A Susceptibility Locus for Epidermodysplasia Verruciformis, an Abnormal Predisposition to Infection with the Oncogenic Human Papillomavirus Type 5, Maps to Chromosome 17qter in a Region Containing a Psoriasis Locus

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Epidermodysplasia verruciformis (EV) is a rare genodermatosis characterized by an abnormal susceptibility to infection with a specific group of related human papillomavirus (HPV) genotypes, including the oncogenic HPV5 associated with the skin carcinomas developing in about half of EV patients. EV is usually considered as an autosomal recessive condition. Taking EV as a model to identify a locus underlying the susceptibility to HPV infections, we performed a genome-wide search for linkage with 255 microsatellite genetic markers in three consanguineous EV families comprising six patients, using the homozygosity mapping approach. Homozygosity restricted to affected individuals was observed for a marker of chromosome 17q (D17S784) in two families and a marker about 17 centiMorgan (cM) distal (D17S1807) in the third family. Ten additional microsatellite markers spanning 29 cM in this region were analyzed. Two-point lod score values greater than 3 were obtained for four markers and multipoint linkage analysis yielded a maximum lod score of 10.17 between markers D17S939 and D17S802. Recombination events observed in two families allowed a candidate region for the EV susceptibility locus to be mapped to the 1 cM region defined by these two markers. The EV locus (named EV1) is included in the 17qter region recently found to contain a dominant locus for the susceptibility to familial psoriasis. It has been shown that patients suffering from psoriasis are likely to constitute the reservoir of HPV5. It is thus tempting to speculate that distinct defects affecting the same gene may be involved in the two skin conditions. ***Key words: consanguineous families/genodermatosis/genome scan/homozygosity mapping/microsatellite markers. J Invest Dermatol 112:259–263, 1999***

Epidermodysplasia verruciformis (EV; MIM#226400) is a rare, life-long skin disease, with a world-wide distribution (Jablonska *et al*, 1972; McKusick, 1990). EV results from a genetically determined, abnormal susceptibility to a specific group of related human papillomavirus (HPV) genotypes and to the oncogenic potential of HPV5, one of these genotypes (Orth *et al*, 1978, 1979; Orth, 1987). Most EV-associated HPV genotypes cause widespread inapparent infections in the general population (Boxman *et al*, 1997; Pfister and ter Schegget, 1997; Astori *et al*, 1998). In EV patients, infection leads to the development of disseminated flat wart-like lesions or pityriasis versicolor-like macules, usually starting in early childhood. Cutaneous carcinomas *in situ* and invasive squamous cell carcinomas develop in about half of the patients, generally during the third or fourth decade. Cancers are usually associated with HPV5 and arise mainly on sun-exposed areas (Jablonska *et al*, 1972; Rueda and Rodriguez, 1976; Orth *et al*, 1978). The patients suffering from EV are unable to reject their skin lesions and this may involve a specific defect of cell-mediated immune responses directed against EV HPV-harboring keratinocytes (Cooper *et al*, 1990; Majewski *et al*, 1990, 1997).

About 10% of EV families have more than one sibling affected and about 10% of EV patients have consanguineous parents (Lutzner, 1978). The proportion of EV siblings in families approaches 25% and the sex ratio for EV is close to one (Lutzner, 1978). These observations support the conclusion that EV is an autosomal recessive disease (Rajagopalan *et al*, 1972; Lutzner, 1978), although, in single EV families, a X-linked recessive (Androphy *et al*, 1985) or an autosomal dominant (McKusick, 1990) transmission have been postulated. This points to a genetic heterogeneity of the disease. The genes involved in EV and their chromosomal localization are unknown as yet.

EV is thus a multifactorial disease involving genetic, immunologic, and environmental factors, in addition to specific HPV genotypes. It represents a model to unravel the poorly understood genetic factors involved in the control of widespread infections with oncogenic HPV genotypes, in particular HPV16 and HPV18 associated with invasive cervical carcinomas, the second cause of cancer death in women, world-wide (zur Hausen, 1996; Favre *et al*, 1997). To identify a locus underlying the susceptibility to HPV infections, we performed a genome-wide search for linkage in three consanguineous EV families. By the use of the homozygosity

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Abbreviation: EV, epidermodysplasia verruciformis.

mapping approach (Lander and Botstein, 1987), we have mapped a disease locus (EV1) to a 1 centiMorgan (cM) region between D17S939 and D17S802 markers of chromosome 17qter. The EV1 locus is located in a region previously shown to contain a locus for the susceptibility to familial psoriasis (Tomfohrde *et al*, 1994; Nair *et al*, 1997).

