

# Evidence for a Nonallelic Heterogeneity of Epidermodysplasia Verruciformis with Two Susceptibility Loci Mapped to Chromosome Regions 2p21–p24 and 17q25

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Epidermodysplasia verruciformis is a rare genodermatosis associated with a high risk of skin cancer. This condition is characterized by an abnormal susceptibility to specific related human papillomavirus genotypes, including the oncogenic HPV5. Epidermodysplasia verruciformis is usually considered as an autosomal recessive disease. We recently mapped a susceptibility locus for epidermodysplasia verruciformis (EV1) to chromosome 17qter within the 1 cM interval between markers D17S939 and D17S802. We report here the genotyping for 10 microsatellite markers spanning 29 cM around EV1 in two consanguineous epidermodysplasia verruciformis families from Colombia (C2) and France (F1) comprising five patients and two patients, respectively. Using homozygosity mapping, linkage with 17qter markers was observed for family C2 only. Multipoint linkage analysis yielded maximum multipoint LOD-score values above 10 between markers D17S1839 and D17S802 encompassing the EV1 locus. A genome-wide search performed in family F1 yielded evidence for linkage between epidermo-

dysplasia verruciformis and the chromosomal 2p marker D2S365. Nine additional microsatellite markers spanning 15 cM in this region were analyzed. Assuming an autosomal recessive inheritance with a complete penetrance, the expected maximum two-point LOD-score value of 1.8 was obtained for three markers and multipoint linkage analysis yielded a maximum LOD-score value of 3.51 between markers D2S2144 and D2S392. Haplotype analysis allowed to map a candidate region for a second epidermodysplasia verruciformis susceptibility locus (EV2) within the 8 cM interval between markers D2S171 and D2S2347 of the 2p21–p24 region. In contrast, linkage with 2p markers was excluded for family C2 and for the three families in which we mapped EV1 previously. The disclosure of two susceptibility loci for epidermodysplasia verruciformis provides evidence for a nonallelic heterogeneity in this disease. **Key words:** genodermatosis/genome scan/homozygosity mapping/human papillomavirus. *J Invest Dermatol* 114:1148–1153, 2000

Epidermodysplasia verruciformis (EV; MIM 226400) is a rare genodermatosis associated with a high risk of skin cancer (Jablonska *et al*, 1972; McKusick, 1998). EV results from an abnormal susceptibility to specific related human papillomavirus (HPV) genotypes and to the oncogenic potential of some of them, mainly HPV5 (Orth *et al*, 1978, 1979; Orth, 1987). Whereas EV-associated HPV (EV HPV) genotypes cause widespread inapparent infections in the general population (Boxman *et al*, 1997; Astori *et al*, 1998), infection leads to the early development of disseminated flat wart-like and pityriasis versicolor-like lesions in EV patients. Patients are unable to reject their lesions and cutaneous Bowen's carcinomas *in situ* and

invasive squamous cell carcinomas develop in about half of them, mainly on sun-exposed areas (Jablonska *et al*, 1972; Rueda and Rodriguez, 1976; Majewski *et al*, 1997). Cancers are usually associated with HPV5 and occasionally with HPV types 8, 14, 17, 20, or 47 (Orth, 1987).

EV is usually considered as an autosomal recessive condition (Rajagopalan *et al*, 1972; Lutzner, 1978). X-linked recessive (Androphy *et al*, 1985) or autosomal dominant (McKusick, 1998) modes of inheritance, however, have been postulated in single families, pointing to a possible genetic heterogeneity of the disease. EV is thus a model to approach the still unknown genes involved in the control of infections with oncogenic HPV genotypes. Recently, a genetic linkage analysis performed on three consanguineous EV families, two originating from Algeria and one from Colombia, allowed us to map a susceptibility locus for EV (EV1) to a 1 cM interval between D17S939 and D17S802 markers of chromosome 17qter (Ramoz *et al*, 1999). We have now extended this study to two additional consanguineous EV families, one from Colombia and the other from France. Linkage with microsatellite markers of the EV1 region was

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Abbreviation: EV, epidermodysplasia verruciformis.

