct evidence for a microbial infection in psoriasis, many up-regulated genes in our psoriasis cases code for antiviral/apoptotic stress signals that appear to be responding to a viral infection or to some other as yet unidentified cellular insult. Of the top 51 up-regulated genes (Table 1), 23 (46%) are antiviral, IFN or immune response genes involved with the regulation of activated NK or T cells, DC signalling and maturation, apoptotic signalling, protein degradation, RNA degradation, mRNA regulation and cell cycle regulation (Fig. 1). Only seven (13.7%) genes appear to be involved directly in the development and maturation of keratinocytes in the epidermis, and three genes, *ARPC1B*, *AIM1* and *TUBB*, are associated with cytoskeleton organization and actin polymerization in various cell types. *CKMT1* is involved in energy transduction in tissues with large, fluctuating energy demands. *P4HB* is a thyroid hormone-binding protein involved in thyroid hormone signalling and Arg/Pro metabolic pathway. Therefore, *P4HB*, like adenosine deaminase, may be a useful molecular marker for assessing the effectiveness and sensitivity of the antithyroid drug propylthiouracil (PTU) as an effective oral treatment for some cases of psoriasis [22]. *EPHA2* is a receptor for EphrinA family members whose immediate role in psoriasis is unclear, although it may participate in angiogenesis (Fig. 1) and vascularization around the region of an apoptotic trigger [23].

Viruses and cellular double-stranded RNA and inhibitor RNAs induce many of the interferon target genes, such as *IFI16*, *IFI35*, *NMI*, *STAT1*, *CIG5*, *OAS1* and *ADAR1*, which are overexpressed in psoriatic affected and unaffected skin (Table 1) [22, 24–28]. At least 15 of the 51 psoriatic up-regulated gene products have been found to interact with the proteins of HIV (for references, visit NCBI using the GeneID numbers listed in Table 1) and other viruses. IFN , IFN , IFN , human cytomegalovirus (HCMV) infection and the HCMV glycoprotein B envelope protein can induce viperin expression, or conversely, viperin can inhibit HCMV infection [26]. The viral and IFN response gene products *ILI30*, *CCL20*, *LAMP3*, *ADAR1*, *CASP4* and *HLA-G* are known to act as signals for promoting or regulating the inflammatory and adaptive immune responses [29–34]. Furthermore, the ubiquitination–proteasome pathway, which is also regulated by IFN, is paramount in the regulation of metabolism, apoptosis, cell cycle, signal transduction, removal of abnormal proteins, stress response and generation of antigenic peptides in the immune response [35–37]. At least 12 of the up-regulated gene products in psoriasis are known to be involved with ubiquitination and peptide processing (protein digestion and transport) and presentation as part of the adaptive immune response, with seven of the proteins (SIAH2, UBE2L6, UBE2N, PSMB10, PSME2, PSMB6, PSMD14) involved specifically in an ATP/ubiquitin-dependent non-lysosomal proteolytic pathway using the immunoproteosome complex, probably to generate antigenic peptides for presentation by MHC class I molecules to activated T cells [38].

It has been proposed that the early or intermediate differentiation markers are overexpressed and late differentiation markers are abolished by premature cell death during keratinization of the epidermis in psoriasis [39]. Our data (Table 1) are consistent with some aspects of this proposal in that the gene markers for early differentiation, such as involucrin, cystatin A, serpinB and transglutaminase 1, were overexpressed in our psoriatic patients. However, we did not find that the gene expression of the late differentiation markers, such as profilaggrin and loricrin, were completely abolished. The psoriatic skin gene markers *FABP5*, *ALOX12B* and *ITGB4BP* were overexpressed in the psoriatic lesions of our patients as previously reported by others [7–10]. In addition, if keratinocytes are programmed for premature cell death in psoriasis as proposed [39], then the overexpression of many ubiquitination and proteosome genes in the skin of our psoriatic patients might also be molecular signatures of enhanced cell death activity in the epidermis.

The transcription factor *JUNB* was at the top of our statistical ranking of up-regulated genes in psoriatic affected skin (Table 1), suggesting that it has an important but as yet undefined role in psoriasis. JunB is a component of the AP-1 transcription regulatory complex [40] and as a *trans*-activating factor (p36) may activate caspase 4 [41] and inhibit proliferation in the epidermis by suppressing the expression of *CCND1*, a cyclin D cell cycle regulator that is essential in the control of the G_1/S transition [42]. In this connection, we found that the *CCND1* gene was down-regulated in the affected skin of three of the four psoriatic patients in our study group (data not shown). The overexpression of *JUNB* in affected psoriatic skin in our study is in accordance with the findings of Johansen et al. [43], who found that the protein and mRNA levels of JunB was increased in lesional psoriatic skin. They also showed that the protein and mRNA expression of the AP-1 subunits c-Fos, fra-1 and c-Jun were decreased in affected psoriatic skin compared with unaffected psoriatic skin. Therefore, JunB overexpression may have an antagonistic effect on the expression of c-Jun and AP-1 binding activity that results in an inappropriate cytokine-regulated mesenchymal–epidermal interaction in skin [44] and the atypical epidermal growth and differentiation observed in psoriasis.