Table I. Characteristics of EV patients[a]

Family, patients[a]	Origin	Sex, age (y)	Age at onset (y)	Age at first cancer (y)	HPV genotypes
A1,IV:1	Algeria	M, 37	6	16	5, 14, 20
A1,IV:4		M, 30	6	18	14, 19, 28
A2,IV:1	Algeria	F, 37	10	–	5, 14, 20
C1,IV:1	Colombia	M, 38	6	–	5, 17
C1,IV:3		F, 29	7	–	5, 17, 20
C1,IV:5		F, 28	7	–	5, 17

[a]As presented in **Fig 1.**

Table II. Two-point lod-scores between EV and 17qter markers

	Recombination fraction (θ)								
Marker	0.00	0.01	0.05	0.1	0.2	0.3	0.4	θ_{max}	Z_{max}
D17S1352	−3.35	−0.42	0.63	0.91	0.87	0.59	0.27	0.14	0.95
D17S1807	−4.47	−0.89	0.78	1.18	1.10	0.72	0.31	0.13	1.23
D17S1839	−0.89	0.40	1.39	1.51	1.18	0.67	0.23	0.09	1.51
D17S1603	3.08	3.00	2.69	2.29	1.53	0.83	0.28	0.00	3.08
D17S785	3.51	3.43	3.10	2.68	1.86	1.09	0.45	0.00	3.51
D17S939	−0.32	0.96	1.92	1.99	1.57	0.97	0.41	0.08	2.01
D17S802	2.16	3.22	3.44	3.16	2.30	1.36	0.52	0.04	3.46
D17S1847	2.19	2.12	1.89	1.59	1.03	0.56	0.21	0.00	2.18
D17S836	0.00	0.67	1.06	1.06	0.79	0.43	0.13	0.07	1.09
D17S1806	2.12	2.75	2.98	2.79	2.09	1.29	0.50	0.04	3.00
D17S784	2.32	2.92	3.15	2.93	2.18	1.30	0.49	0.04	3.16
D17S928	−0.94	−0.26	0.23	0.34	0.30	0.16	0.04	0.12	0.35

MATERIALS AND METHODS

Families Two families from Algeria and one family from Colombia, comprising a total of six individuals suffering from EV (**Table I**), were included in the study. Affected individuals were born from first cousin marriages. The two Algerian families are from Berber origin and live in two places 200 km away. The Colombian family originates from the province of Boyaca and is of Spanish and American Indian ancestry. EV was diagnosed according to established clinical, histologic, and virologic criteria (Jablonska *et al*, 1972; Kremsdorf *et al*, 1984; Orth, 1987). HPV genotypes were identified by Southern blot hybridization in scrapings or biopsy specimens from flat wart-like lesions or pityriasis versicolor-like lesions, and, for one patient, in a biopsy of a basal cell carcinoma of the face as described (Kremsdorf *et al*, 1984). Blood specimens were collected from the six patients, their parents, and eight available healthy sibs. Genomic DNA was extracted from either immortalized lymphoblastoid cell lines or whole blood samples (Sambrook *et al*, 1989). Informed consent was obtained from all individuals.

Genotyping Genotyping was performed as described (Gyapay *et al*, 1996), using fluorescence-labeled primers for 255 highly polymorphic microsatellite markers spanning the 22 autosomes and 10 additional markers located in the 17qter region taken from the Généthon panel (Dib *et al*, 1996).

Linkage analysis Expected and pairwise lod scores were calculated using the SLINK and MLINK programs of the Linkage package (version 5.2) (Lathrop *et al*, 1984) with FASTLINK option (version 3.0) (Schaffer *et al*, 1994). Autosomal recessive inheritance with complete penetrance was assumed for EV. The frequency of 0.001 was assigned to the associated allele disease. Equal allele frequencies for marker values were used because families studied have different ethnic backgrounds. Multipoint lod scores were calculated using the MAPMAKER/HOMOZ program (version 1.0), a computer package especially designed for homozygosity mapping (Kruglyak *et al*, 1995).

