Multiple Self-Healing Squamous Epithelioma in Different Ethnic Groups: More than a Founder Mutation Disorder?

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Multiple self-healing squamous epithelioma (MSSE), also known as Ferguson–Smith Disease, is a rare cancer-associated genodermatosis with an autosomal dominant inheritance. Affected patients suffer from recurrent skin lesions, which clinically and histologically resemble keratoacanthomas or well-differentiated squamous cell carcinomas, but which, if left, undergo spontaneous regression, leaving pronounced scarring. The majority of MSSE cases previously described were of Scottish ancestry and all shared the same at-risk haplotype, suggesting that this disorder was caused by a founder mutation. The candidate locus for MSSE lies in a region of <4 cM in chromosome 9q22, between the markers D9S197 and D9S1809. We recently investigated MSSE families of non-Scottish origin. For every patient of these families, we obtained a detailed clinical history, with particular attention to the age of onset, distribution, and clinical course of their skin lesions. Once confirmed that they were really affected by MSSE, we performed haplotype analysis on them and their families. The haplotypes for polymorphic markers segregating with MSSE in non-Scottish and Scottish families differ, suggesting that MSSE is not caused by a founder mutation and might be more common than originally thought.

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INTRODUCTION

Multiple self-healing squamous epithelioma (MSSE), also known as Ferguson–Smith Disease, is a familial genodermatosis characterized by the occurrence of multiple skin tumors, which histologically cannot be distinguished from well-differentiated squamous cell carcinomas (SCCs), but which undergo spontaneous regression like keratoacanthomas (KAs), resulting in characteristic scar formation. One of the features distinguishing this condition from eruptive KA of the Grzybowski variant is its familial nature (Consigli *et al.*, 2000). MSSE was first described in a 23-year-old Scottish coalminer (Ferguson-Smith, 1934). It is a rare genodermatosis inherited as an autosomal dominant trait. Less than 200 cases have been described so far, the majority originating from West and Central Scotland, traced to two common ancestors around the beginning of the 19th century (Ferguson-Smith *et al.*, 1971).

The age of onset of MSSE is variable with the appearance of first tumors ranging from 8 to 62 years (Goudie *et al.*, 1993). Further tumors appear episodically throughout life. Lesions are typically painless and multiple, with over 100 tumors developing in some affected individuals over their lifetime. Tumors arise predominantly, but not exclusively, on sun-exposed areas of the face, scalp, ears, forearms, and legs. They first appear as red papules and progress to nodules, often with a central keratin plug. Over time the lesions may ulcerate; when ulceration occurs, the edges are typically rolled and undermined. Characteristically, these tumors undergo spontaneous regression within 4–6 months, resulting in scars that are typically deep and pitted on the face, scalp, and ears, although those on the limbs tends to be smoother

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Abbreviations: KA, keratoacanthoma; MSSE, multiple self-healing squamous epithelioma; SCC, squamous cell carcinoma

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and shallower. Considerable morbidity is associated with disfiguring scarring. The tumors invade locally and may be destructive but do not appear to recur following excision. Aggressive local invasion after radiotherapy is very rare, although it has been reported in a patient who underwent radiotherapy (Chakrabarty and Perks, 1996). Treatment with etretinate was found to prevent new lesions from developing in some patients (Wright *et al.*, 1988), but the most successful treatments are surgical excision of the lesions or cryotherapy.

Eleven MSSE families with 49 affected and 104 unaffected individuals originating from West and Central Scotland have previously been subjected to linkage analysis. The candidate locus for MSSE was refined to a region of <4 cM in chromosome 9q22 between the markers D9S197 and D9S1809 (Goudie *et al.*, 1993; Richards *et al.*, 1997). Affected members of nine of the 11 families shared a common haplotype for all the markers in the candidate region, suggesting common ancestry. Although MSSE has been known as a well-defined disorder for more than 60 years, no detailed paper describing the clinical features has been published since 1971 (Ferguson-Smith *et al.*, 1971). Our report includes a description of the common clinical features of the patients studied, the results of linkage analysis in more recently acquired Scottish MSSE families and MSSE patients of non-Scottish origin, and results of the mutations screening in some candidate genes that lie in the MSSE region, between markers D9S197 and D9S1809.

RESULTS

Histological analysis of the tumors from the MSSE patients

Histological analysis was conducted on several tumors from each MSSE patient reported in this paper. In all cases, the lesions were confirmed to be KAs or well-differentiated SCCs.

Figure 1 shows a low power micrograph of a skin lesion removed from the forearm of a 44-year-old MSSE female patient from the Scottish kindred. The lesion lacks the collarette of epidermis on either edge, and the distinctive eosinophilic cytoplasm observed in a KA. When viewed at higher power, there is no obvious epithelial atypia, there are few mitoses in the epithelium and the inflammatory infiltrate is mixed. A single microabcess is seen. This lesion has the features of a well-differentiated SCC.