A possible limitation of our study is that the changes detected in RNA expression in psoriatic skin by microarray profiling may be non-correlative with protein activity or function and the pathophysiological state of disease. Nevertheless, our gene expression results, like those of others, provide strong evidence that psoriasis is a chronic IFN- and T-cell-mediated disorder of the skin where the imbalance in

epidermal cellular structure, growth and differentiation arises when the cellular and molecular stress signals initiate inappropriate immune responses in genetically predisposed people. As part of our future studies of psoriasis, we intend to undertake quantitative PCR, in situ hybridization and immunohistochemistry to confirm the expression of genes in this study that had not been previously reported to be either up- or down-regulated. In addition, we propose to further characterize those genes that appear to be useful positive markers for the diagnosis of psoriatic unaffected (symptomless) and affected skin.

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Identification and characterization of novel variants of the thioredoxin reductase 3 new transcript 1 *TXNRD3NT1*

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Abstract

We have identified and characterized a new gene sequence, *TXNRD3NT1*, whose transcripts, corresponding to the EST AA430236, were found by Affymetrix DNA chip analysis to be significantly down regulated in affected psoriatic tissue. The full-length cDNA of *TXNRD3NT1* was isolated and characterized by combining 5′- and 3′-RACE (rapid amplication of cDNA ends) with screening a keratinocyte cDNA library, designing appropriate PCR primers, cloning amplified products, sequencing, and sequence analysis. Because part of this gene overlaps the previously described *thioredoxin reductase 3* (*TXNRD3*) gene, we have named it *TXNRD3NT1* (*TXNRD3* new transcript 1). The full-length *TXNRD3NT1* cDNA has 1133 nucleotides with a 251-bp 3-UTRand 2 poly(A)signal variants and 2 poly (A) sites. The *TXNRD3NT1* cDNA ORF encodes for 133 amino acids, with the first four residues coding for a tubulin-β mRNA autoregulation signal. Mapping the cDNA nucleotide sequence to the human genome sequence revealed that the *TXNRD3NT1* gene has 4 exons located on Chromosome 3, at position 3q21. Exons 1 and 2 of the *TXNRD3NT1* gene overlap with exons 15 and 16 of the *thioredoxin reductase 2* gene which has different ORFs to that of *TXNRD3NT1*. The translation initiation codon ATG was found in exon 3 of the *TXNRD3NT1* gene. RT-PCR showed that the full-length variant of the *TXNRD3NT1* gene was expressed in only four issues (pancreas, esophagus, bone marrow, and keratinocytes) of the 30 different tissues tested. In most other tissues, an aberrant and truncated form of the transcript (i.e., missing exon 3 and part of exon 4) was detected. The result of a preliminary association study between psoriasis and single microsatellite marker of the *TXNRD3NT1* gene suggests that it may not be a significant genetic determinant of psoriasis. However, we cannot exclude the possibility that other sequence variants may still exist within the *TXNRD3NT1* gene. Sequence analysis of the *TXNRD3NT1* gene from 8 psoriasis patients and 8 healthy controls revealed a number of synonymous SNPs that may be useful markers for future disease association studies.

The thioredoxin–thioredoxin reductase system is one of the key thio-disulfide redox regulation processes in cells and tissues that respond to environmental changes and stresses such as reactive oxygen species (ROS), ultraviolet (UV) light, gamma rays, and heat shock (Holmgren 1985; Nakamura et al. 1997). Thioredoxin (TRX) is a 12-kDa thiol reductase with 2 redox-active cysteine residues in its consensus sequence that serves as a general disulfide oxidoreductase (Holmgren 1985). Its classical function is to act as a hydrogen donor for ribonucleotide reductase in DNA synthesis (Laurent et al. 1964; Reichard 1993). TRX, however, has other multifunctional roles, e.g., as a powerful antioxidant against ROS, H_2O_2- and TNF-α-induced cytotoxicity,

GenBank accession number for human *TXNRD3NT1* cDNA is AB113648.

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Table 1. Oligonucleotide primers used for 5′ and 3′ RACE of the human *TXNRD3NT1* gene

Primer names	*Oligonucleotide primer sequences (5′ to 3′)*	*Nested oligonucleotide primer sequences (5′ to 3′)*
3′ RACE_01[a]	GGATGATGTTTGCCCAGGC	GATGATGTTTGCCCAGGCATG
5′ RACE_01[a]	TTCCATGTGAGAGATCATCCGC	CATGTGAGAGATCATCCGCTGATAC

[a]These primers were designed on EST sequences, AA430236.

and as a biological regulator of signal transduction and binding of transcription factors such as NF-kB, TFIIC, estrogen, and glucocorticoid receptors and AP-1 activity via ref-1 (Cromlish and Roeder 1989; Bannister et al. 1991; Toledano and Leonard 1991; Matthews et al. 1992; Hirota et al. 1997; Hayashi et al. 1997; Tanaka et al. 1999). TRX also has cytokine-like functions when secreted outside the cell and it stimulates the proliferation of lymphoid cells, fibroblasts, and tumor cell lines and inhibits HIV expression in macrophages (Wakasugi et al. 1990; Ericson et al. 1992; Rubartelli et al. 1992; Biguet et al. 1994; Newman et al. 1994). It also is an inhibitor of apoptosis signal-regulating kinase 1 (ASK-1) and a regulator of cytokine- and stress-induced apoptosis (Saitoh et al. 1998).

