(degree and duration of gluten restriction or dapsone/sulfapyridine dose); however, future studies should attempt to correlate levels of IgA anti-eTG with parameters of disease activity, including degree and duration of gluten restriction, and dapsone/sulfapyridine dose. The patient presented in Table 2 shows that the levels of each Ab assay decreases over time with adherence to a gluten-free diet. Although definitive conclusions cannot be made from one patient, these results suggest that the levels of IgA against eTG correlate with dietary gluten intake. Prospective studies should be designed to evaluate the levels of IgA anti-eTG in DH patients over time after initiation of a gluten-free diet.

IgA anti-eTG was present in only 11.1% of pediatric CD, which is significantly lower than that reported in adult CD (~50%) (Hull *et al.*, 2008). These results are similar to our previously published data showing a low prevalence of IgA anti-eTG in pediatric CD patients. This occurred in the setting of significantly elevated concentrations of IgA anti-tTG (90.7%). The majority of the six pediatric CD patients with IgA anti-eTG$^+$ results showed low titers (13, 13, 14, 15, 20, and 96 Units) and all were duodenal biopsy positive (1 of 18 Marsh 3a (5.6%), 1 of 15 Marsh 3b (6.7%), and 4 of 16 Marsh 3c (25.0%)). All six pediatric CD patients were also positive for IgA anti-tTG (6 of 6), for IgA anti-deamidated gliadin peptides (6 of 6), and for IgG anti-deamidated gliadin peptides (5 of 6). We will follow-up pediatric CD patients with IgA anti-eTG$^+$ results to find out whether they develop symptoms of DH.

In conclusion, IgA anti-eTG was more sensitive in detecting DH than any other marker associated with gluten-sensitive enteropathy and its prevalence is significantly lower in pediatric CD than that which has been reported in adult CD. In a patient suspected of having DH, the present data support testing for IgA anti-eTG, which may help in screening and monitoring the response to a gluten-free diet. We then recommend a biopsy of uninvolved, perilesional skin in an area of grouped vesicles or erosions for direct immunofluorescence, which is the gold standard for diagnosis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Troy D. Jaskowski[1], Tracy Hamblin[2], Andrew R. Wilson[1], Harry R. Hill[1,3,4,5], Linda S. Book[6], Laurence J. Meyer[2,7], John J. Zone[2] and Christopher M. Hull[2]

[1]Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology, Salt Lake City, Utah, USA; [2]Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah, USA; [3]Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah, USA; [4]Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, USA; [5]Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah, USA; [6]Department of Pediatric Gastroenterology, University of Utah School of Medicine, Salt Lake City, Utah, USA and [7]Salt Lake City Department of Veterans Affairs, Salt Lake City, Utah, USA
E-mail: jaskowtd@aruplab.com

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The Absence of *BRAF*, *FGFR3*, and *PIK3CA* Mutations Differentiates Lentigo Simplex from Melanocytic Nevus and Solar Lentigo

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TO THE EDITOR

Lentigo simplex (LS) is a benign skin lesion preferentially observed in younger people, although it may occur at any age. The lesion measures a few millimeters in diameter, is brown to black, and is clinically indistinguishable from a melanocytic nevus. Histopathologically, LS shows a moderate elongation of the epidermal rete ridges and basal hyperpigmentation. The number of melanocytes in the basal epidermal layer may be slightly increased. In contrast to LS, solar lentigo displays markedly elongated rete ridges and profound actinic elastosis in the dermis. It has been proposed that LS may

Abbreviations: LS, lentigo simplex

evolve into a lentiginous/junctional melanocytic nevus when melanocytes start proliferating and aggregating to form small nests in the junctional zone, whereas solar lentigo may be a precursor lesion of seborrheic keratosis (Ackerman and Ragaz, 1984). In the past few years, it has become well known that melanocytic nevi frequently show the *BRAF* V600E hotspot mutation (Pollock *et al.*, 2003). The genetic basis of LS, however, remains unknown.