**Table I. Characteristics of EV patients<sup>a</sup>**

Family, patients <sup>a</sup>	Origin	Sex, age (y)	Age at onset (y)	Age at first cancer (y)	HPV genotypes <sup>b</sup>
C2, IV:6	Colombia	M, 32	7–10	–	38, 3
C2, IV:8		M, 23	8	–	8, 3
C2, IV:10		F, 34	unknown	–	20, 3
C2, IV:11		F, 33	8	30	8, 3
C2, IV:12		F, 30	unknown	–	EV-related, 3, <i>RTRX5</i> , <i>RTRX9</i>
F1, IV:2	France	M, 18	5	–	12, 20, 3
F1, IV:4		F, 7	4	–	20

<sup>a</sup>As presented in Fig 1.<sup>b</sup>Genotypes in italics were identified by sequencing polymerase chain reaction products.**Table II. Two-point LOD-scores between EV and 17qter markers in two EV families**

		Recombination fraction ( $\theta$ )							$\theta_{\max}$	$Z_{\max}$
Marker		0.00	0.01	0.05	0.1	0.2	0.3	0.4		
D17S1352	C2	– $\infty$	–1.35	–0.19	0.19	0.35	0.24	0.08	0.19	0.35
	F1	– $\infty$	–3.84	–1.91	–1.15	–0.50	–0.23	–0.08	0.50	0.00
D17S1839	C2	3.54	3.46	3.17	2.78	1.98	1.17	0.44	0.00	3.54
	F1	– $\infty$	–0.95	–0.32	–0.12	0.00	0.01	0.00	0.50	0.00
D17S1603	C2	3.42	3.35	3.05	2.68	1.89	1.09	0.39	0.00	3.42
	F1	– $\infty$	–2.72	–1.51	–1.03	–0.60	–0.35	–0.15	0.50	0.00
D17S785	C2	3.72	3.65	3.36	2.97	2.17	1.34	0.55	0.00	3.72
	F1	– $\infty$	–2.11	–0.85	–0.40	–0.09	–0.09	0.00	0.40	0.00
D17S939	C2	3.53	3.45	3.12	2.71	1.88	1.07	0.38	0.00	3.53
	F1	–1.48	–1.09	–0.62	–0.42	–0.27	–0.21	–0.13	0.50	0.00
D17S802	C2	3.03	2.95	2.66	2.30	1.58	0.89	0.32	0.00	3.03
	F1	– $\infty$	–2.49	–1.35	–0.88	–0.49	–0.30	–0.15	0.50	0.00
D17S1847	C2	3.03	2.95	2.66	2.30	1.58	0.89	0.32	0.00	3.03
	F1	– $\infty$	–0.73	–0.16	–0.02	0.03	0.02	0.00	0.50	0.00
D17S836	C2	– $\infty$	–3.67	–1.25	–0.43	0.05	0.07	0.00	0.00	0.43
	F1	–2.24	–1.23	–0.53	–0.24	–0.04	0.00	0.00	0.30	0.00
D17S1806	C2	– $\infty$	–1.28	–0.09	0.27	0.40	0.28	0.11	0.00	0.40
	F1	–1.70	–0.66	–0.04	0.18	0.26	0.19	0.09	0.19	0.26
D17S928	C2	– $\infty$	–6.12	–2.83	–1.59	–0.64	–0.30	–0.13	0.00	0.17
	F1	–2.08	–1.61	–0.82	–0.48	–0.17	–0.06	–0.01	0.50	0.00

only observed for the Colombian family. A genome-wide search was performed in the French family. Using homozygosity mapping strategy (Lander and Botstein, 1987), we have mapped a second EV susceptibility locus (EV2) to the 2p21–p24 chromosomal region. Our results provide evidence for a nonallelic heterogeneity in EV.

#### MATERIALS AND METHODS

**Families** The study included a large family from Colombia and a family from France, comprising a total of seven individuals suffering from EV (Table I). All affected individuals were born from first cousin marriages. The Colombian family, referred as C2, originates from the region of Bucaramanga and is of Spanish and American Indian ancestry. It is unrelated to the Colombian family, referred as C1, previously studied (Ramos *et al*, 1999). The parents and grandparents of the French EV patients were born in southern Italy. EV was diagnosed according to established clinical, histologic, and virologic criteria (Rueda and Rodriguez, 1976; Orth, 1987; Majewski *et al*, 1997). HPV genotypes were identified in DNA preparations obtained from scrapings or biopsy specimens from flat wart-like or pityriasis versicolor-like lesions, both by Southern blot hybridization as described (Kremsdorf *et al*, 1984) and by sequencing amplimers obtained using a nested polymerase chain reaction method (Berkhout *et al*, 1995), as described (Favre *et al*, 1998). For the patient referred as F1-IV:4, the only DNA preparation available for HPV typing was obtained from tissue sections of a formaldehyde-fixed, paraffin-embedded flat wart-like lesion (Wright and Manos, 1990). Blood specimens were collected from a total of 27 affected and unaffected individuals. Genomic DNA was extracted from either immortalized lymphoblastoid cell lines or whole blood samples (Sambrook *et al*, 1989).