The activity of TRX is dependent on the redox status of a number of selenoproteins, known as thioredoxin reductases (TRXRD), and on the activity of the selenocysteine residue that is a cellular redox sensor at their carboxyl termini encoded by a UGA codon (Gasdaska et al. 1995; Tamura and Stadtman 1996). The human has at least three TRXRD isoenzymes, TRXRD1, 2, and 3, that have more than 50% sequence similarity and two active-site redox cysteines in the NH_2 terminal region, the NADH and FAD binding residues and a dimer interface domain (Sun et al. 1999). TRXRD is highly expressed on the surface of human melanocytes and keratinocytes, regulates pigmentation biology, and correlates with different skin phototypes. Together with TRX it provides the skin's first epidermal and dermal defenses against the free radicals generated by UV light (Didier et al. 2001; Schallreuter and Wood 2001; Oh et al. 2004).

In this article we describe the isolation, characterization, and genome mapping of the novel gene, *thioredoxin reductase 3 new transcript 1* (*TXNRD3NT1*), whose cDNA sequences corresponded in part to an expressed sequence tag (EST) that was differentially downregulated in affected psoriatic skin tissues. The *TXNRD3NT1* cDNA sequences were found to be two different intragenic variants of the *thioredoxin reductase 3* (*TXNRD3*) gene (alias TR2, TRXR3) located on the chromosome position 3q21 (Ensembl cytogenic band), a location that was identified previously to be a psoriasis candidate region in association studies (Enlund et al. 1999; Hewett et al. 2002). *In silico* translation of the cDNA sequences revealed that TXNRD3NT1 is a much smaller protein than TXNRD3 and that it has a unique tubulin-β autoregulation signal. On the basis of positional and functional information, the TXNRD3NT1 transcripts and proteins may have a role in either the regulation of thioredoxin or TXNRD3 and in the maintenance of epithelia and development of psoriasis. In order to better understand the association study between *TXNRD3NT1* and psoriasis, we also performed an association study using a single microsatellite marker specific to the *TXNRD3NT1* and *TXNRD3*.

Materials and methods

5′- and 3′-RACE (rapid amplification of cDNA ends). By microarray assay several ESTs whose expression was up- or downregulated in the affected skin in patients with psoriasis vulgaris were identified (unpublished data). Full-length and partial clones of the EST sequence (accession number AA430236), whose expression was significantly reduced in the psoriatic skin (unpublished data), were prepared from a human keratinocyte cDNA library by employing the 5′ and 3′ RACE as previously described (Matsuzaka et al. 2002; Tsuji et al. 2003). The cDNA library was prepared from total RNA of the cultured keratinocyte cell line, HEK, using the SMART RACE cDNA amplification kit according to the manufacturer's instructions (Clontech, Palo Alto, CA). The 5′- and 3′-RACE was performed using a total of 10 ng of keratinocyte cDNA and PCR amplification of the transcript ends using the Universal Primer Mix (UPM), long primer (0.4 mM): 5′-CTAATACGACTCACTATAGGGCAAGCAGTG GTAACAACGCAGAGT-3′, and short primer (2 mM): 5′-CTAATACGACTCACTATAGGGC-3′, and either of the gene-specific primers for the 5′-terminal exon and for the 3′-terminal exon (Table 1). The PCR products were then amplified in a secondary PCR using the Nested Universal Primer (NUP); 5′-AAGCAGTGGTAACAACGCAGAGT-3′, and the primer for 5′-terminal exon or for 3′-terminal exon (Table 1). PCR products were purified using the QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA) and then ligated into the pGEM-T

Table 2. Oligonucleotide primers used for PCR amplification of the human *TXNRD3NT1* gene

Location	*Forward sequences (5′ to 3′)*	*Reverse sequences (5′ to 3′)*	*Sequencing primers (5′ to 3′)*	*Annealing temp. (C)*	*Product size (bp)*
Promoter-exon 1	GCCCTAGAGTTATTCCA ATGTTGGAC	GACTTTGTGATTTCCAA AGTCGTG	GCCCTAGAGTTATTC CAATGTTGGAC GACTTTGTGATTTCC AAAGTCGTG	60	1040
Exon 1-2	GATCGGGTGATAGGAT TTCATATTCTTG	AAGCTAACTTAGGTGAA TGGTGCCAC	GATCGGGTGATAGG ATTTCATATTCTTG AAGCTAACTTAGGTG AATGGTGCCAC	60	1067
Exon 3	GAGAAAGGCACAGACC TGTCTCC	TTCCATGTGAGAGATCA TCCGC	GAGAAAGGCACAGAC CTGTCTCC TTCCATGTGAGAGATC ATCCGC	60	861
Exon 4	GGATGATGTTTGCCCA GGC	AGCTCAGCATAGTGAGT GGCATG	CAGCAGCTGACCTCTG GAAG GGATGATGTTTGCCC AGGC	60	600
			AGCTCAGCATAGTGA GTGGCATG		

easy vector using the TA cloning system (Promega, Madison, WI). To further clone the 5′ region of this gene, 5′ RACE was performed using various primer sets (Table 1) and the same conditions as described previously (Matsuzaka et al. 2002; Tsuji et al. 2003).