To investigate a possible common genetic background of LS and melanocytic nevi, we analyzed both LS and melanocytic nevi for the *BRAF* V600E mutation. We retrieved 58 LS samples (from 30 males, 28 females; mean age 34.6 years) and 46 melanocytic nevi (from 21 males, 25 females; mean age 38.7 years) from the histological files of the Department of Dermatology at the University of Regensburg, Germany. The characteristics of the patients and lesions are shown in Table 1. Written, informed consent was obtained from all patients according to the guidelines of the local ethics committee and the Declaration of Helsinki, and the study was approved by the ethics committee of the University Clinic of Regensburg. The samples were manually microdissected from formalin-fixed paraffin-embedded material, and DNA was isolated using standard protocols. For the analysis of the *BRAF* V600E hotspot mutation, a highly sensitive allele-specific PCR (PCR amplification of specific alleles, PASA) was used as described previously (Stoehr *et al.*, 2004). None of the 58 LS specimens exhibited the *BRAF* V600E mutation (Table 1, Figure 1a). The HT29 colon carcinoma cell line with a known *BRAF* V600E mutation was used as a positive control

Table 1. Analysis of *BRAF*, *FGFR3*, and *PIK3CA* in lentigo simplex and melanocytic nevus

No.	Sex	Age	Diagnosis	*BRAF*	*FGFR3*	*PIK3CA*
1	f	34	Lentigo simplex	wt	wt	wt
2	f	28	Lentigo simplex	wt	wt	wt
3	f	55	Lentigo simplex	wt	wt	wt
4	m	23	Lentigo simplex	wt	wt	wt
5	f	33	Lentigo simplex	wt	wt	wt
6	f	35	Lentigo simplex	wt	wt	wt
7	f	45	Lentigo simplex	wt	wt	wt
8	m	16	Lentigo simplex	wt	wt	wt
9	m	18	Lentigo simplex	wt	wt	wt
10	m	15	Lentigo simplex	wt	wt	wt
11	m	40	Lentigo simplex	wt	wt	wt
12	m	17	Lentigo simplex	wt	wt	na
13	f	27	Lentigo simplex	wt	wt	wt
14	m	29	Lentigo simplex	wt	wt	wt
15	f	38	Lentigo simplex	wt	wt	na
16	m	39	Lentigo simplex	wt	wt	wt
17	f	35	Lentigo simplex	wt	wt	wt
18	f	21	Lentigo simplex	wt	wt	wt
19	f	32	Lentigo simplex	wt	wt	wt
20	f	72	Lentigo simplex	wt	wt	wt
21	m	38	Lentigo simplex	wt	wt	wt
22	f	27	Lentigo simplex	wt	wt	na
23	f	42	Lentigo simplex	wt	wt	na
24	m	30	Lentigo simplex	wt	wt	na
25	m	56	Lentigo simplex	wt	wt	wt
26	f	42	Lentigo simplex	wt	wt	wt
27	m	22	Lentigo simplex	wt	wt	na
28	m	19	Lentigo simplex	wt	wt	wt
29	m	35	Lentigo simplex	wt	wt	na
30	f	28	Lentigo simplex	wt	wt	na