Clinical assessment of the patients

The clinical features of 37 patients of Scottish origin and of 19 affected persons of non-Scottish origin were examined and found to be similar, in almost every aspect, to the cases described by Ferguson-Smith (1934) and Ferguson-Smith *et al.* (1971).

Of the 37 Scottish patients, 11 were males (29.73%) and 26 were females (70.27%). The mean age of onset was 25.1 years (Figure 2): 26.3 years in male subjects (range, 12–50) and 24.6 years in female subjects (range, 8–63). A total of 97 tumors were observed in the 11 male patients and the average number of tumors was 8.82 (range, 3–15). A total of 637 tumors were observed in the 26 female patients, and the average number of tumors per patient was 24.5 (range, 1–133). All patients had at least one tumor on the head. In both male and female patients, the majority of tumors (82% and 72%, respectively) were located on the head with fewer tumors on the trunk and limbs. The tumor distribution, according to anatomical location, is illustrated in Table 1.

Two cases, MA1.2 and MA2.1, are here described in more detail. MA1.2 is a 78-year-old female born in west Scotland, who emigrated to Australia aged 51. She developed her first lesion, aged 50, on her right lateral malleolus, which cleared spontaneously after 1 year, leaving a 1 cm scar. A second tumor was excised on the dorsum of her left hand a few months later. Subsequently, more than 25 tumors developed on her face, nose, and ears. These were either surgically removed or left untreated to heal spontaneously (within an average of 6 months). Her father died of intra-oral SCC.

MA2.1, the son of MA1.2, who is in his fifties, developed his first lesion on his left eyebrow aged 15. This spontaneously resolved, but, in the following year, several other lesions appeared on his lower face and in the lobes of both his ears. All resolved spontaneously, within 7 weeks on average. When MA2.1 was in his early 20s, he developed several lesions on both his cheeks and ears, on his left eyebrow and on his jaw line; all these lesions were surgically removed. Similar lesions came and went in the same areas in the following years, and six of them were surgically removed. A bigger benign tumor developed on his tongue on his late 20s, it was left untreated and still has not resolved. In total,

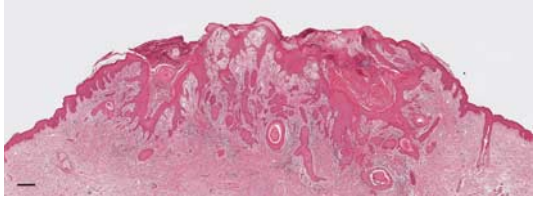

Figure 1. Histological section from a typical MSSE lesion. The symmetry and architecture of this lesion can resemble a KA, but the collarette of epidermis on the edge and the eosinophilic cytoplasm, typical of a KA, are absent. When examined at higher power magnification, epithelial atypia is not prominent, fewer than normal mitoses are present, and the inflammatory infiltrate is mixed. A single microabcess is seen. (hematoxylin and eosin; Bar: 100 μm)

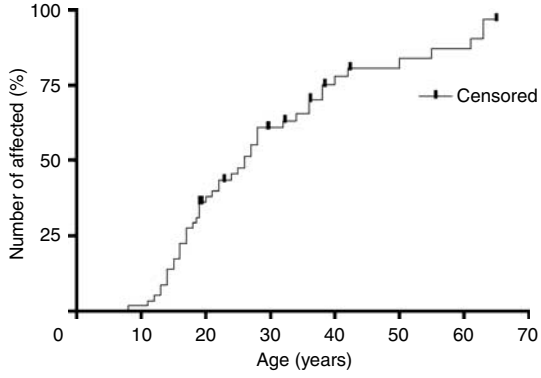

Figure 2. Age of MSSE onset in the Scottish patients. Fifty percent of the Scottish patients were affected by the age of 26 years. By the age of 65, 97% of the patients were affected. Censored patients are shown by the vertical line.

Table 1. Tumor distribution in the Scottish patients

		Head	Upper limbs	Lower limbs	Trunk
Males	No. of tumors (range)	80 (3–14)	6 (0–3)	5 (0–2)	6 (0–4)
	% of tumors	82.47%	6.19%	5.15%	6.19%
	No. of males	*n*=11 (100%)	*n*=4 (36.36%)	*n*=2 (18.18%)	*n*=3 (27.27%)
Females	No. of tumors (range)	464 (1–97)	62 (0–17)	107 (0–24)	4 (0–3)
	% of tumors	72.84%	9.73%	16.80%	0.63%
	No. of females	*n*=26 (100%)	*n*=13 (50%)	*n*=17 (65.38%)	*n*=2 (7.69%)