DNA sequencing and database mining. The plasmid recombinant DNA, containing the cloned insert, was purified using the QIAprep minicolumns (Qiagen) and sequenced by dideoxy sequencing using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an ABI 377 or 3700 DNA sequencer. DNA and deduced amino acid sequences were compared with the nonredundant nucleotide and protein sequence databases using BLASTN and BLASTP (http://www.ncbi.nlm.nih.gov/genome/seq/page.cgi?F=HsBlast.htmlORG=Hs), respectively (Altschul et al. 1990). The gene loci were mapped onto the human chromosomes by using Mapview (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/hum_srch).

Reverse transcriptase-polymerase chain reaction (RT-PCR). Human poly(A)$^+$ RNA (10 ng) purified from various tissues (TOYOBO) and 1 μl (10 mM) of the gene-specific primer RT-BF (5′-AAATACCTCTGCTCTCTGAACAGCC-3′) were denaturated at 65 C for 5 min and then quenched on ice. The first-strand cDNA was synthesized using THERMOSCRIPT RT (GIBCO BRL, Rockville, MD) at 60 C for 1 h as previously described (Matsuzaka et al. 2002. Tsuji et al. 2003). The first-strand cDNA was amplified by PCR with Ampli *Taq* Gold with the following paired RT_001F and RT-001R primers: TXNRD3NT1-RT-001F: 5′-GGACTGCTTCCTTGACGCCTTAG-3′, TXNRD3NTI-RT-001R: 5′-CTGAACAGCCTTTTCCTTCTGCC-3′.

Subjects. A total of 85 unrelated Japanese patients with psoriasis and 110 healthy controls were investigated in this study. Details of the collection of the blood samples from the patients with psoriasis and healthy controls have been described previously (Oka et al. 1999). Informed consent was obtained by explaining the details of this study prior to collection of peripheral blood.

Detection of TXNRD31T1 sequence polymorphisms. In order to carry out direct sequencing analysis of *TXNRD3NT1*, 4 pairs of oligonucleotide PCR primers for *TXNRD3NT1* that allow amplification of 4 exons, respectively, were designed from the intron sequences on either side of the exon and promoter regions (Table 2). The reaction mixture (20 μl) contained 2 μl of dNTP (2.5 mM each), genomic DNA (5 μL; 2 ng/μl), 2 μl of 10 × buffer (100 mM Tris-HCl, pH 8.3, 500 mM KC1, 15 mM $MgCl_2$), 20 pmol of forward and reverse primers, and 0.5 U Ampli *Taq* Gold polymerase (Applied Biosystems Japan Co.). PCR amplification was performed in an automated thermal cycler, Gene Amp PCR system 9600 (Applied Biosystems Japan Co.). PCR reaction conditions consisted of an initial denaturation for 10 min at 96 C, annealing for 1 min at various temperatures depending on primers used (Table 2), and an extension for 1 min at 72 C followed by 30 amplification cycles of 45 sec at 96 C, 45 sec at the temperatures for annealing, and 1 min at 72 C with a final

extension of 7 min at 72 C. Each PCR product was purified by exonuclease I and then sequenced using an ABI 3700 automated sequencer (Applied Biosystems Japan Co.).

Analysis of repeat polymorphisms within a microsatellite near the TXNRD3NT1 gene. The PCR primers used for the amplification of the TXNRD3NT1 microsatellite were TXNRD3NT 1_MSM01F: GGAGATGAGAATCTAGGTAGAAGAGCAG, and TXNRD3NT1_MSM01R: HEX-CCATCAGTAGTGCTGGAAGCTAATTC. PCR amplification of the microsatellite and detection with GenScan were carried out as previously described (Matsuzaka et al. 2000).

Statistical analysis. Allele and genotype frequencies were determined by direct counting. The significance of the distribution of alleles between the patients and controls was tested by the Fisher's exact probability test (p-value test). A level of $p < 0.05$ was accepted as statistically significant.

Results

Isolation of* TXNRD3NT1 *cDNAs. cDNA clones corresponding in sequence to the EST, accession No. AA430236, were isolated and, by aligning the sequences of a number of overlapping partial cDNA clones, the sequence of a full-length cDNA of 1133 bp was determined. This full-length cDNA sequence contained the 251-bp 3′ untranslated region (UTR), two polyadenylation signal variant sequences—AATAAT at nucleotide position 1077 and AATGAA at nucleotide position 1098—and a polyadenylation site in the 51-bp and 31-bp downstream regions, respectively. According to the Open reading frame (ORF) assignment, the putative full-length cDNA sequence encodes a protein of 133 amino acids that has a tubulin-β mRNA autoregulation signal at amino acid positions 1–4 (Fig. 1).

A BLASTN search of GenBank with the full-length cDNA (1133 bp) revealed that only 382 bp of the TXNRD3NT1 full-length sequence had a 100% identity with the thioredoxin reductase 3 (TXNRD3) mRNA sequences (accession Nos. BC050032, AF133519, AF171055) that were 2165 bp or more. The full-length TXNRD3NT1 sequence (1133 bp), however, also had 100% identity hit with the BAG RP11-390G14 (accession No. AC024558) sequence from Chromosome 3.

