the pathogenesis of both CD and HS. Early detection and treatment of CD in patients with HS may prevent late complications. Therefore, clinicians should consider whether HS patients may have undiagnosed IBD, especially CD, and suggest an appropriate screening and treatment.

CONFLICT OF INTEREST

ADC served as an advisor, investigator, or speaker for Abbvie, BI Dexcel Pharma, Janssen, Novartis, Perrigo, Pfizer, and Rafa. The authors state no conflict of interest.

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Somatic Embryonic *FGFR2* Mutations in Keratinocytic Epidermal Nevi

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TO THE EDITOR

Keratinocytic epidermal nevi (KEN) frequently harbor somatic mutations in *FGFR3*, *PIK3CA*, *HRAS*, *NRAS*, and *KRAS*. We performed whole exome sequencing (WES) to discover additional causes of KEN in lesions that were wild type for hotspot codons of these genes. We now report somatic mutations in fibroblast growth factor receptor 2 (*FGFR2*) in 4 of 23 KEN. Existing evidence and computational analyses of the predicted mutations found in KEN strongly support their pathogenic role. We estimate that 5–10% of KEN are caused by embryonic *FGFR2* mutations. Our findings emphasize the notion that multiple genes involved in cell signaling can contribute to KEN.

Epidermal nevi (EN) are hamartomatous proliferations of the skin epithelium, including keratinocytes, sebocytes, pilosebaceous units, and eccrine or apocrine glands. EN includes nonorganoid KEN, nevus sebaceous, and nevus comedonicus. Approximately 40% of KEN harbor postzygotic activating mutations in *FGFR3* and *PIK3CA* (Hafner et al., 2007; Hernandez et al., 2007) and an additional 40% are caused by postzygotic activating *RAS* mutations, with a predominance of the *HRAS* G13R substitution (Hafner et al., 2012).

To identify additional genes causing KEN, we performed WES of the skin lesion and blood leukocytes from three patients whose KEN was wild type for mutational hotspots of *FGFR3, PIK3CA*, and *RAS* (Hafner et al., 2012). The study was approved by the ethics committees of the participating institutions and performed according to the Declaration of Helsinki. All patients, or their legal representative if minor, gave written informed consent to the study and gave permission to publish images and case histories. WES disclosed two different *FGFR2* mutations in the lesions of two of three patients that were undetectable in leukocyte DNA at the sequencing depth used. Patient 1 harbored a p.Y376C (c.1127A>G) (exon 9) mutation, whereas patient 2 harbored a p.S252W (c.755C>G) (exon 6) mutation (Figure 1). Additional details of the WES analyses are provided as Supplementary Materials online. Both mutations and their somatic nature were validated using Sanger sequencing of both strands of amplicons (Figure 1C and G, Supplementary Figure S1 online). The p.Y376C (c.1127A>G) and p.S252W (c.755C>G) mutations are predicted as damaging according to several bioinformatics algorithms (i.e., sorting intolerant from tolerant prediction tool, Polyphen-2, Mutation

Abbreviations: EN, epidermal nevi; FGFR2, fibroblast growth factor receptor 2; KEN, keratinocytic epidermal nevi; WES, whole exome sequencing

