British Journal of Dermatology 1998; 139: 974–977.

Evidence for loss of heterozygosity in human psoriatic lesions

G.ZACHOS,*† E.KOUMANTAKI,† A.VARELTZIDIS‡ AND D.A.SPANDIDOS*†

**Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Ave, Athens 11635, Greece*
†Division of Virology, Medical School, University of Crete, Heraklion, 71409, Greece
‡Medical School, University of Athens, Department of Dermatology, 'A. Sygros' Hospital, Athens, Greece

Accepted for publication 30 April 1998

Summary Psoriasis, a disease of human skin, is characterized by abnormal differentiation and hyperproliferation of keratinocytes; it has a genetic background. Using 11 highly polymorphic microsatellite markers on eight chromosome arms, we performed an allelotype analysis in 14 psoriatic plaques, in order to reveal any chromosome deletions involved in the development of the disease. We detected loss of heterozygosity (LOH) on at least one microsatellite marker in nine of 14 (64%) cases. We also observed particular genetic loci altered with LOH, on chromosomes 3p, 7p/q and 8p. Our results suggest that LOH is an important phenomenon in the development of psoriatic plaques, providing evidence for deletion of regulatory genes.

Psoriasis affects approximately 2–3% of the European population and presents with red scaly patches on the skin. The major pathogenic features of psoriasis include abnormalities in differentiation, hyperproliferation of keratinocytes and infiltration of inflammatory components into the skin.[1] Epidemiological data and familial association suggest a hereditary base for transmission of psoriasis; however, it does not appear to follow simple autosomal dominant or recessive patterns. This may be due to multifactorial inheritance or to inheritance only of a predisposition to disease that requires an environmental stimulus for expression, such as stress, drugs, viral infection or dietary factors.[2–5]

The molecular genetic basis of the disorder was investigated. Evidence that a locus for familial psoriasis susceptibility maps to the distal end of chromosome 17q was reported; however, it proved to be genetically heterogeneous and is possibly involved in familial psoriasis under a polygenic model.[6,7] Other potent genes for familial psoriasis were mapped to chromosome 6p21, within the major histocompatibility complex (MHC) and close to the class 1 human leucocyte associated antigen (HLA) loci, and to chromosome 4q.[8,9] Altered expression of proto-oncogenes, which are regulators of the cell cycle, was proposed as a mechanism of hyperproliferation and abnormal differentiation of keratinocytes. However, the proto-oncogenes tested (c-*fos*, c-*jun*, c-H-*ras*, c-*erb*B2, c-*myc*, c-*raf*) were not implicated in the pathogenesis of psoriatic epidermal hyperplasia, either with quantitative, or qualitative changes.[10–12] A role in the regulation of epidermal proliferation for genes on chromosome 1q21, which are co-ordinately overexpressed in psoriatic lesions, was proposed; aberrantly regulated genes in diseased skin included mitochondrial genes, junction proteins and protease inhibitors.[13,14]

The aim of our investigation was to perform an allelotype analysis in 14 psoriatic plaques, in order to reveal any chromosome deletions involved in the development of the disease. This analysis could provide information about the inactivation of regulatory genes. Such an inactivation might result in hyperproliferation and abnormal differentiation of psoriatic cells, a model analogous to the inactivation of tumour suppressor genes in cancer development, thus also extended to non-cancerous lesions.[15,16] We performed loss of heterozygosity (LOH) analysis of eight chromosome arms using 11 highly polymorphic microsatellite markers. Our results suggest that LOH is a detectable phenomenon in psoriatic plaques and contributes to the molecular background of the disease.

Materials and methods

Biological material and DNA extraction

Fourteen specimens (keratinocytes from punch biopsies of patients with psoriasis) were obtained from the Department of Dermatology, 'A.Sygros' Hospital, Athens. Biopsies confirmed the histopathological phenotype of

Correspondence: Professor D.A.Spandidos.
Present address: G.Zachos, Institute of Virology, University of Glasgow, Church St, Glasgow G11 5JR, U.K.