For the patient referred as C2-IV:6, who died during the course of this study, and for the patient referred as C2-IV:10, the only DNA preparations available for genotyping experiments were obtained from biopsy specimens. Informed consent was obtained from all individuals or their parents.

**Genotyping** Genotyping was performed as described (Gyapay *et al*, 1996), using fluorescent-labeled primers for 10 microsatellite markers spanning the 17qter region containing the EV1 locus (Table II), 10 markers of the 2p21–p24 region (Table III), 255 highly polymorphic microsatellite markers spanning the 22 autosomes, and additional markers of the chromosome 3qter (D3S1305, D3S3642, D3S1265, D3S1311, D3S1272), 16q21–q22 (D16S512, D16S3018, D16S515, D16S3116, D16S3097), and 18pter (D18S1105, D18S481, D18S63, D18S52, D18S1132) taken from the Génethon panel (Dib *et al*, 1996). Genotyping for the markers of the 2p21–p24 region was also performed for the 20 individuals, including six EV patients, from the two Algerian families (referred as A1 and A2) and the Colombian family C1 previously studied (Ramos *et al*, 1999).

**Linkage analysis** Expected pairwise and maximum LOD-scores were calculated using the SLINK, MLINK, and LODSCORE programs of the Linkage package (version 5.2) (Lathrop *et al*, 1984) with FASTLINK option (version 4.0) (Schaffer *et al*, 1994). An autosomal recessive model of inheritance with complete penetrance was assumed using a disease associated allele frequency of 0.001. Equal allele frequencies for marker values were used because families 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).

**Table III. Two-point LOD-scores between EV and 2p21–p24 markers in two EV families**

Marker		Recombination fraction ( $\theta$ )							$\theta_{\max}$	$Z_{\max}$
		0.00	0.01	0.05	0.1	0.2	0.3	0.4		
D2S2221	C2	–∞	–3.61	–1.86	–1.11	–0.51	–0.25	–0.10	0.50	0.00
	F1	–∞	0.06	0.60	0.70	0.61	0.42	0.21	0.11	0.71
D2S171	C2	–2.19	–1.76	–1.16	–0.82	–0.45	–0.24	–0.10	0.50	0.00
	F1	–∞	0.06	0.60	0.70	0.62	0.43	0.22	0.11	0.71
D2S2303	C2	–∞	–3.22	–1.34	–0.64	–0.13	0.01	0.02	0.37	0.03
	F1	0.70	0.68	0.59	0.48	0.28	0.12	0.02	0.00	0.70
D2S2144	C2	–2.21	–1.32	–0.07	–0.43	–0.19	–0.08	–0.03	0.50	0.00
	F1	1.80	1.76	1.59	1.38	0.98	0.61	0.28	0.00	1.80
D2S174	C2	0.89	0.86	0.76	0.64	0.44	0.26	0.11	0.00	0.89
	F1	0.70	0.69	0.63	0.55	0.40	0.26	0.12	0.00	0.70
D2S365	C2	–2.19	–1.76	–1.16	–0.82	–0.45	–0.24	–0.10	0.50	0.00
	F1	1.80	1.76	1.59	1.38	0.98	0.61	0.58	0.00	1.80
D2S392	C2	–∞	–1.36	–0.69	–0.42	–0.17	–0.05	0.00	0.40	0.00
	F1	1.80	1.76	1.59	1.38	0.98	0.61	0.58	0.00	1.80
D2S2383	C2	–∞	–2.75	–1.53	–0.99	–0.50	–0.26	–0.11	0.50	0.00
	F1	0.22	0.21	0.18	0.14	0.08	0.03	0.01	0.00	0.22
D2S2347	C2	–∞	–3.08	–1.21	–0.50	–0.01	0.08	0.05	0.30	0.08
	F1	–∞	–0.24	0.31	0.44	0.40	0.27	0.13	0.42	0.12
D2S367	C2	–∞	–2.88	–0.99	–0.32	0.11	0.17	0.10	0.28	0.17
	F1	–∞	–1.01	–0.38	–0.17	–0.04	–0.01	0.00	0.40	0.00