The entire TXNRD3NT1 amino acid sequence was used to BLASTP search protein databases for a homologous sequence. The most significant hit was with an *E. coli* ATP binding protein (accession

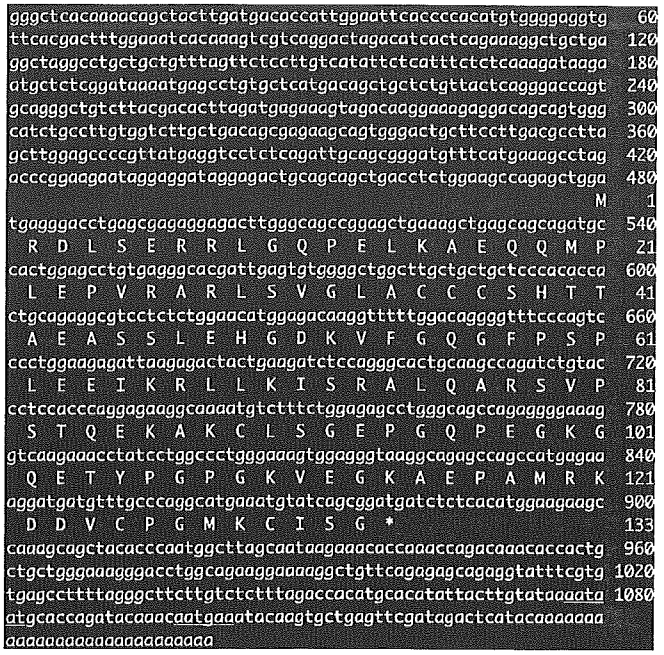

Fig. 1. Nucleotide and deduced amino acid sequences of *TXNRD3NT1*. Nucleotide and amino acid sequences are numbered to the right of the sequence. The putative polyadenylation signals are underlined. The sequence data have been deposited in the DDBJ/EMBL/GenBank Data Libraries under accession No. AB113648.

number Z2278). The amino acid comparison of the *E. coli* ATP binding protein with that encoded by the human TXNRD3NT1 is shown in Fig. 2. Although there was an overall low sequence similarity between the two proteins, 50% sequence similarity was found for the first 26 residues, including the amino acid group LGxxxLKxxExQMPLE.

***Genome organization of* TXNRDC3NT1.** The TXNRD3NT1 cDNA sequence was found to overlap with the *TXNRD3* (aliases TR2, TRXR3) gene on the genome sequence of the BAG clone accession No. AC024558. The genomic sequence of this BAG clone was initially mapped to the long arm of

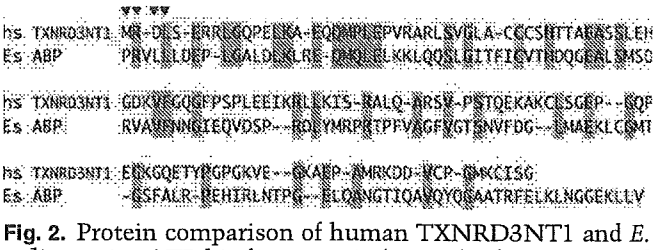

Fig. 2. Protein comparison of human TXNRD3NT1 and *E. coli* putative ATP-binding protein (Z2278). The amino acid sequences are numbered to the right. Dashes indicate gaps introduced to maximize sequence similarity. Identical amino acids are highlighted by shading. Inverted triangles show the tubulin-β autoregulation signal.

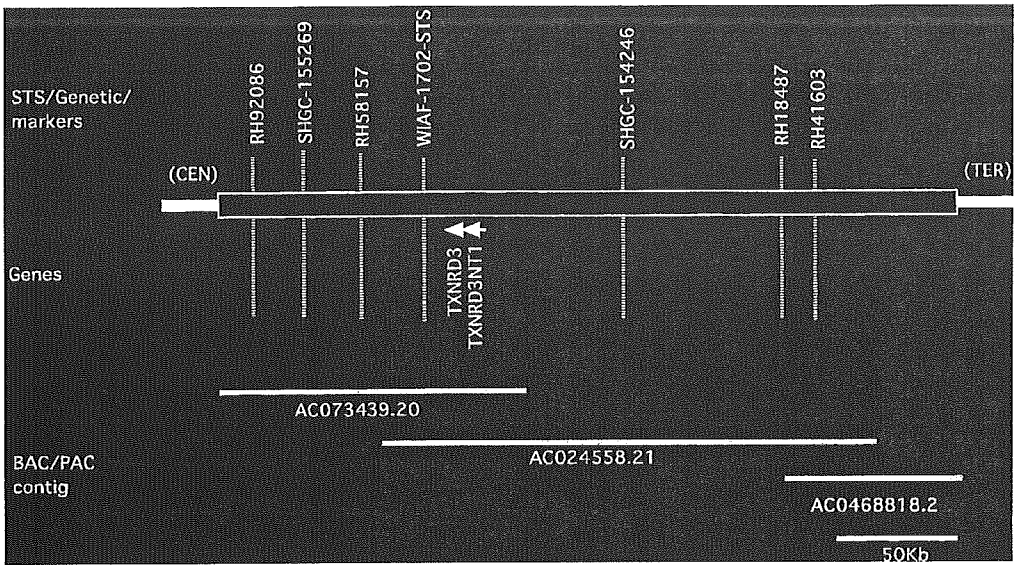

Fig. 3. Physical and transcript maps of a 300-kb region including the *TXNRD3NT1* gene on 3q21. Locations of novel gene, *TXNRD3NT1* and known gene *TXNRD3* are shown as arrows which represent the transcription orientation. A BAC/PAC contig available from the public database (GenBank) is shown as solid lines. Positions of STS/genetic markers are drawn as vertical dotted lines.