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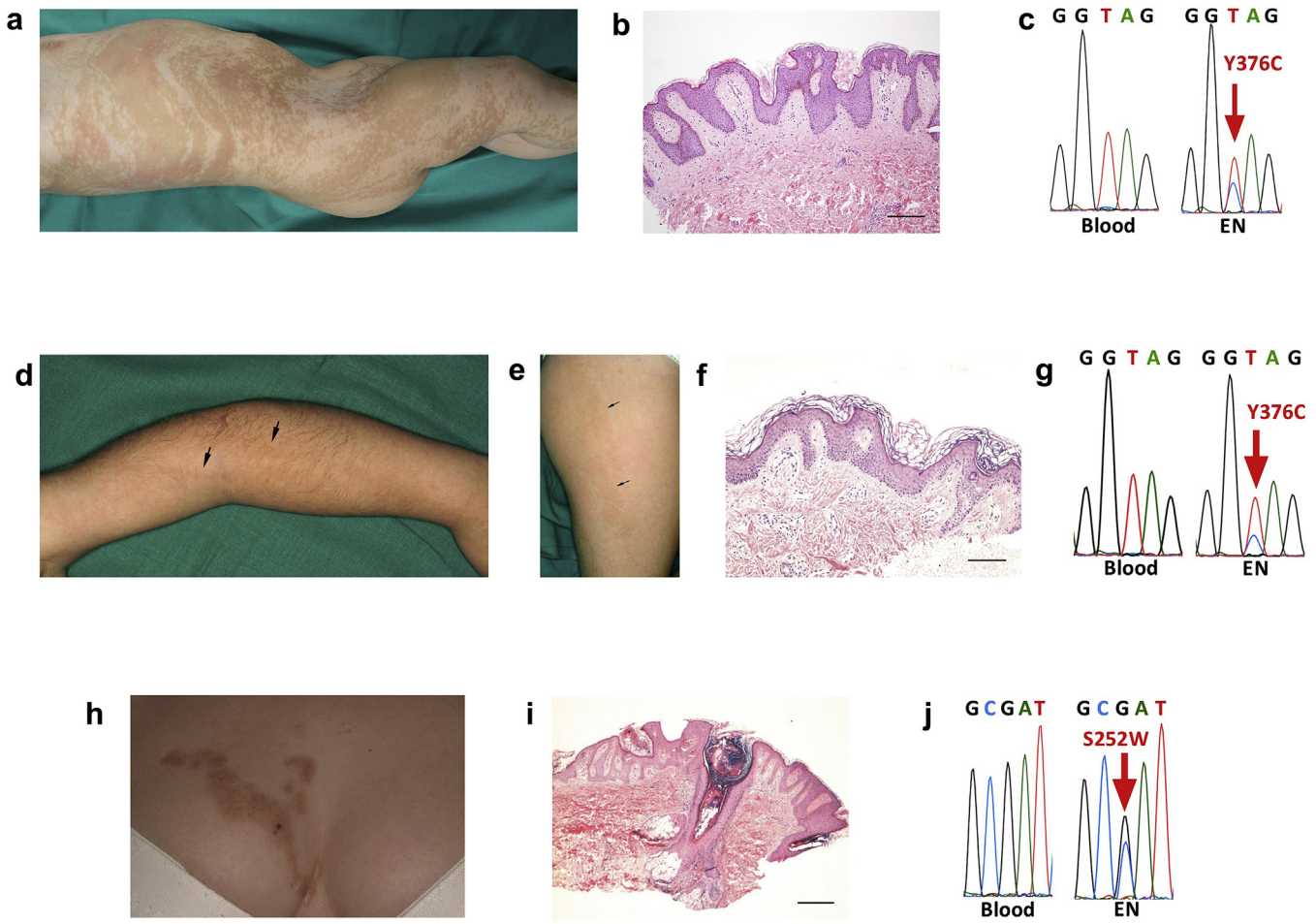

Figure 1. Clinical, histological, and molecular features of patients 1, 2, and 4. Patient 1 (**a**–**c**): 5-year-old girl with a widespread keratinocytic epidermal nevus (KEN) involving the head, extremities, and trunk. She also had twisted hair and a bulging right eye (**a**). Histologically conventional KEN; scale bar = 250 μm (**b**). Whole exome sequencing (WES) and Sanger sequencing revealed an *FGFR2* p.Y376C (c.1127A>G) mutation in the skin (**c**). Patient 4 (**d**–**g**): 12-year-old girl with an extensive epidermal nevi (EN) involving the right leg, the left arm, the whole right arm, and the chest. She also showed bilateral syndactyly of the second and third toes. Multiple cutaneous capillary vascular malformations could be observed in the philtrum, glabella, upper lip, and cervical and sacral regions. She also had macrocephaly. Thus, she fulfilled diagnostic criteria of macrocephaly-capillary malformation syndrome (**d**, **e**). Histologically conventional KEN; scale bar = 100 μm (**f**). Sanger sequencing revealed a *FGFR2* p.Y376C (c.1127A>G) mutation in the skin (**g**). Patient 2 (**h**–**j**) 27-year-old woman with a KEN involving the right mammary region (**h**). Histopathological analysis showed epidermal acanthosis, papillomatosis, and a dilated follicular infundibulum filled with keratin; scale bar = 500 μm (**i**). WES and Sanger sequencing revealed an *FGFR2* p.S252W (c.755C>G) mutation (**j**).

Assesor, and functional analysis through hidden Markov models) (Supplementary Table S1 online).