## RESULTS

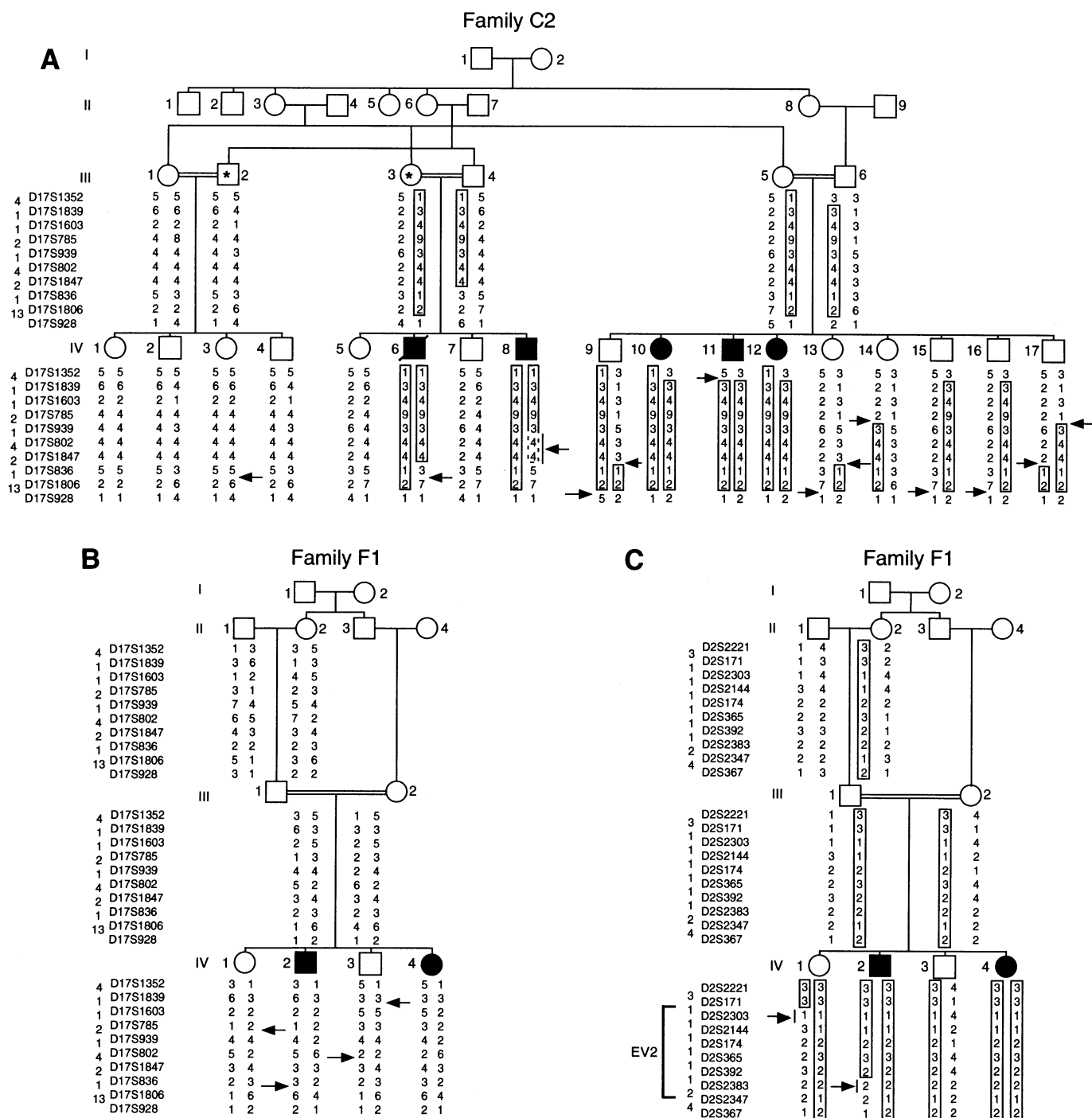
**Clinical evaluation** A total of seven EV patients born from first cousin marriages were studied (**Table I**). Five patients were born from two of three first cousin marriages in a large Colombian family referred to as C2 (**Fig 1A**). Two patients were from a French family referred to as F1 (**Fig 1B**). EV was diagnosed on the basis of an early onset, the presence of disseminated flat wart-like lesions mostly located on the face and the dorsa of the hands and, for patients C2-IV:6 and C2-IV:11, the occurrence of pityriasis versicolor-like lesions on the trunk, the observation of histologic features specific for EV, and the detection of EV-specific (EV-HPV) or EV HPV-related HPV genotypes (Rueda and Rodriguez, 1976; Orth, 1987; Berkhout *et al*, 1995; Majewski *et al*, 1997). The oncogenic HPV5 was not detected but HPV8 and HPV20 also, albeit less frequently, associated with EV carcinomas (Orth, 1987) were found in five patients. One of them had developed Bowen's carcinomas *in situ* and invasive squamous cell carcinomas on the face (**Table I**). In patient F1-IV:2, wart-like lesions started to appear following the treatment of Burkitt's lymphoma of abdominal lymph nodes with chemotherapy and systemic steroids. Patient C2-IV:6 and unaffected individual C2-IV:16 were severely mentally retarded and almost all their siblings showed some degree of mental retardation. Neurologic disorders characterized by a progressive paralysis starting at about 20 y of age were observed in the four siblings (C2-IV:1–4) born from the first cousin marriage with no EV patients in the Colombian family.