Table 2. Tumor distribution in the non-Scottish patients

	Current age	Age of onset	Total no. of lesions	No. of lesions in the head	No. of lesions in the limbs	No. of lesions in the trunk	No. of lesions excised	Radio-therapy
COI.2	64	10s	>20	>20	0	2	21	Yes
COII.2	41	18	22	20	0	2	19	No
COIII.1	13	8	2	1	1	0	2	No
DKI.1	Deceased	67	4	4	0	0	3	Yes
DKII.2	Deceased	75	1	1	0	0	0	Yes
DKII.3	93	81	1	0	1	0	0	No
DKII.6	Deceased	41	2	2	0	0	2	No
DKII.8	73	60	2	0	2	0	2	No
DKIII.3	57	43	23	10	13	0	15	Yes
DKIII.5	52	50	1	1	0	0	0	No
DKIII.7	44	30	7	5	2	0	4	No
DKIII.8	57	50	1	0	1	0	0	No
DKIII.9	53	48	1	0	1	0	0	No
DKIII.11	41	34	1	1	0	0	1	No
HO1.1	52	30s	>20	>10	0	>10	1	Yes
HO1.2	49	46	>20	>20	0	0	0	No
JP1	39	27	>40	>20	>20	0	0	No
JP2	33	30	1	1	0	0	1	No
BE	27	9	>20	>10	>10	0	0	No

MA2.1 has developed more than 50 lesions on his face, ears, and neck, as his first one was diagnosed, with new ones appearing every few months. The most uncomfortable tumors were excised, whereas the others were left to heal spontaneously. Histology is not yet available on this subject.

The non-Scottish patients assessed included three members from an Italian family (Family CO, Table 2, Figure 3), 11 patients from a Danish family (Family DK, Table 2, Figure 4), two siblings from an English family (HO1.1 and HO1.2, Tables 2 and 3), one American (BE, Tables 2 and 4) and two unrelated Japanese patients (JP1 and JP2 Tables 2 and 4).

The CO family includes three patients, from three generations (Figure 3). The mean age of onset is 12 years (range, 8–18). A total of more than 50 tumors were observed in the patients and the average number of tumors per patient was more than 20. Tumors were located on their nose, front, ears, lower lip, chin, neck, and anterior chest, and most of them were excised (Table 2). These lesions were confirmed to have the histological appearances of a KA or a well-differentiated SCC.

The DK family comprises 11 patients, from three generations (Figure 4). The mean age of onset is 52.6 years (range, 30–81). A total of 44 tumors were observed in the patients

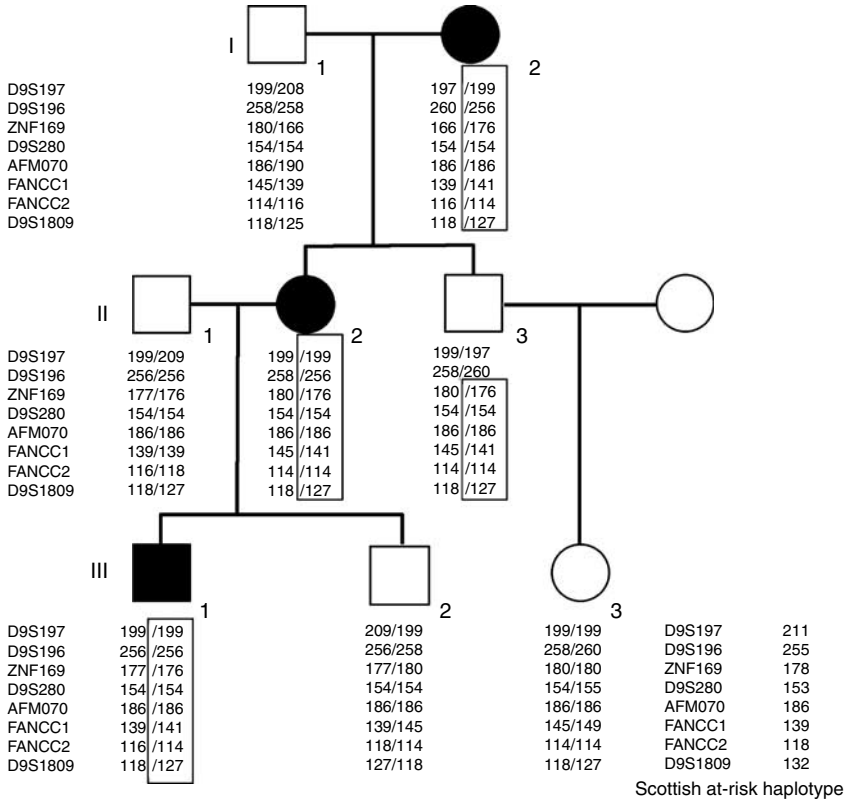

Figure 3. Haplotype analysis of an Italian MSSE family (CO) with eight informative markers. All the affected individuals have the same at-risk haplotype (boxed), which is not shared by unaffected members of the family.

and the average number of tumors per patient was four (range, 1–23). Tumors were located equally on the head (45.94%) and limbs (54.06%). The majority of tumors were excised (61.36%). Histological analysis was performed on 32 lesions and 21 of them were classified as KAs, whereas the remaining ones were described as well-differentiated SCCs.