We then assessed the prevalence of *FGFR2* mutations in 20 additional KEN that were wild type for the genes known to cause EN. We used Sanger sequencing to interrogate exons 6, 9, 12, and 14, where the most common hotspot somatic mutations have been reported in human cancer (http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=FGFR2#histo). The clinical-pathological characteristics of these patients are summarized in Table 1. Two additional *FGFR2*-mutant KEN were identified. Patient 4 had a widespread KEN, with a phenotype consistent with macrocephaly-capillary malformation, and harbored a somatic p.Y376C (c.1127A>G) *FGFR2* mutation (Figure 1G). Patient 5 had a conventional KEN, involving the head and neck region, harboring a p.P286S (c.857C>T) somatic mutation (Supplementary Figure S2 online). Altogether, we identified *FGFR2* mutations in 4 of the 23 samples tested.

To assess whether the somatic mutations were present at lower allelic frequency in leukocyte DNA, we used targeted resequencing of *FGFR2* in three of the mutant cases at >1,000 depth. In patients 2 and 4, no allelic variants were detected. By contrast, in patient 1 the c.1127A>G substitution was found at allelic frequencies of 35.85% and 3.37% in DNA from EN and leukocytes, respectively, indicating wider tissue mosaicism (Supplementary Table S2 and Supplementary Figure S3 online).

The mutations identified, or their equivalent positions in other FGFR genes/isoforms, have been reported to occur in cancer (Supplementary Table S3 online), supporting their damaging potential. To gain further insight into their putative functional effects, we mapped the predicted amino acid substitutions onto a structural model of the extracellular ligand-binding domains of a dimeric 2:2:2 FGFR2IIIb:FGF10:heparin ternary complex (Supplementary Materials for details). FGF ligands interact with the second and third Ig domains (hereafter referred to as D2 and D3) and with the

Table 1. Clinical characteristics of the KEN cases included in this study and mutation findings

Patient	Age	Gender	Histology	Localization	Extracutaneous anomalies	Exons sequenced	Sequence
1 (ATN 10)	5	F		Head, trunk, extremities (right > left)	YES[1]	All (WES)	p.Y376C[3](c.1127A>G)
2 (ATN 30)	27	F	KEN with comedos	Breast	NO	All (WES)	p.S252W(c.755C>G)
3 (ATN 5)	8	F	Verrucous KEN	Left hemiface	NO	All (WES)	WT
4 (ATN 29)	12	F		External area right leg, internal area left arm, right arm, chest	YES[2]	6, 9	p.Y376C(c.1127A>G)
5 (EN 4120/96)	16	F	KEN	Trunk (Blaschko)	NO	6, 9	p.P286S(c.857C>T)
6 (13084/07)	22	F	KEN	Trunk (Blaschko)	NO	6, 9, 12, 14	WT
7 (EN 12003/08)	16	M	Pigmented KEN	Neck		6, 9, 12, 14	WT
8 (EN 20162/01)	17	M	Pigmented KEN	Head	na	6, 9, 12, 14	WT
9 (EN 1173/98)	28	F	KEN	Neck	na	6, 9, 12, 14	WT
10 (EN 18563/00)	7	M	KEN	Head	na	6, 9, 12, 14	WT
11 (35901-08)	7	F	KEN	Head	na	6, 9, 12, 14	WT
12 (31753-08)	11	M	KEN with lymphatic vessel	Trunk	na	6, 9, 12, 14	WT
13 (256812-05)	15	M	Verrucous KEN	Head	na	6, 9, 12, 14	WT
14 (249388-05)	13	M	Verrucous KEN	Head	na	6, 9, 12, 14	WT
15 (242030-05)	1	F	Verrucous KEN	Arm	na	6, 9, 12, 14	WT
16 (20002-01)	16	F	KEN	Neck	na	6, 9, 12, 14	WT
17 (180780-05)	18	F	Verrucous KEN	Lower leg	na	6, 9, 12, 14	WT
18 (15012-99)	9	F	Irritated KEN	Head	NO	6, 9, 12, 14	WT
19 (13897-04)	16	F	na	Right arm	NO	6, 9, 12, 14	WT
20 (15190-99)	1	F	KEN	Head	NO	6, 9, 12, 14	WT
21 (567449-09)	13	F	Verrucous KEN	Head	na	6, 9, 12, 14	WT
22 (256812-05)	15	M	Verrucous KEN	Head	na	6, 9, 12, 14	WT

Abbreviations: KEN, keratinocytic epidermal nevus; na, no available information; WES, whole exome sequencing; WT, wild type.
[1]Twisted hair, right eye bulging.
[2]Macrocephaly + Arnold Chiari type II malformation + toe syndactyly + scoliosis + overgrowth of hands and feet + capillary malformation.
[3]The amino acid numbering refers to the FGFR2IIIb isoform, RefSeq ID: NM_022970.3, and Ensembl Transcript ID: ENST00000457416, which is the main transcript expressed in KEN.