**Linkage between EV and 17qter markers in the Colombian family C2** Seven patients born from three first cousin marriages (two within family C2 and one within family F1), five of their parents, 10 available unaffected siblings, the paternal grandparents in family F1, and the four unaffected individuals born from another first-cousin marriage in family C2, as well as their mother, were genotyped for 10 highly polymorphic microsatellite markers spanning 29cM in the 17qter region encompassing the EV1 locus. Using homozygosity mapping (Lander and Botstein, 1987), linkage with markers of the EV1 region was only found for family C2. A homozygous haplotype restricted to the five affected individuals was observed in this family, whereas none of the markers were found homozygous in the two EV patients of family F1. (**Fig 1A, B**). Pairwise linkage analysis performed in family C2 yielded maximum two-point LOD-score values above 3 for six markers, at a recombination fraction of 0.0 (**Table II**). Multipoint

linkage analysis yielded LOD-score values above 10 between markers D17S1839 and D17S802, as determined using the MAPMAKER/HOMOZ program (**Fig 2**). Haplotype analysis allowed to delineate the disease linkage interval to a 15cM region, between D17S1352 and D17S836. This region contains the EV1 locus previously mapped between markers D17S939 and D17S802 (**Fig 1A**). By combining the data obtained for family C2 and for the three families previously studied (Ramoz *et al*, 1999), a maximum multipoint LOD-score value of 20.52 was obtained between these markers (**Fig 2**), further demonstrating the strength of the linkage between EV and the 17qter region. Two-point and multipoint linkage analyses failed to reveal any evidence of linkage between the 17qter region and the EV phenotype in family F1 (**Table II; Fig 2**).

**Linkage between EV and 2p markers in the French family F1** Failure to show a linkage between EV and chromosome 17qter in family F1 prompted us to perform a genome-wide search using 255 highly polymorphic autosomal markers from the Génethon panel of microsatellite markers, with an average distance of 15cM (Dib *et al*, 1996). Homozygosity restricted to affected individuals was found with markers of chromosomes 2p21–p24 (D2S365), 3qter (D3S1265), 16q21–q22 (D16S515), and 18pter (D18S63). When four additional microsatellites encompassing each of these markers over 4–8cM intervals were genotyped, a homozygous haplotype restricted to EV patients was observed for 2p markers only. By analyzing five additional microsatellites for this region, the 10 markers studied spanned 15cM on the 2p21–p24 region (**Fig 1C**). Assuming an autosomal recessive inheritance with a complete penetrance, the expected maximum two-point LOD-score value of 1.8 was obtained for three markers (**Table III**). Multipoint linkage analysis over the 2p21–p24 region yielded LOD-score values above 3 between markers D2S2303 and D2S2383, with a maximum LOD-score of 3.51 between D2S2144 and D2S392 (**Fig 3**). Genotyping of the paternal grandmother helped to establish the ancestral haplotype segregating with EV in family F1 and critical recombination events observed in the unaffected individual F1-IV:1 and her affected brother F1-IV:2 allowed to map the candidate region for a second EV locus, EV2, to the 8cM interval between D2S171 and D2S2347 (**Fig 1C**).