RESULTS

Clinical evaluation Three consanguineous EV families comprising a total of six patients were studied (**Table I**). Two families (A1, A2) originated from Algeria and one (C1) from Colombia. All EV patients were born from first cousin marriages. EV was diagnosed on the basis of an early onset, the presence of persistent flat wart-like lesions and pityriasis versicolor-like lesions disseminated on the face, neck, and limbs (families A1 and A2) or all over the body

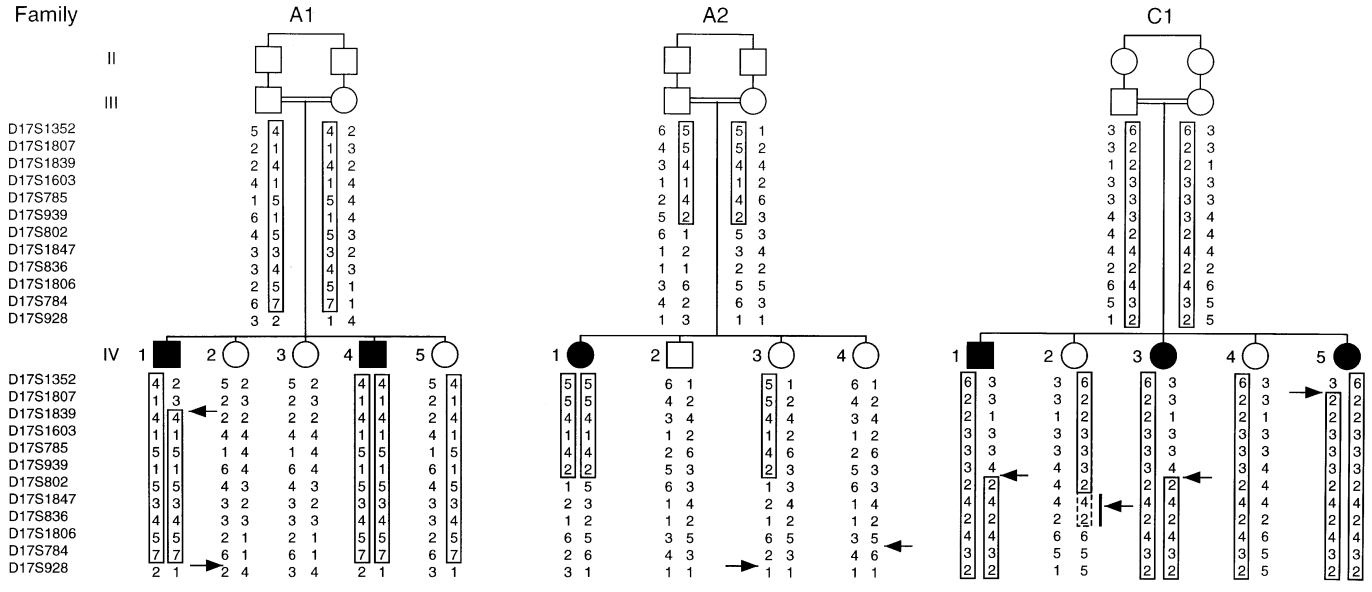

Figure 1. Simplified pedigrees of EV families and haplotype analysis of 17qter markers. Individuals in generation IV are numbered according to their age order and affected individuals are shown as filled black symbols. Haplotypes or portions of haplotypes presumed to be associated with EV in each family are boxed. Parents of family C1 share the same alleles for eight heterozygous markers, seven of which are homozygous in patient IV:5, allowing the identification of the parental haplotypes. Alleles of the centromeric marker D17S1352 are assigned arbitrarily. Recombination events are indicated by *arrows*. Recombination occurring in individual C1-IV:2 cannot be mapped more precisely because markers D17S1847 and D17S836 are homozygous in both parents (interrupted box).