The HO family includes two siblings, HO1.1 and HO1.2, who developed their first tumors in their late 30s and middle 40s, respectively. So far, they both have had more than 20 lesions, mainly located on their face and trunk (Table 2). Biopsies of some of these lesions showed histologic features of KA or well-differentiated SCC. The father of HO1.1 and HO1.2 was diagnosed with a metastatic SCC, with an unknown primary, at age 70.

The clinical features and histological analysis of JP1, JP2, and BE have been already presented and discussed in previous publications (Koga *et al.*, 2003; Kato *et al.*, 2004; Feldman and Maize, 2007). A brief summary is reported in Table 2.

If we compare the median age of onset in the different MSSE families, we can observe that family CO has the earliest age (12 years; Table 2), followed by the Scottish families (25.1 years; Figure 2). Both the families, DK and HO, have a much later median age of onset (52.6 and 40 years, respectively; Table 2). The distribution of the tumors is also quite different, with no lesion in the limbs reported in families CO and HO (Table 2), and no lesion in the trunk in family DK, in JP1 and BE. The number of lesions developed is also quite variable, with only one or two lesions reported for most family DK patients (with the exception of DKI.1, DKIII.3, DKIII.7; Table 2) and more than 20 lesions found in the affected patients of families CO and HO (Table 2).

Response to radiotherapy in the MSSE patients

Six of the MSSE patients described in this report underwent radiotherapy (MA1.2, COI.2, HO1.1, DKI.1, DKII.2, and DKIII.3).

COI.2 underwent radiation therapy on her face, but no records are available regarding the outcome of this treatment.

None of the three patients of family DK who underwent radiotherapy (DKI.1, DKII.2, and DKIII.3) had any sign of aggravation after the treatment.

In contrast, patients MA1.2 and HO1.1 developed multiple skin tumors in the radiation field. MA1.2 had a rapidly growing, moderately well differentiated, SCC removed from her left cheek in 1992. Although the excision margins were quite clear, lymphatic permeation was noted and radial radiotherapy was given, to prevent metastatic spread. Within two months, MA1.2 developed myriads of squamo-proliferative lesions in the irradiated area of the skin, which included her left cheek, the left side of her neck and her left pinna. The lesions were treated unsuccessfully with interferon alpha/2B and major reconstructive surgery was required following