interconnecting D2-D3 linker region of FGFR. The p.S252W (c.755C>G) mutation is localized in the D2-D3 linker and can alter the ligand-binding specificity, being classified as an activating damaging mutation. The p.P286S (c.857C>T) mutation—which has not been reported previously—is also positioned in D3 in the ligand-binding site and is in a position equivalent to that of the p.P283S mutation in FGFR3b. The structure-PPi system (http://structureppi.bioinfo.cnio.es/Structure) suggests that this mutation could also have an activating effect through changes in ligand-binding specificity. Pro286 is a strictly conserved residue among members of the human FGFR family and the p.P286S (c.857C>T) mutation is predicted to destabilize the structure of the D3 domain in FGFR2 and to increase the binding affinity of FGF10 (Supplementary Figure S4 online). The p.Y376C (c.1127A>G) mutation localizes to a segment between D3 and the transmembrane region that is not included in the structural model. Bioinformatics analysis classified this mutation as harmful (Supplementary Table S1).

The experimental evidence, in silico modeling, and genotype/phenotype correlations strongly support the notion that the three FGFR2 mutations that we have found in KEN are pathogenic (Supplementary Table S1, Supplementary Figure S4). Moreover, these—or equivalently positioned—mutations are recurrently found in several cancers, such as endometrial, breast, and bladder cancers (Byron et al., 2012) (Supplementary Table S3).

Patient 4 fulfilled criteria of macrocephaly-capillary malformation [OMIM 602501]. This syndrome consists of megalencephaly, neonatal hypotonia, growth dysregulation with asymmetry, vascular anomalies, distal limb malformations (syndactyly and polydactyly), cortical malformation, and a mild connective tissue dysplasia. Although infrequent, KEN may occur in patients with macrocephaly-capillary malformation (Katugampola et al., 2008). Postzygotic mosaic mutations in several genes involved in the PI3K pathway (*AKT3*, *PIK3CA,* and *PIK3R2*) have recently been reported in overgrowth syndromes (Riviere et al., 2012). However, 25% of patients with macrocephaly-capillary malformation lack mutations in these genes (Riviere et al., 2012) and our findings support the concept that somatic mutations in genes coding for upstream PI3K activators may lead to a similar clinical phenotype.

Patient 1 had a KEN, unilateral exophthalmia, and a p.Y376C (c.1127A>G) mutation in mosaicism in the skin and blood (Supplementary Table S4 online). This mutation has been reported in the germline in Beare-Stevenson syndrome [OMIM 123790] that associates craniosynostosis, ocular proptosis, cutis gyrata, acanthosis nigricans, prominent umbilical stump, furrowed palms and soles, and

anogenital anomalies. Thus, the patient may be considered a mosaic form of the syndrome.

Patient 2 had a KEN carrying the p.S252W (c.755C>G) mutation (Figure 1h–j). This mutation causes Apert syndrome [OMIM 101200] when present in the germline and can also be found in its mosaic state, unilateral/segmental acneiform nevus (Munro's acne nevus) (Melnik et al., 2008; Munro and Wilkie, 1998). Both conditions show acneiform lesions. Intriguingly, the lesion in patient 2 showed histological features reminiscent of acne, with dilated follicular infundibula (Melnik et al., 2008). A 13-year-old boy with acne within a pre-existing KEN, with no genotype studies, has been reported (Hivnor et al., 2007). These observations suggest that some KEN share histological and genetic changes with mosaic acne and Apert syndrome.

The phenotypic pleiotropy described above for the *FGFR2* mutations parallels previous observations regarding *HRAS* mutations that can occur both in KEN and in Costello syndrome (frequently showing acanthosis nigricans) (Gripp et al., 2006; Hafner et al., 2011). Altogether, these findings suggest that the cellular compartments (i.e., progenitor vs. stem cells) and cell types (epithelial and/or mesenchymal) affected by the mutation may determine, among others, the clinical presentation.

In conclusion, we report that 5–10% of EN harbor embryonic postzygotic *FGFR2* activating mutations. Overall, the genetic cause of 80–90% of KEN is thus identified as an activating mutation in an oncogene. The products of these genes are positioned from the cell membrane to various tiers of intracellular signaling. Therefore, we propose to extend the concept of "mosaic RASopathy" to one of "mosaic signalopathy" as the general cause of KEN and related conditions.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
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