(family C1) (Jablonska *et al*, 1972; Rueda and Rodriguez, 1976; Lutzner, 1978), and the detection of several EV-specific HPV genotypes. These included the oncogenic HPV5, found in five patients, HPV14, HPV17, and HPV20 (Orth, 1987) (**Table I**). HPV17 had been originally cloned and characterized from one of the Colombian patients (referred as C1-IV:1) (Kremsdorf *et al*, 1984). Two Algerian patients had already developed skin carcinomas and HPV5 genomes (about five copies per diploid cell DNA content) were detected in a basal cell carcinoma specimen obtained from one of them (patient A1-IV:1).

Genetic mapping and haplotype analysis Six patients, their parents, and eight available healthy sibs were genotyped for 255 highly polymorphic autosomal markers from the Généthon panel of microsatellite markers (Dib *et al*, 1996), with an average distance of 15 cM. We used the homozygosity mapping approach that represents a simple and efficient strategy to map rare human recessive traits (Lander and Botstein, 1987). Homozygosity restricted to affected individuals was observed for a marker of chromosome 17q (D17S784) in families A1 and C1 and for the adjacent marker about 17 cM distal (D17S1807) in family A2. Assuming an autosomal recessive inheritance model with a complete penetrance, two-point lod score expected values of 2.15 (family A1), 2.64 (family C1), and 1.55 (family A2) were obtained when consanguinity loops were taken into account. Ten additional microsatellite markers encompassing the D17S784 and D17S1807 markers and spanning 29 cM in this region were analyzed (**Fig 1**). Haplotypes were established for parents by minimizing the number of recombination events, and ancestral haplotypes associated with EV were deduced. Significant evidence of linkage with several markers was obtained by pairwise lod score analysis (**Table II**). Maximum two-point lod scores (Z_{max}) values were above 3 for markers D17S1603 (Z_{max} = 3.08) and D17S785 (Z_{max} = 3.51) at a recombination fraction (θ) of 0.00 and markers D17S802 (Z_{max} = 3.46) and D17S784 (Z_{max} = 3.16) at a recombination fraction of 0.04 (**Table II**). Multipoint lod score analysis over the 17qter region yielded a maximum lod score value of 10.17 between markers D17S939 and D17S802, as determined by homozygosity mapping using the MAPMAKER/HOMOZ program (**Fig 2**).

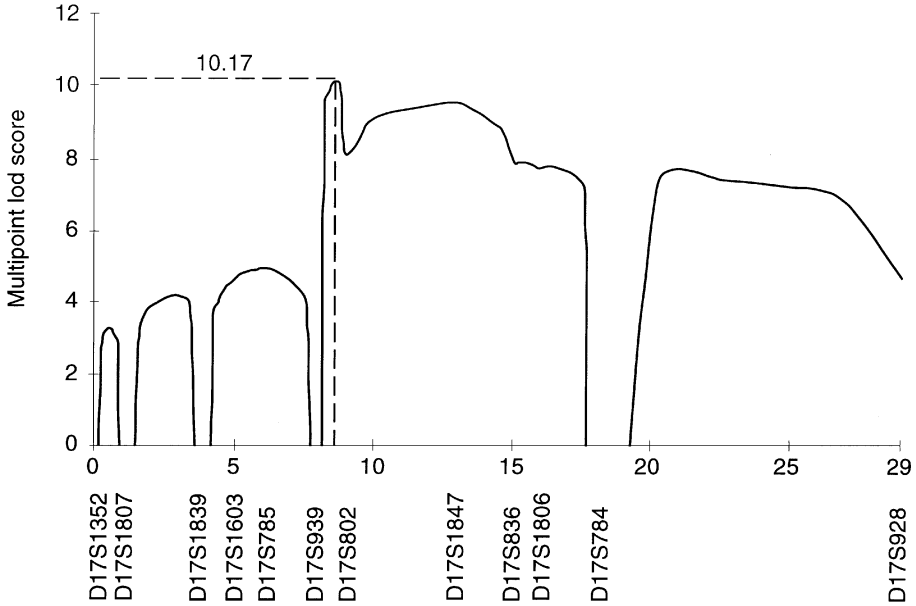

Figure 2. Multipoint linkage analysis between EV and chromosome 17qter markers. D17S1352, the marker closer to the centromere, was taken arbitrarily as the origin of the map. Markers are ordered according to the sex-average distance determined with CEPH pedigree data (Dib *et al*, 1996).