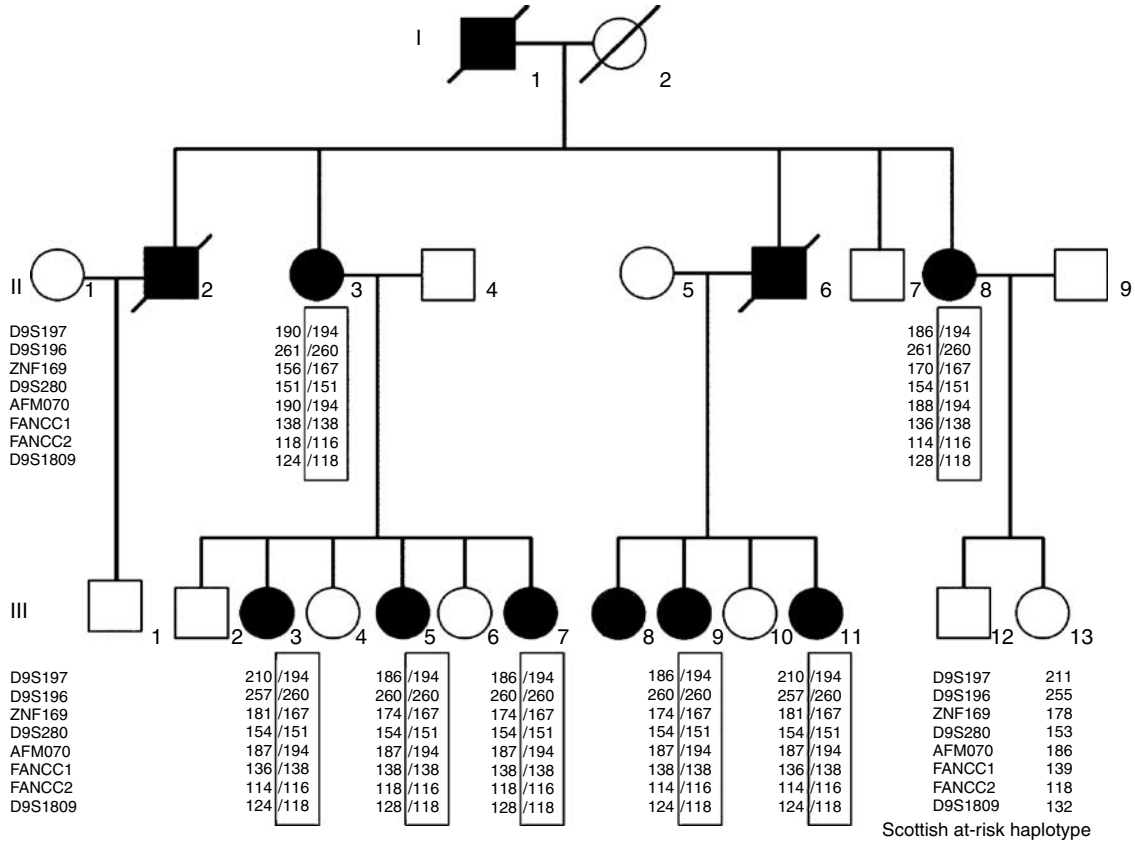

Figure 4. Haplotype analysis of a Danish MSSE family (DK) with eight informative markers. All the affected individuals have the same at-risk haplotype (boxed), which is not shared by unaffected members of the family.

Table 3. Haplotype analysis of two related Scottish patients (MA1.2 and MA2.1), with eight informative markers

	Scottish at risk haplotype	MA1.2	MA2.1
D9S197	211	208/210	208/210
D9S196	255	259	259
ZNF169	178	162	162
D9S280	153	153	153
AFM070	186	188/190	188/190
FANCC1	139	141	141
FANCC2	118	114	114
D9S1809	132	132	132

MA1.2 and MA2.1 share an identical at-risk haplotype, which is not the same of the other Scottish families (where the at-risk allele for a marker could not be determined, both alleles have been indicated).

excision of the affected area. Histology showed *de novo* SCCs rather than metastatic lesions.

At 49 years, HO1.1 developed a keratotic lesion inside his left nostril and underwent radiotherapy, which caused the development of multiple keratotic and ulcerated papules and plaques in the radiotherapy field as well as a yellowish plaque over the left cheek close to the site of the previous SCC excision. The patient started treatment with an oral RAR retinoid, acitretin (50 mg/day), 3 months after completion of radiotherapy, resulting in a complete remission.

None of the Scottish MSSE patients sharing the same at-risk haplotype have had radiation therapy in recent years.

Haplotype analysis in one MSSE family of Scottish origin

Haplotype analysis of a woman of Scottish origin and her son (MA1.2 and MA2.1, respectively), using the eight markers D9S197; D9S196; ZNF169; D9S280; AFM070; FANCC1; FANCC2; and D9S1809, demonstrated that their disease associated haplotype was different from the shared haplotype of the affected individuals in the other Scottish families (Table 3).

This could suggest that MSSE is not only caused by a single founder mutation in the Scottish population.

Haplotype analysis in MSSE families of non-Scottish origin

We performed haplotype analysis on MSSE families of non-Scottish origin with the eight markers D9S197; D9S196; ZNF169; D9S280; AFM070; FANCC1; FANCC2; and D9S1809. In particular, we genotyped family CO (a three-generation family from Italy) (Figure 3), seven of the affected members of family DK (a three-generation Danish family) (Figure 4), two affected siblings from London (HO 1.1 and

Table 4. Haplotype analysis of two British MSSE patients (HO1.1 and HO1.2), on two unrelated Japanese patients (JP1 and JP2), and an American patient (BE), with eight informative markers

	Scottish at-risk haplotype	HO1.1	HO1.2	JP1	JP2	BE
D9S197	211	209	209	198/208	198/208	208/209
D9S196	255	260	260	259/261	258/258	257/259
ZNF169	178	167	167	171/182	171/178	167/180
D9S280	153	153	153	153/153	135/153	149/153
AFM070	186	190/192	190/192	186/192	186/186	186/196
FANCC1	139	141/147	141/147	141/143	147/147	141/143
FANCC2	118	114	114	116/116	114/118	114/118
D9S1809	132	118/127	118/127	118/127	128/132	118/127

MSSE, multiple self-healing squamous epithelioma.
Note that none of the five patients share the same at-risk haplotype of the Scottish families. HO1.1 and HO1.2, who are siblings, share an identical at-risk haplotype (where the at-risk allele for a marker could not be determined, both alleles have been indicated).