Table 1. Loss of Heterozygosity analysis in human psoriatic lesions

Patient no.	D2S138	D2S147	D3S1478	D3S1298	FGA	D7S478	D7S519	ANK1	D8S7	D9S290	D21S1245
1	−	−	+	−	−	−	−	−	−	−	−
2	−	−	−	+	−	+	+	+	+	−	−
3	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	+	−	+	+	+	+	−	−
5	−	−	−	−	−	−	−	−	−	−	−
6	−	−	+	+	−	+	−	+	+	−	−
7	−	−	+	+	−	−	−	−	+	−	−
8	−	−	−	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−	−	−
10	−	−	−	+	−	−	−	−	+	−	−
11	−	−	−	−	−	−	−	−	−	−	−
12	−	−	−	−	−	−	−	−	+	−	−
13	−	−	+	−	−	−	−	−	−	−	−
14	−	−	+	−	−	−	+	−	+	−	−

psoriasis. Ten patients were females and four males. Twelve patients (numbers 1–12) had a family history of the disease and/or an onset below the age of 40. Patients 13 and 14 had a late onset of the disease and no family history. Matched blood samples were used as negative controls. DNA was extracted from psoriasis plaque specimens and blood samples using standard methods.[15]

Microsatellite analysis

Eleven microsatellite markers were used (Table 1).[17] Markers D2S138 and D2S147 are located on chromosomes 2q21–q33 and 2p14–q13, respectively. Markers D3S1478 and D3S1298 are located on chromosomes 3p21.3–p21.2 and 3p24.2–p22, within the hMlh1 gene. FGA lies on chromosome 4q28. Markers D7S478 and D7S519 are located on chromosome 7p15–q22, within the Pms2 gene. ANK1 and D8S7 lie on chromosome regions 8p21.1–p11.2 and 8p23, respectively. Finally, markers D9S290 and D21S1245 are located on chromosomes 9q32–q34.1 and 21q22.2–qter, respectively.

PCR reactions were performed in a 50 μL reaction volume, containing 100 ng of genomic DNA, 200 μmol/L dNTPs, 10 pmol of each forward and reverse primer, 10 mmol/L Tris–HCl, pH 8·8, 1·5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0·1% Triton X-100 and 2 U Dynazyme™ II DNA polymerase (Finnzymes OY, Espoo, Finland). The reactions were denatured at 98 °C for 3 min, prior to the addition of the polymerase, and the DNA was subsequently amplified for 30 cycles, at 94 °C, 54–58 °C,

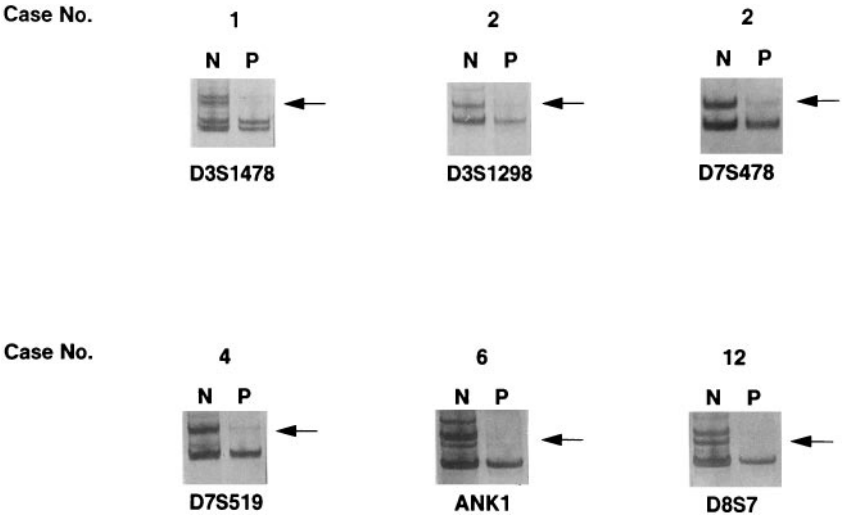

Figure 1. Examples of specimens exhibiting loss of heterozygosity (LOH). Arrows indicate the position of the deleted allele. N, normal; P, psoriatic plaque.