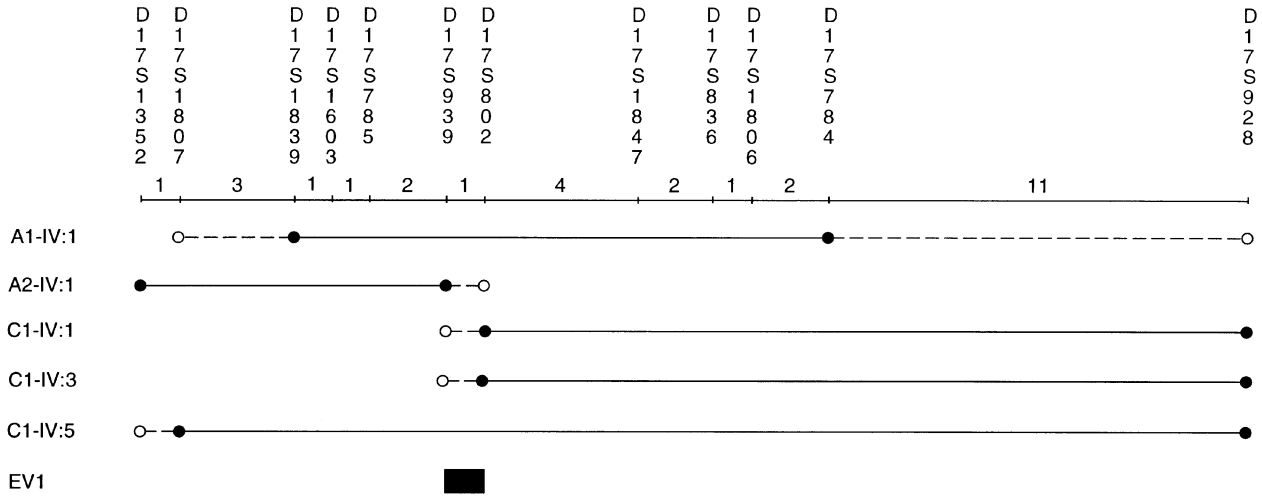

Figure 3. Chromosomal localization of the EV1 locus as defined by recombination events in the patients. The map of the markers is shown on the top and the distances between markers are given in cM (Dib *et al*, 1996). Affected individuals from families in which a recombination event occurred (**Fig 1**) are indicated on the left. Solid lines represent the homozygous haplotypes found in each affected individual and dotted lines the region where a crossover took place. Filled circles correspond to homozygous markers and open circles to heterozygous markers. The EV1 locus is indicated by a black box.

Identification of critical recombination events Recombination events were observed in families (**Fig 1**). Patients referred as A2-IV:1, C1-IV:1, and C1-IV:3 allowed to define a candidate region for the EV susceptibility locus (named EV1) extending on a 1 cM interval between D17S939 and D17S802 markers (**Fig 3**).

DISCUSSION

The results provide significant evidence for the presence of an epidermodysplasia verruciformis susceptibility locus on chromosome 17qter in a 1 cM interval flanked by D17S939 and D17S802. EV is characterized by an abnormal susceptibility to the infection with specific, phylogenetically related HPV genotypes, including the oncogenic HPV5 (Orth, 1987). The function of the mutated genes underlying predisposition to the EV HPV infections is unknown. These genes could encode proteins involved in the intracellular control of infection, acting either on the expression of the viral genome or on the activity of the nonstructural viral proteins E1, E2, E6, or E7 (Orth, 1987; Favre *et al*, 1997). These genes could also play a part in the immunologic control of EV HPV infections (Majewski *et al*, 1997). Numerous genes and EST have been assigned to the 17qter region containing EV1 (Plummer *et al*, 1997). Among these genes, some are likely to play a role in the control of the expression of the biologic properties of EV HPV, such as those encoding transcription factors like ILF1 (interleukin enhancer binding factor 1) or proteins involved in signal transduction like GRB2 (growth factor receptor binding protein 2) or the protein-kinase C α subunit. None of them, however, have been mapped accurately.