HO 1.2; Table 4), one American patient (BE, Table 4) and two unrelated Japanese patients (JP1 and JP2; Table 4). The at-risk haplotypes for families CO (Figure 3, in box), DK (Figure 4, in box), and HO (Table 4) are different from each other and, more importantly, they are all different from the shared haplotype of the affected individuals in the Scottish families. The disease-associated haplotypes in BE, JP1, and JP2 could not be determined, because they were the only affected individuals of their families from whom we could obtain DNA samples. None of the three, however, share common alleles with the Scottish families for most of the markers analyzed, indicating that the disease has also arisen independently in these patients (Table 4). MSSE cannot therefore be solely caused by a single founder mutation and it is probably less rare than originally thought.

The three affected individuals of family CO share the same allele for the eight polymorphic markers linked to the MSSE locus. However, a recombination event between ZNF169 and D9S196 (Figure 3) suggests that the MSSE locus is most likely to lie proximal to ZNF169. Individual II-3 is in his early 40s and still unaffected. He has inherited the high-risk haplotype at ZNF169; D9S280; AFM070; FANCC1; FANCC2; D9S1809; and more distal markers, and the low-risk haplotype at D9S197; D9S196; and more proximal markers. Considering that 78% of affected individuals have developed a skin tumor by 40 years of age (Figure 2), and that CO II-3 is in his early 40s and is still unaffected, it is probable that this individual is unaffected and thus that the MSSE locus is proximal to ZNF169. This recombination event suggests that the MSSE locus may lie in an interval of <1 Mb (776 343 bp) between D9S197 and ZNF169.

The seven affected members analyzed in family DK all share the same haplotype for the eight markers listed above (Figure 4). There is no evidence of recombination between these makers and the MSSE locus. The segregation of the microsatellite markers in family DK is compatible with linkage to the MSSE locus (maximum lod score of 1.46 for marker ZNF169 at recombination fraction 0.0).

Identification of candidate genes in the MSSE locus and mutation analysis

We are currently sequencing 14 known genes (*NINJ1*, *WNK2*, *C9orf10*, *PHF2*, *BARX1*, *PTP9Q22*, *ZNF169*, *Q9UFB1*, *HIATL1*, *FBP2*, *FBP1*, *C9orf3*, *FANCC*, and *PTCH*) (http://genome.ucsc.edu/ and http://www.ensembl.org) from the MSSE candidate region between markers D9S197 and D9S1809 (Table 5 and Figure S1), in a group of nine patients representing MSSE families with different disease associated haplotypes (Sc1&2, MA1.2, COI.2, DKII.2, HO1.1, BE, JP1, and JP2).

We have already analyzed 12 of the candidate genes, including *NINJ1*, *WNK2*, *c9orf10*, *PHF2*, *BARX1*, and *PTP9Q22*, which are located within the narrower MSSE region, proximal to ZNF169, identified by the recombination event in COII-3. For each gene, we have sequenced every exon and at least 100 bp of the flanking introns (primers sequences provided as Table S1) in the nine members of the selected group. We have not detected a mutation, but we have identified several polymorphisms (45 in total) in most of the candidate genes (Table 5). Large deletions spanning a polymorphism can be excluded in families with an affected member heterozygous for those rearrangements.

We have also sequenced a cluster of three miRNA genes (*Let-7a-1*, *Let-7f-1*, or *Let-7d*) that lie in the interval between D9S196 and ZNF169, but did not identify any abnormality.

DISCUSSION

The great majority of MSSE cases have been attributed to a founder mutation that occurred in the west of Scotland at the end of the 18th century. Nine Scottish MSSE families were identified and linkage analysis showed that they were carrying the same at-risk haplotype, confirming the common origin of their mutation. We recently acquired patients of non-Scottish origin whose symptoms were very similar to those reported for MSSE. An exhaustive clinical and histological analysis further suggested that their clinical phenotype was effectively indistinguishable from MSSE. Linkage analysis confirmed that their at-risk haplotype