Chromosome 3, at position 3q21.1 (Fig. 3). Comparison of the genomic and cDNA sequences revealed that the gene spans approximately 37 kb of the *TXNRD3NT1* gene and is organized into four exons. All the intron/exon boundaries conformed to the GT–AG rule (Table 3). Exon 3 contains the putative translation initiation codon ATG, and exon 4 contains the translation termination codon TGA. The first four amino acids of the translated cDNA sequence contain the tubulin-β autoregulation signal, MRDL, which is one of the possible alternatives of the consensus pattern MR(DE)(IL) provided at the PROSITE database (http://us.expasy.org/prosite/). A part of exon 1 and exon 2 of *TXNRD3NT1* overlaps with exon 15 and exon 16 of the *TXNRD3* gene sequence. However, the *TXNRD3NT1* cDNA has distinctly different open reading frames than those of the *TXNRD3* sequence. Therefore, the cDNA sequence that we have cloned and characterized was named *TXNRD3NT1* (thioredoxin reductase 3 new transcript 1).

Tissue expression profile and alternative splicing variants of the TXNRD3NT1 gene. No transcripts were detected in any of the tissues examined by the Northern blot hybridization technique, including the spleen, thymus, prostate, uterus, small intestine, colon, and peripheral blood leukocytes (data not shown). However, RT-PCR sequences amplified from a part of the exon 2 to exon 4 region of the gene generated the expected product size of 664 bp in pancreas, esophagus, bone marrow, and keratinocytes (Fig. 4A). In contrast, a 177-bp amplification product that was generated from an alternative splicing product was observed in the other 27 tissues including skin, skin fibroblasts, and fetal skin (Fig. 4B). The nucleotide sequence of each PCR product was determined. As a result, the 177-bp amplification product lacks the exon 3 sequence. This alternative splicing results in a premature termination codon and, consequently, the truncated transcript size (Fig. 4B). No RT-PCR product was amplified from skeletal muscle or the negative control.

Table 3. Exon–intron organization of the human *TXNRD3NT1* gene

Exon No./size (bp)				*5′* Splice donor	*Intron No./size (bp)*	*3′* Splice acceptor			
ex. 1 (57)	TGT	GGG	GAG	gtaggaaaca...	int. 1 (525)	...tcttgaacag	GTG	TTC	ACG
ex. 2 (323)	CGT	TAT	GAG	gtgagccaag...	int. 2 (35009)	...tctaccgaag	GTC	CTC	TCA
ex. 3 (487)	ATG	AAA	T	gtaagtgatg...	int. 3 (110)	...cccacaacag	GT	ATC	AGC
	Met	Lys	Cys					Ile	Ser
ex. 4 (266)	GAC	TCA	TAC						