**Lack of evidence for linkage to EV2 in families with linkage to EV1** When the 10 microsatellites spanning the 2p21–p24



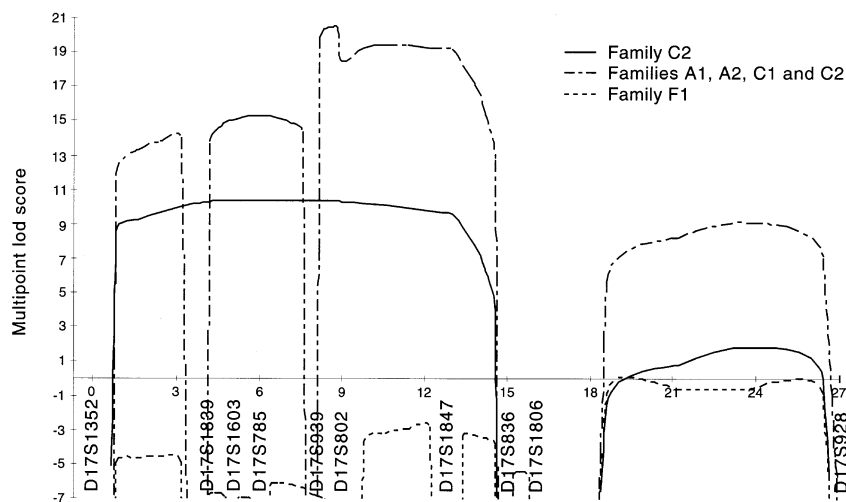
**Figure 1. Haplotype analysis of microsatellite markers in simplified pedigrees of families.** Genotyping of 17qter markers in families C2 (A) and F1 (B), and 2p21-p24 markers in family F1 (C). Sex-average distances between microsatellites are given in cM on the left (Dib *et al*, 1996). Affected individuals are shown as filled black symbols. Haplotypes or portions of haplotypes associated with EV in each family are boxed (or indicated by an interrupted box in individual C2-IV:8). Recombination events are indicated by arrows or a line when they cannot be exactly positioned. The EV2 locus is indicated by a bracket (C). Asterisks indicate individuals with a deduced genotype.

region were genotyped in family C2, no evidence for linkage between the EV phenotype and the 2p region was observed. Negative two-point LOD-score values were obtained (Table III) and a multipoint linkage analysis yielded LOD-score values below -9 between markers D2S171 and D2S2347 delimiting EV2 (Fig 3). In the genome-wide search previously performed on Algerian families A1 and A2 and on the Colombian family C1, no linkage had been found between EV and microsatellites D2S365 (included in EV2), D2S131 (25 cM telomeric) and D2S177 (12 cM centromeric). When the seven markers encompassing the EV2 locus from D2S171 to D2S2347 were genotyped in these three

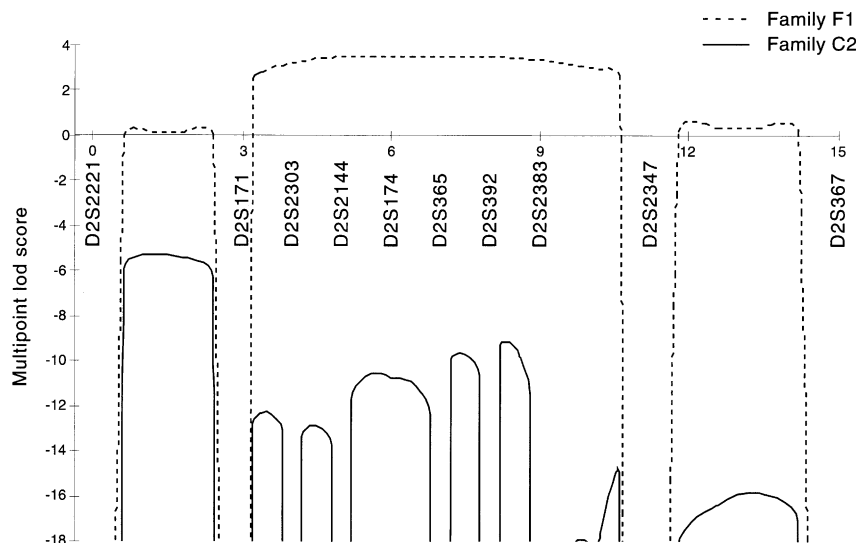
families, no haplotype segregation with the disease was observed and multipoint linkage analysis over the region yielded LOD-score values below -2 in each family.