Table 5. Mutation screening of the candidate genes for MSSE

Gene	Function	Size	Polymorphism	Heterozygotes
NINJ1	Nerve injury-induced protein 1	12 791 bp (four exons)	A→G intron 3 A→G* exon 4 C→T* exon 4	MA, CO MA JP
WNK2	Protein kinase	135 462 bp (29 exons)	G→C intron 3 G→A* exon 7 A→T intron 8 G→A* exon 11 G→A* exon 11 C→T* exon 11 C→T intron 12 T→C intron 13 C→T* exon 19 G→A intron 26	MA, JP1, BE CO, BE Sc1&2, CO, JP1, HO BE Sc1&2, JP1, BE Sc1&2 CO CO BE BE
C9orf10	DNA polymerase	112 598 bp (18 exons)	NO polymorphism detected	
PHF2	PHD finger protein	102 750 bp (22 exons)	G→A intron 2 G→A intron 2 C→T intron 3 C→T exon 4 G→A intron 5 C→T intron 6 A→G intron 6 T→C intron 11 G→A intron 13 C→T intron 13 C→T intron 15 A→G intron 17 T→C intron 17 in frame del in exon 21 polyG exon 22 C→A exon 22 A→G exon 22 C→T exon 22 C→A exon 22	CO, MA, BE CO, HO, DK Sc1&2, CO, HO MA, BE MA Sc1&2, CO, HO, DK HO CO, BE, JP1, HO HO HO, DK MA, BE Sc1&2, CO, JP2 Sc1&2, CO, JP2 Sc1&2, Be, Co Sc1&2, CO, JP1 JP1, MA Sc1&2, CO, JP1, HO BE, MA Sc1&2, CO, HO
BARX1	Homeobox protein barh-like1	3431 bp (four exons)	T→C exon 4 G→A exon 4	BE, HO CO
PTP9Q22	Protein tyrosine phosphatase	79 060 bp (10 exons)	C→G intron 2	Sc1&2
ZNF169	Zinc finger protein	42 115 bp (six exons)	delCG exon 1 C→T* exon 4 C→G exon 6	MA MA Sc1&2
Q9UFB1	DNA binding protein	5988 bp (six exons)	A→G exon 6	Sc1&2, CO
HIATL1	Hippocampus abundant transcript-like1	19 898 bp (eight exons)	A→G intron 1 A→T intron 4 G→A intron 7	BE BE BE, HO
FBP2	Fructose 1,6-bisphosp iso2	35 071 bp (seven exons)		
FBP1	Fructose 1,6-bisphosphatase	36 381 bp (seven exons)		
C9orf3	Aminopeptidase	171 252 bp (15 exons)	NO polymorphism detected	
FANCC	Fanconi anemia group C protein	147 585 bp (15 exons)	NO polymorphism detected	
PTCH	Patched protein homolog 1	63 743 bp (25 exons)	C→G intron 2 C→G intron 11 delT exon 25	Sc1&2 Sc, MA Sc1&2, MA,

MSSE, Multiple self-healing squamous epithelioma.
Fourteen genes are mapped within the MSSE candidate locus. The 12 genes we have analyzed are in bold. Sc1&2 are a father and a son from one of the Scottish families with the same at-risk haplotype. The nucleotide changes marked with an asterisk are non-synonymous. Novel single nucleotide polymorphisms are underlined.

segregated with the condition. The at-risk haplotype of family CO, however, was different from the one of family DK and HO, and, more importantly, they were all different from the shared haplotype of the affected individuals in the original Scottish families. MSSE is therefore not solely caused by a founder mutation and is less rare than originally thought.

The diverse genetic basis of this condition has been further confirmed by haplotype analysis of an additional family with the MSSE phenotype originating from the west of Scotland (MA1.2 and MA2.1), who has a different disease-associated haplotype. It seems therefore that MSSE is not after all caused by a single founder mutation even among the Scottish population. This could explain why two of the 11 Scottish families used for linkage analysis have different at-risk alleles for some of the markers analyzed in previous studies (Goudie *et al.*, 1993, Richards *et al.*, 1997).

The disease-associated haplotypes in the American and Japanese patients pedigrees could not be determined unambiguously, because samples for molecular genetic analysis were only available from one affected member of each family. JP1, JP2, and BE, however, have typical MSSE clinical features and they are most likely to have independent mutations in the MSSE gene, although genetic heterogeneity cannot be ruled out. JP1 and JP2 do not share common alleles at most marker loci, providing little evidence for a founder mutation in Japan.

We think it is extremely important for physicians to be aware that MSSE is more common, with a wider geographical distribution than was originally thought, and that the condition may be under recognized. It is indeed very difficult to distinguish between an individual skin lesion caused by MSSE and a KA or a SCC. The literature is divided as to whether MSSE lesions have unique distinguishing histological features or lie on a spectrum between a KA and a well-differentiated SCC (Clausen *et al.*, 2006). Features may vary depending on the stage of the lesion at the time of excision and on the body site (reviewed by McKee et al., 2005; Weedon, 2002). Early lesions are in keeping with well-differentiated SCC. Within 2–4 months, lesions regress and the infiltrating columns may become increasingly keratinized and discharge a keratin plug or they may undergo necrosis. A dense infiltrate of lymphocytes may be seen, but their role in relation to regression is unknown. Ultimately a fibrous scar forms. Local invasion occurs in vertical columns, keratinization is prominent, mitoses are present in low numbers and lymphatic invasion is rare.