Loci for the susceptibility to other skin diseases have been mapped to region 17qter. A susceptibility locus for nonepidermolytic palmoplantar keratoderma associated with esophageal cancer has been mapped between D17S1839 and D17S1603 markers (Kelsell *et al*, 1996), centromeric to EV1 (**Fig 3**). A frequent loss of heterozygosity of the D17S785 marker located close to EV1 has been reported in actinic keratoses and squamous cell carcinomas of the skin (Quinn *et al*, 1994; Rehman *et al*, 1996), suggesting that this region contains a gene important in the development of squamous neoplasia. A dominant susceptibility locus for familial psoriasis has been recently mapped near the D17S802 marker, i.e., between D17S802 and D17S928 (Tomfohrde *et al*, 1994) and between D17S785 and D17S802 markers (Nair *et al*, 1997). This region contains EV1 (**Fig 3**).

It is worth stressing that patients suffering from psoriasis have recently been shown to constitute the long-searched for reservoir of HPV5, the genotype associated with EV carcinomas (Favre *et al*, 1998; Majewski *et al*, 1998). Using nested polymerase chain reaction methods, HPV5 was detected in more than 90% of patients with psoriasis, in contrast with the rare detection of this particular EV HPV genotype in skin specimens from normal individuals or patients with skin cancers (Boxman *et al*, 1997; Pfister and ter Schegget, 1997; Astori *et al*, 1998). Furthermore, antibodies against HPV5 capsid proteins were found in a significant proportion of psoriatic patients, which indicates the occurrence of a complete HPV5 life cycle in some epidermal cells (Favre *et al*, 1998; Majewski *et al*, 1998). This suggests that the host restriction towards HPV5 infection is somewhat less efficient in patients suffering from psoriasis than in the general population, and raises the question of the role of HPV5 in the immunopathogenesis of psoriasis (Majewski *et al*, 1998). Patients suffering from psoriasis or EV are not known to be prone to EV or psoriasis, respectively; however, it is tempting to speculate that distinct defects affecting the same gene on chromosome 17qter may be involved in the two skin conditions.

Little is known on the host genes underlying the susceptibility to infectious diseases (Bellamy and Hill, 1998). The EV1 locus represents the first locus known so far for a gene controlling the susceptibility to an oncogenic HPV genotype. The isolation of this gene should be of major interest because it should allow to disclose the relationships, if any, between the susceptibility to EV and to psoriasis.

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REFERENCES

Androphy EJ, Dvoretzky I, Lowy DR: X-linked inheritance of epidermodysplasia verruciformis: Genetic and virologic studies of a kindred. *Arch Dermatol* 121:864–868, 1985

Astori G, Lavergne D, Benton C, Hockmayr B, Egawa K, Garbe C, deVilliers EM: Human papillomaviruses are commonly found in normal skin of immunocompetent hosts. *J Invest Dermatol* 110:752–755, 1998

Bellamy R, Hill AVS: Genetic susceptibility to mycobacteria and other infectious pathogens in humans. *Curr Opin Immunol* 10:483–487, 1998

Boxman ILA, Berkhout RJM, Mulder LHC, Wolkers MC, Bavinck JNB, Vermeer BJ, ter Schegget J: Detection of human papillomavirus DNA in plucked hairs from renal transplant recipients and healthy volunteers. *J Invest Dermatol* 108:712–715, 1997

Cooper KD, Androphy EJ, Lowy D, Katz SI: Antigen presentation and T-cell activation in epidermodysplasia verruciformis. *J Invest Dermatol* 94:769–776, 1990

Dib C, Faure S, Fizames C, *et al*: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–154, 1996

Favre M, Orth G, Majewski S, Baloul S, Pura A, Jablonska S: Psoriasis: a possible reservoir for human papillomavirus type 5, the virus associated with skin carcinomas of epidermodysplasia verruciformis. *J Invest Dermatol* 110:311–317, 1998

Favre M, Ramoz N, Orth G: Human papillomaviruses: general features. *Clin Dermatol* 15:181–198, 1997