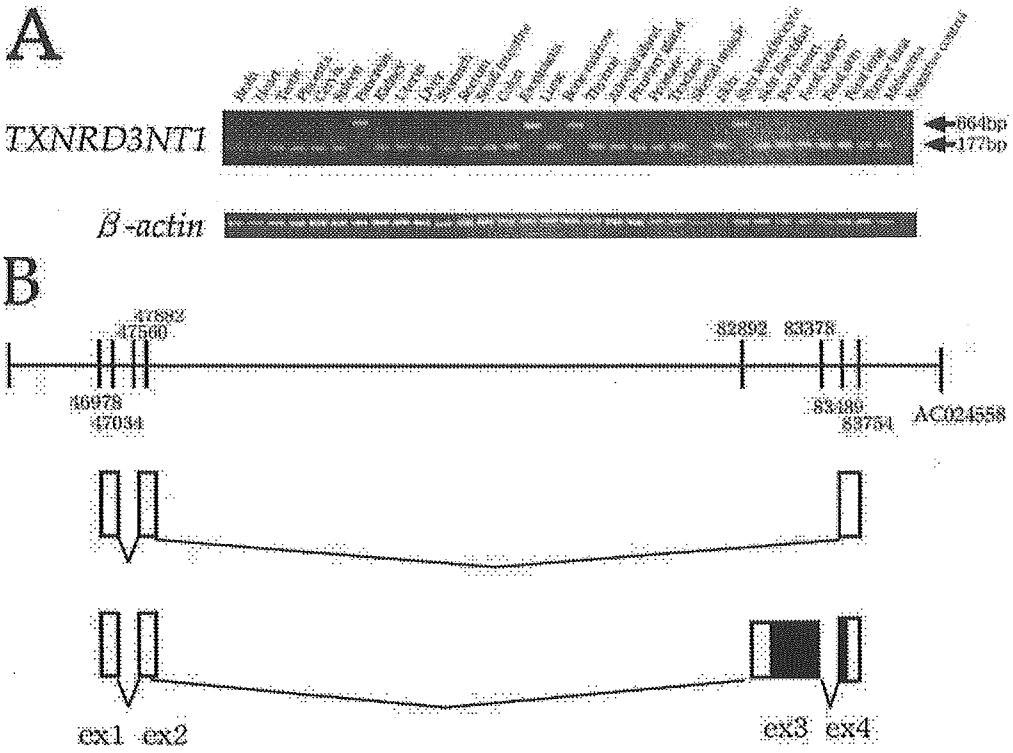

Fig. 4. Tissue expression of the *TXNRD3NT1* gene as determined by RT-PCR (**A**) and the schematic representation of alternative splicing variants of the *TXNRD3NT1* gene (**B**). (**A**, top) RT-PCR using 10 ng of poly(A)$^+$ RNA prepared from the indicated tissues was performed with the primer sets specific for the *TXNRD3NT1* gene. RT-PCR products were separated on 2.0% agarose gels and stained with ethidium bromide. The bottom panel shows the RT-PCR results using a pair of primers specific for the β-*actin* gene as a positive control. (**B**). Each exon of the *TXNRD3NT1* gene is aligned with the genomic sequence of a BAG clone, AC024558. Numbering follows the genomic sequence (AC024558). The putative coding regions are in closed boxes and the putative noncoding regions are in open boxes.

Polymorphisms within TXNRD3NT1. The chromosomal location of *TXNRD3NT1* at position 3q21 appears to correspond closely to the psoriasis candidate regions previously reported (Enlund et al. 1999; Hewett et al. 2002). We determined the genomic nucleotide sequence variations in the *TXNRD3NT1* gene that may alter the concentration or activity of these proteins and ultimately contribute to the development of psoriasis. In order to detect genetic polymorphisms in the genomic sequences of the entire exons and 5′-promoter region of *TXNRD3NT1*, PCR products were separately amplified from 8 patients with psoriasis and 8 healthy controls, and their nucleotide sequences were determined by direct sequencing. Two silent single nucleotide polymorphisms (SNPs) (A/G) were identified within the 5′-untranslated region (UTR) of exon 1 at the nucleotide positions 23 and 26 (Table 4). One polymorphic site (GGC/DEL) was found within intron 4 at nucleotide position 36889. The two SNP sites (T/C) in the 5′-promoter region were identified at nucleotide positions -203 and -196.

Table 4. Nucleotide variations within the *TXNRD3NT1* gene

		Polymorphisms	
Location	*Relative distance*[a] (kb)	*Common allele*	*Variant allele*
Promoter	−203	T	C
Promoter	−196	T	C
Exon 1	23	A	G
Exon 1	26	A	G
Intron 4	36889	GGC	DEL

[a]Relative distances are from the transcriptional start site of *TXNRD3NT1* gene.

Table 5. Association study using a microsatellite marker

Location	No. of alleles	Allele name	Allele frequency[b] Patients (2n = 170)	Allele frequency[b] Controls (2n = 220)	OR	χ^2	p-value[a]
Centromeric (11.4 kb)/TXNRD3NT1_exon3	5	271	0.97	0.95	1.98	1.33	0.248

[a]Determined by Fisher's exact test.
[b]Allele with significantly higher frequency in patients than in controls is listed.

Association of TXNRD3NT1 polymorphisms with psoriasis. A total of 85 Japanese patients with psoriasis and 110 control patients were enrolled for the association analysis between psoriasis and the polymorphism of the single microsatellite marker, TXNRD3NTl-71460_MS, of the *TXNRD3NT1* gene. As shown in Table 5, this polymorphic microsatellite locus was not associated significantly with psoriasis (allele 271, $p = 0.248$).

Discussion

We have identified, isolated, and characterized a new gene, *TXNRD3NT1*, whose transcripts, corresponding to the EST AA430236, were found to be significantly downregulated in affected psoriatic tissue (unpublished data). The full-length cDNAs of *TXNRD3NT1* were isolated and characterized by combining 5′- and 3′- RACE with screening a keratinocyte cDNA library, designing appropriate PCR primers, cloning amplified products, sequencing, and sequence analysis with database searches. The full-length *TXNRD3NT1* cDNA was found to have 1133 nucleotides with a 251-bp 3′ -UTR and 2 poly(A) signal variants and 2 poly(A) sites and to code for 133 amino acids. Its nucleotide sequence was found to spread across four exons of the *TXNRD3* gene that has been localized on Chromosome 3q21 according to the ENSEMBL cytogenetic band or 3p13–q13.33 according to the LOCUSLINK cytogenetic band. Exons 1 and 2 of the *TXNRD3NT1* gene overlapped with exons 15 and 16 of the *TXNRD3* gene which has 17 exons. The translation initiation codon ATG was found in exon 3 of the *TXNRD3NT1* gene. RT-PCR showed that the full-length variant of the *TXNRD3NT1* gene was expressed in only four tissues (pancreas, esophagus, bone marrow, and keratinocytes) of the 30 tissues tested. In most other tissues, an aberrant and truncated form of the transcript (i.e., missing exon 3 and part of exon 4) was detected.