## DISCUSSION

Our study of consanguineous families with EV showing an autosomal mode of inheritance provides evidence for a nonallelic heterogeneity within the disease. Indeed, the presence of an EV susceptibility locus (EV1) on chromosome 17q25 in a 1 cM region previously mapped between D17S939 and D17S802 markers



**Figure 2. Multipoint linkage analysis between EV and chromosome 17qter markers.** Filled and dotted lines are LOD-score values for families C2 and F1, respectively. Interrupted lines indicate LOD-score values obtained by combining data from family C2 and the families A1, A2, and C1 previously studied (Ramos *et al*, 1999). The centromeric marker D17S1352 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. Multipoint linkage analysis between EV and chromosome 2p21-p24 markers.** Filled and dotted lines are LOD-score values for families C2 and F1, respectively. The telomeric marker D2S2221 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).

(Ramos *et al*, 1999) was further substantiated in an additional family. In another family in which the 17q locus was excluded as the site for the disease-causing gene, a second EV susceptibility locus was disclosed on chromosomal region 2p21-p24, in an 8 cM interval between D2S171 and D2S2347 markers.

The genetic heterogeneity of EV had already been postulated from the study of single families showing an X-linked recessive mode of transmission (Androphy *et al*, 1985) or suggesting a dominant autosomal inheritance (McKusick, 1998). It remains to determine to what extent a phenotypic variability of EV corresponds to the genetic heterogeneity of the disease. The five families included in this report (families C2 and F1) and in a previous study (families A1, A2, and C1) (Ramos *et al*, 1999) are characterized by the early onset of the disease, by a disseminated infection by EV-specific HPV genotypes, including at least one of the potentially oncogenic genotypes (HPV types 5, 8, 14, 17, or 20), and by lesions of the skin showing the cytopathic effect pathognomonic of EV. Significant variations were observed, however, in the HPV genotypes detected, the extension of the lesions and the development of skin carcinomas. HPV5, the genotype most frequently associated with EV cancers (Orth, 1987), was detected only in families A1, A2, and C1, whereas the less oncogenic HPV20, a HPV5-related genotype (Kremsdorf *et al*, 1984; Yutsudo *et al*, 1994), was found in the five families. HPV3 or the related HPV28, that cause flat warts in the general population and are frequently detected in EV patients, were found in families

A1, C2, and F1. Skin precancers and cancers were observed in two of the five families, only. In the French family F1, the young age of the patients and an infection with HPV20 instead of HPV5 could account for the absence of malignant transformation. Worth stressing is that no common disease haplotype was observed in the two Colombian and the two Algerian families showing a linkage with the 17q25 region. This renders unlikely a common founder mutation event and may suggest the existence of multiple allelic forms of the disease linked to EV1 locus. In the Colombian family C2, an EV patient and a nonaffected cousin were severely mentally retarded and most of their siblings, including four EV patients, showed a moderate mental retardation. In a review of 147 EV patients from 125 families, mental retardation was reported in 8% of the EV patients (Lutzner, 1978). A parallel had been suggested between this form of EV and a form of another rare autosomal recessive skin disease, xeroderma pigmentosum, associated with mental deficiency (De Sanctis-Cacchione syndrome) (Lutzner, 1978). In family C2, EV and mental retardation do not cosegregate, which renders unlikely the hypothesis that the two diseases correspond to the pleiotropic expression of a same gene defect.

The genes involved in EV are unknown (Orth, 1987; Ramos *et al*, 1999). Numerous genes, including genes encoding transcription factors or proteins involved in signal transduction, as well as numerous expressed sequence tags, have been assigned to regions 2p21-p24 and 17qter (Plummer *et al*, 1997; Deloukas *et al*, 1998).

An integrated physical and partial transcript map of the chromosomal region 17q25 encompassing the EV1 locus has been reported recently (Kuhlenbäumer *et al*, 1999). So far, no likely candidate gene has been identified. As for the EV2 locus, a map of the 1 cM region telomeric to D2S174 marker has been described (Yasunaga *et al*, 1999). Among the five genes mapped within this region (Yasunaga *et al*, 1999), the gene encoding the centromere protein A could be involved in chromosomal instability.