72 °C each step. Ten microlitres of the PCR product were electrophoresed in a 10% polyacrylamide gel and stained with silver. LOH was scored when a more than 70% reduction in the intensity of one allele in the heterozygous specimens was observed in DNA from psoriasis plaques, compared with the corresponding blood samples. The remaining faint bands of the lost allele could be due to contamination with non-lesional skin. The analysis in the LOH positive cases was repeated at least twice and the results were highly reproducible.

Results

Nine of 14 (64%) cases tested exhibited LOH on at least one microsatellite marker (Table 1, Fig. 1). Allelic imbalance was observed for the locus 3p24–p21.2 (markers D3S1478, D3S1298) in eight of 14 (57%) cases, for the locus 7p15–q22 (markers D7S478, D7S519) in four of 14 (28%) cases, and for the region 8p21.1–p11.2 and 8p23, encompassing almost the entire 8p chromosome arm (markers ANK1, D8S7), in seven of 14 (50%) cases. Retention of heterozygosity was observed for the remaining chromosome loci tested, in all cases.

Discussion

Little is still known about the genetic background of the disorder. We therefore tested for LOH genetic loci dispersed in multiple chromosome regions. Furthermore, possible candidates like the mismatch repair genes hMlh1 and Pms2, the VHL onco-suppressor and the 4q region, previously linked to psoriasis, were included in our study. The incidence of LOH was investigated on eight chromosome arms (2p, 2q, 3p, 4q, 7p/q, 8p, 9q, 21q) using 11 microsatellite markers. Loss of heterozygosity of particular chromosome regions was reported in the development of human neoplasia, indicating the presence of onco-suppressor genes.[18] Our experimental results suggest that LOH represents an important phenomenon in the development of human psoriatic lesions as well, as the majority of the patients (nine of 14, 64%) exhibited LOH on at least one microsatellite marker. Moreover, allelic imbalance was observed for regions on the chromosome arms 3p (eight of 14, 57%), 7p/q (four of 14, 28%) and 8p (seven of 14, 50%). This suggests that genes important for suppressing cell cycle alterations are located in these particular chromosome regions; the mechanism may be similar to that seen in neoplastic disorders.

The region in chromosome 3p tested in our study, is deleted in many solid tumours and is considered as the location of a lung cancer suppressor gene[19] and of the VHL onco-suppressor, which is implicated in renal cell carcinomas and haemangioblastomas.[20] In addition, allele loss of the hMlh1 mismatch repair gene located in this region, is implicated in human cancers exhibiting high frequency of somatic mutations.[21] Moreover, allelic loss on chromosome 7q is observed in human tumours.[22] The Pms2 mismatch repair gene is located in this region and is a likely candidate for the allelic imbalance that we detected.[23] Furthermore, a frequently deleted region on chromosome 8p, overlapping the locus tested in our study, was established in studies of colorectal and bladder tumours.[24]

In conclusion, we identified chromosome loci where genes regulating cell differentiation, growth and proliferation may be located, and suggest that LOH contributes to the development of human psoriatic lesions. To our knowledge, this is the first report of LOH in a chronic inflammatory disease with a genetic background. Extension of our observations in more chromosome arms, as well as a detailed mapping of the deleted regions using additional sets of microsatellite markers, may be useful in understanding the molecular basis of the disorder.