Gyapay G, Ginot F, Nguyen S, Vignal A, Weissenbach J: Genotyping procedures in linkage mapping. *Methods* 9:91–97, 1996

zur Hausen H: Papillomavirus infections: a major cause of human cancers. *Biochim Biophys Acta* 1288:F55–F78, 1996

Jablonska S, Dabrowski J, Jakubowicz K: Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis. *Cancer Res* 32:583–589, 1972

Kelsell DP, Risk JM, Leigh IM, *et al*: Close mapping of the focal non-epidermolytic palmoplantar keratoderma (PPK) locus associated with oesophageal cancer (*TOC*). *Hum Mol Genet* 5:857–860, 1996

Kremsdorf D, Favre M, Jablonska S, *et al*: Molecular cloning and biochemical characterization of the genomes of nine newly recognized human papillomavirus types associated with epidermodysplasia verruciformis. *J Virol* 52:1013–1018, 1984

Kruglyak L, Daly MJ, Lander ES: Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. *Am J Hum Genet* 56:519–527, 1995

Lander ES, Botstein D: Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. *Science* 236:1567–1570, 1987

Lathrop GM, Lalouel JM, Julier C, Ott J: Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443–3446, 1984

Lutzner MA: Epidermodysplasia verruciformis. An autosomal recessive disease characterized by viral warts and skin cancer. A model for viral oncogenesis. *Bull Cancer* 65:169–182, 1978

Majewski S, Favre M, Orth G, Jablonska S: Is human papillomavirus type 5 the putative autoantigen involved in psoriasis? *J Invest Dermatol* 111:541–542, 1998

Majewski S, Jablonska S, Orth G: Epidermodysplasia verruciformis. Immunological and nonimmunological surveillance mechanisms: role in tumor progression. *Clin Dermatol* 15:321–334, 1997

Majewski S, Malejczyk J, Jablonska S, Misiewicz J, Rudnicka L, Obalek S, Orth G: Natural cell-mediated cytotoxicity against various target cells in patients with epidermodysplasia verruciformis. *J Am Acad Dermatol* 22:423–427, 1990

McKusick VA: *Mendelian Inheritance in Man*. Baltimore, London: The Johns Hopkins University Press, 1990

Nair RP, Henseler T, Jenisch S, *et al*: Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. *Hum Mol Genet* 6:1349–1356, 1997

Orth G. Epidermodysplasia verruciformis. In: Howley PM, Salzman NP (eds). *The Papillomaviruses*, Vol 2. New York: Plenum Press, 1987, pp. 199–243

Orth G, Jablonska S, Favre M, Croissant O, Jarzabek-Chorzelska M, Rzesa G: Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. *Proc Natl Acad Sci USA* 75:1537–1541, 1978

Orth G, Jablonska S, Jarzabek-Chorzelska M, Rzesa G, Obalek S, Favre M, Croissant O: Characteristics of the lesions and risk of malignant conversion as

related to the type of the human papillomavirus involved in epidermodysplasia verruciformis. *Cancer Res* 39:1074–1082, 1979

Pfister H, ter Schegget J: Role of HPV in cutaneous premalignant and malignant tumors. *Clin Dermatol* 15:335–348, 1997

Plummer SJ, Simmons JA, Adams L, Casey G: Mapping of 228 ESTs and 26 genes into an integrated physical and genetic map of human chromosome 17. *Genomics* 45:140–146, 1997

Quinn AG, Sikkink S, Rees JL: Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. *Cancer Res* 54:4756–4759, 1994

Rajagopalan K, Bahru J, Tay CH, Chin KN, Tan KK: Familial epidermodysplasia verruciformis of Lewandowsky and Lutz. *Arch Dermatol* 105:73–78, 1972

Rehman I, Takata M, Wu YY, Rees JL: Genetic change in actinic keratoses. *Oncogene* 12:2483–2490, 1996

Rueda LA, Rodriguez G: Verrugas humanas por virus papova. Correlacion clinica, histologica y ultraestructural. *Med Cutanea Ibero Lat Am* 2:113–136, 1976

Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989

Schaffer AA, Gupta SK, Shriram K, Cottingham RW: Avoiding recomputation in linkage analysis. *Hum Hered* 44:225–237, 1994

Tomfohrde J, Silverman A, Barnes R, *et al*: Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. *Science* 264:1141–1145, 1994