The EV1 locus is included in a major susceptibility locus for familial psoriasis (PSORS2) (Tomfohrde *et al*, 1994; Nair *et al*, 1997; Ramoz *et al*, 1999). Recent data suggest that the host restriction towards HPV5 and other EV HPV is somewhat less stringent in patients suffering from psoriasis than in the general population (Favre *et al*, 1998; Weissenborn *et al*, 1999). This led us to speculate that distinct defects affecting the same, as yet unknown, gene on chromosome 17qter could be involved in EV and psoriasis (Majewski *et al*, 1999; Ramoz *et al*, 1999). As for region 2p21–p24, a suggestive linkage has been reported recently between familial psoriasis and the D2S177 marker (Bhalerao and Bowcock, 1998). This marker is localized 10 cM centromeric to D2S2347, one of the markers delimiting the EV2 locus. It is worth mentioning that the 2p21–p22 region is homologous to the region of the mouse chromosome 17 that harbors the flaky skin mutation conferring a phenotype similar to human psoriasis (Beamer *et al*, 1995; Sundberg *et al*, 1997). The potential susceptibility locus for psoriasis in chromosomal region 2p has to be confirmed before further speculation on the possible link between psoriasis and EV.

Identification of EV-associated genes in chromosomal regions 17q and 2p requires refinement of the genetic interval containing these genes. This should involve the analysis of other EV families and the identification of new markers within these regions. Host genes conditioning susceptibility or resistance to oncogenic HPV genotypes are still unknown. The identification of two susceptibility loci for EV provides an opportunity to decipher the multiplicity of the controls involved, as revealed by the genetic heterogeneity of the disease.

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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
- Beamer WG, Pelsue SC, Shultz LD, Sundberg JP, Barker JE: The flaky skin (*fsn*) mutation in mice: map location and description of the anemia. *Blood* 86:3220–3226, 1995
- Berkhout RJM, Tieben LM, Smits HL, Bouwes Bavinck JN, Vermeer BJ, ter Schegget J: Nested PCR approach for detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients. *J Clin Microbiol* 33:690–695, 1995
- Bhalerao J, Bowcock AM: The genetics of psoriasis: a complex disorder of the skin and immune system. *Hum Mol Genet* 7:1537–1545, 1998
- Boxman ILA, Berkhout RJM, Mulder LHC, Wolters 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
- Deloukas P, Schuler GD, Gyapay G, *et al*: A physical map of 30,000 human genes. *Science* 282:744–746, 1998
- 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
- Gyapay G, Ginot F, Nguyen S, Vignal A, Weissenbach J: Genotyping procedures in linkage mapping. *Methods* 9:91–97, 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
- 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
- Kuhlenb  umer G, Schirmacher A, Meuleman J, *et al*: A sequence-ready BAC/PAC contig and partial transcript map of approximately 1.5 Mb in human chromosome 17q25 comprising multiple disease genes. *Genomics* 62:242–250, 1999
- 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, Jablonska S, Orth G: Epidermodysplasia verruciformis. Immunological and nonimmunological surveillance mechanisms: role in tumor progression. *Clin Dermatol* 15:321–334, 1997
- Majewski S, Jablonska S, Favre M, Ramoz N, Orth G: Papillomavirus and autoimmunity in psoriasis. *Immunol Today* 20:475–476, 1999
- McKusick VA: *Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders*. The Johns Hopkins University Press, Baltimore, London, 1998
- 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, Rzaea 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, Rzaea 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
- 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
- Rajagopalan K, Bahru J, Tay CH, Chin KN, Tan KK: Familial epidermodysplasia verruciformis of Lewandowsky and Lutz. *Arch Dermatol* 105:73–78, 1972
- Ramoz N, Rueda L-A, Bouadjar B, Favre M, Orth G: 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. *J Invest Dermatol* 112:259–263, 1999
- 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
- Sundberg JP, France M, Boggess D, Sundberg BA, Jensen AB, Beamer WG, Shultz LD: Development and progression of psoriasiform dermatitis and systemic lesions in the flaky skin (*fsn*) mouse mutant. *Pathobiology* 65:271–286, 1997
- 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
- Weissenborn SJ, H  pfel R, Weber F, Smola H, Pfister HJ, Fuchs PG: High prevalence of a variety of epidermodysplasia verruciformis-associated human papillomaviruses in psoriatic skin of patients treated or not treated with PUVA. *J Invest Dermatol* 113:122–126, 1999
- Wright DK, Manos MM. Sample preparation from paraffin-embedded tissues. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). *PCR Protocols: a Guide to Methods and Applications*. San Diego: Academic Press Inc., 1990, pp 153–158
- Yasunaga S, Grati M, Cohen-Salmon M, *et al*: A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. *Nature Genet* 21:363–369, 1999
- Yutsudo M, Tanigaki T, Kanda R, *et al*: Involvement of human papillomavirus type 20 in epidermodysplasia verruciformis skin carcinogenesis. *J Clin Microbiol* 32:1076–1078, 1994