The phenotypic variability among the MSSE families, including age of onset, tumors number, and distribution, might imply the existence of mutations of different severity within the same MSSE gene. But the condition can also vary in severity between affected members of the same family, suggesting that other non-genetic factors can affect the phenotype. The age of onset of the condition, for example, is very variable in the Scottish families with the same at-risk haplotype, where 20% of patients have not developed their first tumor by 50 years of age (Figure 2). MSSE patients in family DK have a milder phenotype, with a late age of onset and, in some cases, only one lesion (DKII.2, DKII.3, DKIII.5, DKIII.8, DKIII.9, DKIII.11), but they all share the same at-risk haplotype, compatible with linkage to the MSSE locus. Late age of onset is typical of the Grzybowski type of KA, but this is a rare sporadic disorder, characterized by hundreds of eruptive follicular papules (Consigli *et al.*, 2000, Haas *et al.*, 2002).

It should be noted that six of the patients described in this report (MA1.2, COI.2, HO1.1, DKI.1, DKII.2, and DKIII.3) had radiotherapy and that two of them (MA1.2 and HO1.1) subsequently developed multiple skin tumors in the radiation field (no records are available for patient COI.2). Radiotherapy might therefore aggravate the MSSE phenotype, as previously indicated by a case of fatal local invasion in a patient who underwent radiation therapy (Chakrabarty and Perks, 1996). Although family DK appears to have a milder phenotype and have not had adverse reactions to radiotherapy, linkage studies suggest that their condition is attributable to a mutation in the MSSE locus. None of the Scottish MSSE patients have had radiation therapy in recent years.

It is our current priority to identify the candidate gene for MSSE, which could be a tumor suppressor gene, as indicated by studies on loss of heterozygosity within the MSSE region (Bose *et al.*, 2006). We have sequenced a cluster of three miRNA genes (*Let-7a-1*, *Let-7f-1*, or *Let-7d*) and the coding regions of 12 of the 14 genes within the 4 cM locus of MSSE (*NINJ1*, *WNK2*, *C9orf10*, *PHF2*, *BARX1*, *PTP9Q22*, *ZNF169*, *Q9UFB1*, *HIATL1*, *C9orf3*, *FANCC*, and *PTCH*), and found several rearrangements. Large deletions spanning a polymorphism could be excluded in families with an affected member heterozygous for those rearrangements.

We hope that the here reported description of the common clinical features in MSSE, including the type, number, and location of tumors, will serve to aid and improve the accuracy of its diagnosis.

MATERIALS AND METHODS

Patients and samples

All aspects of this study were carried out in accordance with the Declaration of Helsinki Principles and complied with the requirements of the local institutional ethical review boards. All individuals gave their fully informed consent for blood and tissue sample collection. DNA was extracted from 186 individuals, including 64 affected and 110 unaffected or at-risk members from 11 Scottish MSSE families, one Italian (CO), one Danish (DK), and one English family (HO), plus 12 isolated MSSE cases. All the affected individuals were examined for signs of active or healed skin tumors by one of the authors. A detailed family history and a history of the age of onset, distribution and clinical course of skin lesions were obtained from each patient.

Histological analysis of MSSE tumors

Skin biopsies were obtained with informed consent from the MSSE patients involved in this study, and processed by standard methods through formalin fixation and embedding in paraffin wax. Sections (5 μm) were cut, mounted on slides, and stained with hematoxylin and eosin.

Linkage studies and haplotype analysis

DNA from patients and their families was amplified for the microsatellite markers D9S197; D9S196; ZNF169; D9S280; AFM070; and D9S1809 (Figure S1) using published primers fluorescently labelled (http://www.gdb.org). PCR reactions were performed in a total volume of 12.5 μl containing 75 ng of DNA,

42 ng of each primer, 2 mM deoxynucleoside triphosphates, and 1 U of HotStarTaq DNA Polymerase (QIAGEN Ltd, West Sussex, UK). After 15 minutes of denaturation at 94°C, samples were amplified for 40 cycles of 30 seconds at 94°C, 1 minute at 58°C, and 2 minutes at 72°C, using a Biometra PCR machine (Whatman-Biometra, Goettingen, Germany). Two CA repeats in intron 1a and intron 2 of the *FANCC* gene (Savoia *et al.*, 1996) were amplified using the above reagents and PCR conditions. PCR products were directly sequenced using BigDye terminator (Applied Biosystems, Warrington, UK) and analyzed by capillary electrophoresis on an ABI 3100 Genetic Analyzer (PerkinElmer, Boston, MA). Lod score on family DK was calculated using the MLINK algorithm version of LINKAGE 5.1.

Mutation analysis

Using the program OLIGO 4.0, we designed forward and reverse intronic primers to amplify every exon and at least 100 bp of the flanking introns of all the candidate genes. Primers sequences and annealing temperatures are provided as Supplementary Data (Table S1). PCR reactions were performed as above indicated for the haplotype analysis. PCR products were sequenced with an ABI 3100 Genetic Analyzer (PerkinElmer, Boston, MA), using their respective forward and reverse primers.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. List of primers used for mutation analysis in the MSSE region.

Figure S1. The MSSE candidate region.

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