Since the *TXNRD3NT1* gene overlaps with *thioredoxin reductase 3*(*TXNRD3*), it may also have some role in thioredoxin regulation. Thioredoxin (OMIM 187700) is a major regulator of the cell redox state with many cellular functions, including cell growth, activation, differentiation, and apoptosis (Gadaska et al. 1995; Hirota et al. 2002). Abnormal regulation of thioredoxin could have a critical influence on skin pathogenesis and psoriasis. TXNRD3 is a sensor of reactive oxygen species that maintains theoredoxin in a reduced state (Sun et al. 1999). One or another of the TXNRD3NT1 variants may regulate the TRX system by either binding directly to the TRX protein or by competing with the TXNRD3 at the transcription and translation levels. These possibilities warrant future studies in normal and disease tissues.

Although it is not known if TXNRD3NT1 also regulates thioredoxin, another thioredoxin-interacting protein, TXNIP, that binds to thioredoxin and inhibits its reducing activity (Nishiyama et al. 1999; Junn et al. 2000), was also significantly downregulated in the psoriatic affected tissue that we (unpublished finding) and others have observed (Champliaud et al. 2003). Because TXNIP inhibits thioredoxin's reducing activity (Junn et al. 2000), the downregulation of TXNIP, and/or TXNRD3NT1, may lead to the misregulation of thioredoxin as well as to aberrant cellular growth, differentiation, activation, and apoptosis in psoriasis. In addition, TXNRD3NT1 may regulate its own or heterologous mRNA stability as the first four amino acids of the protein carry the tubulin-β mRNA autoregulation signal for RNA degradation coupled to ribosome attachment and translation (Yen et al. 1988). Interestingly, the tubulin-β mRNA was significantly upregulated while the *TXNRD3NT1* mRNA was significantly downregulated in the affected psoriatic tissue (unpublished data).

Genomewide linkage analyses and nonparametric linkage analyses of a large number of affected sib pairs have identified many psoriasis-susceptibility loci (reviewed by Henseler 1998) including chromosome position 3q21 (PSORS5; OMIM 604316) (Enlund et al. 1999; Hewett et al. 2002). We have mapped the newly identified gene, *TXNRD3NT1*, also to chromosome position 3q21. Therefore, based on its genomic location within the psoriasis candidate loci and downregulation in affected psoriatic tissue,

TXNRD3NT1 may be a genuine psoriatic gene. In order to evaluate the association between *TXNRD3NT1* and psoriasis, we performed an association study between a single microsatellite marker in *TXNRD3NT1* and psoriasis. The microsatellite marker revealed no statistically significant difference in allele or genotype frequency distributions between psoriasis patients and healthy controls. However, we cannot exclude the possibility that other sequence variants may still exist within the 5′ regulatory noncoding region and the coding regions of *TXNRD3NT1* gene that we have not identified or studied so far. In this regard, we detected some new SNP markers around the *TXNRD3NT1* gene that warrant future analyses to determine whether these mutations might be associated with psoriasis or other diseases.

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Telomere length of normal leukocytes is affected by a functional polymorphism of hTERT

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Abstract

Transcriptional regulation of human telomerase reverse transcriptase (hTERT), a catalytic subunit of telomerase, is essential for telomerase activity associated with telomere length. In this study, we investigated the effects of a $^{-1327}$T/C polymorphism within the hTERT promoter region on the hTERT promoter activity and leukocyte telomere length in normal individuals. The promoter activity in the $^{-1327}$T-sequence was significantly higher than that in the $^{-1327}$C-sequence ($p = 0.0004$). For leukocyte telomere length, the $^{-1327}$T-allele carriers had significantly longer than the $^{-1327}$T-allele non-carriers ($p = 0.0007$). Also, there was no age-related shortening in leukocyte telomere length in the $^{-1327}$T/T ($p = 0.6633$) and $^{-1327}$T/C subjects ($p = 0.1691$), whereas there was clear age-related telomere shortening in the $^{-1327}$C/C subjects ($p = 0.0117$). These findings suggest that the functional $^{-1327}$T/C polymorphism of hTERT is associated with leukocyte telomere length in normal individuals.

Keywords: Human telomerase reverse transcriptase; Polymorphism; Telomere length

Telomerase synthesizes telomeric repeats for addition to the end of linear chromosomes, although replication of the telomeric end is sometimes incomplete [1,2]. Thus, telomere shortening occurs after repeated cell divisions and has a key role in cellular senescence, differentiation, immortalization, and transformation [3]. A recent study showed that telomere shortening is assumed to contribute to mortality in older subjects or age-related diseases [4].

Telomere length is mainly regulated by telomerase activity associated with transcriptional activity of human telomerase reverse transcriptase (hTERT), a subunit of telomerase [5–7]. The hTERT promoter region located with the 1375 bp upstream of the transcription-starting site is rich in transcription factor binding sites [8,9]. Although the regulation of hTERT transcription has been widely studied, little is known about the genetic variations in relation to hTERT transcriptional activity.

In this study, the hTERT promoter region was sequenced for screening of genetic polymorphisms in a healthy population. A T to C transition 1327 bp upstream of the transcription-starting site of hTERT ($^{-1327}$T/C) was frequently observed (nucleotide numbering according to Horikawa et al.) [9]. Further, we investigated the association between the $^{-1327}$T/C polymorphism and (a) hTERT transcriptional activity in normal human umbilical vein endothelial cells (HUVECs), and (b) telomere length and telomerase activity in peripheral leukocytes in normal individuals.

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