References

1 Ortonne J-P. Aetiology and pathogenesis of psoriasis. *Br J Dermatol* 1996; 135 (Suppl. 49): 1–5.
2 Harvima RJ, Viinamaki H, Harvima IT *et al*. Association of psychic stress with clinical severity and symptoms of psoriatic patients. *Acta Derm Venereol (Stockh)* 1996; 76: 467–71.
3 Gold MH, Holy AK, Roenigk HH. Beta-blocking drugs and psoriasis. *J Am Acad Dermatol* 1988; 19: 837–41.
4 Coopman SA, Johnson TM, Platt R *et al*. Cutaneous disease and drug reactions in HIV infection. *N Engl J Med* 1993; 328: 1670–4.
5 Naldi L. Psoriasis. *Dermatol Clin* 1993; 13: 635–47.
6 Tomfohrde J, Silverman A, Barnes R *et al*. Gene to familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. *Science* 1994; 264: 1141–5.
7 Nair RP, Guo SW, Jenisch S *et al*. Scanning chromosome 17 for psoriasis susceptibility: lack of evidence for a distal 17q locus. *Hum Hered* 1995; 45: 219–30.
8 Trembath RC, Clough RL, Rosbotham JL *et al*. Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. *Hum Mol Genet* 1997; 6: 813–20.
9 Matthews D, Fry L, Powles A *et al*. Evidence that a locus for familial psoriasis maps to chromosome 4q. *Nat Genet* 1996; 14: 231–3.
10 Basset-Seguin N, Escot C, Moles JP *et al*. c-*fos* and c-*jun* proto-oncogene expression is decreased in psoriasis: an in situ quantitative analysis. *J Invest Dermatol* 1991; 97: 672–8.
11 Elder JT, Klein SB, Tavakkol A *et al*. Growth factor and proto-oncogene expression in psoriasis. *J Invest Dermatol* 1990; 95: 7–9S.

12 Takahashi H, Iizuka H, Katagiri M. No evidence for the mutation of *ras* gene in psoriatic epidermis. *Arch Dermatol Res* 1990; 282: 8–11.
13 Hardas BD, Zhao X, Zhang J *et al*. Assignment of psoriasin to human chromosomal band 1q21: coordinate overexpression of clustered genes in psoriasis. *J Invest Dermatol* 1996; 106: 753–8.
14 Rivas MV, Jarvis ED, Morisaki S *et al*. Identification of aberrantly regulated genes in diseased skin using the cDNA differential display technique. *J Invest Dermatol* 1997; 108: 188–94.
15 Hatzistamou J, Kiaris H, Ergazaki M, Spandidos DA. Loss of heterozygosity and microsatellite instability in human atherosclerotic plaques. *Biochem Biophys Res Commun* 1996; 225: 186–90.
16 Spandidos DA, Sourvinos G, Kiaris H, Tsamparlakis J. Microsatellite instability and loss of heterozygosity in human pterygia. *Br J Ophthalmol* 1997; 81: 493–6.
17 Murray JC, Buettow KH, Weber JL *et al*. A comprehensive human linkage map with centimorgan density. Cooperative Human Linkage Center (CHLC). *Science* 1994; 265: 2049–54.
18 Lasco D, Cavenee W, Nordenskjold M. Loss of constitutional heterozygosity in human cancer. *Annu Rev Genet* 1991; 25: 281–314.
19 Wei MH, Latif F, Bader S *et al*. Construction of a 600-kilobase cosmid clone and generation of a transcriptional map surrounding the lung cancer tumor suppressor gene (TSG) locus on chromosome 3p21.3: progress toward the isolation of a lung cancer TSG. *Cancer Res* 1996; 56: 1487–92.
20 Prowse AH, Webster AR, Richards FM *et al*. Somatic inactivation of the VHL gene in Von Hippel-Lindau disease tumors. *Am J Hum Genet* 1997; 60: 765–71.
21 Thibodeau SN, French AJ, Roche PC *et al*. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res* 1996; 56: 4836–40.
22 Kerr J, Leary JA, Hurst T *et al*. Allelic loss on chromosome 7q in ovarian adenocarcinomas: two critical regions and a rearrangement of the PLANH1 locus. *Oncogene* 1996; 13: 1815–18.
23 Narayanan L, Fritzell JA, Baker SM *et al*. Elevated levels of mutation in multiple tissues of mice deficient in the DNA mismatch repair gene Pms2. *Proc Natl Acad Sci USA* 1997; 94: 3122–7.
24 Macoska JA, Trybus TM, Benson PD *et al*. Evidence for three tumor suppressor gene loci on chromosome 8p in human prostate cancer. *Cancer Res* 1995; 55: 5390–5.