Table 1 Continued on the following page

Table 1. Continued

No.	Sex	Age	Diagnosis	*BRAF*	*FGFR3*	*PIK3CA*
31	m	24	Lentigo simplex	wt	wt	na
32	f	24	Lentigo simplex	wt	wt	wt
33	m	25	Lentigo simplex	wt	wt	na
34	m	52	Lentigo simplex	wt	wt	wt
35	m	51	Lentigo simplex	wt	wt	wt
36	f	23	Lentigo simplex	wt	wt	wt
37	m	26	Lentigo simplex	wt	wt	wt
38	m	43	Lentigo simplex	wt	wt	wt
39	f	40	Lentigo simplex	wt	wt	wt
40	f	60	Lentigo simplex	wt	wt	wt
41	m	62	Lentigo simplex	wt	wt	na
42	m	21	Lentigo simplex	wt	wt	wt
43	m	14	Lentigo simplex	wt	wt	wt
44	m	75	Lentigo simplex	wt	wt	wt
45	f	44	Lentigo simplex	wt	wt	wt
46	m	42	Lentigo simplex	wt	wt	wt
47	f	23	Lentigo simplex	wt	wt	wt
48	f	37	Lentigo simplex	wt	wt	wt
49	f	40	Lentigo simplex	wt	wt	wt
50	m	34	Lentigo simplex	wt	wt	wt
51	m	27	Lentigo simplex	wt	wt	wt
52	f	12	Lentigo simplex	wt	wt	wt
53	m	39	Lentigo simplex	wt	wt	wt
54	m	36	Lentigo simplex	wt	wt	wt
55	f	32	Lentigo simplex	wt	wt	wt
56	f	22	Lentigo simplex	wt	wt	wt
57	f	60	Lentigo simplex	wt	wt	wt
58	m	30	Lentigo simplex	wt	wt	wt
1	m	51	Lentiginous melanocytic nevus	wt	wt	wt
2	m	43	Lentiginous melanocytic nevus	wt	wt	wt
3	m	67	Lentiginous melanocytic nevus	wt	wt	wt
4	m	39	Lentiginous melanocytic nevus	wt	wt	wt
5	m	28	Lentiginous melanocytic nevus	wt	wt	wt
6	f	17	Lentiginous melanocytic nevus	wt	wt	wt
7	m	65	Lentiginous melanocytic nevus	V600E	wt	wt
8	f	29	Lentiginous melanocytic nevus	V600E	wt	wt
9	m	31	Lentiginous melanocytic nevus	wt	wt	wt
10	m	58	Junctional melanocytic nevus	wt	wt	wt
11	f	24	Junctional melanocytic nevus	wt	wt	wt
12	m	43	Junctional melanocytic nevus	wt	wt	wt
13	m	48	Compound melanocytic nevus	wt	wt	wt
14	f	60	Compound melanocytic nevus	wt	wt	wt
15	f	12	Compound melanocytic nevus	V600E	wt	wt

Table 1 Continued on the following page

Table 1. Continued

No.	Sex	Age	Diagnosis	*BRAF*	*FGFR3*	*PIK3CA*
16	f	60	Compound melanocytic nevus	V600E	wt	wt
17	m	42	Compound melanocytic nevus	wt	wt	wt
18	f	26	Compound melanocytic nevus	wt	wt	wt
19	f	52	Compound melanocytic nevus	V600E	wt	wt
20	m	23	Compound melanocytic nevus	V600E	wt	wt
21	f	35	Compound melanocytic nevus	V600E	wt	wt
22	m	32	Compound melanocytic nevus	wt	wt	wt
23	f	22	Compound melanocytic nevus	V600E	wt	wt
24	m	34	Dermal melanocytic nevus	V600E	wt	wt
25	f	36	Dermal melanocytic nevus	V600E	wt	wt
26	f	10	Dermal melanocytic nevus	V600E	wt	wt
27	f	73	Dermal melanocytic nevus	V600E	wt	wt
28	f	56	Dermal melanocytic nevus	V600E	wt	wt
29	m	54	Dermal melanocytic nevus	wt	wt	wt
30	f	56	Dermal melanocytic nevus	V600E	wt	wt
31	m	26	Dermal melanocytic nevus	V600E	wt	wt
32	m	48	Dermal melanocytic nevus	V600E	wt	wt
33	f	24	Dermal melanocytic nevus	V600E	wt	wt
34	f	19	Dermal melanocytic nevus	V600E	wt	wt
35	f	29	Dermal melanocytic nevus	V600E	wt	wt
36	f	12	Dermal melanocytic nevus	V600E	wt	wt
37	m	36	Dermal melanocytic nevus	V600E	wt	wt
38	f	16	Dermal melanocytic nevus	wt	wt	wt
39	m	64	Dermal melanocytic nevus	V600E	wt	wt
40	m	36	Dermal melanocytic nevus	V600E	wt	wt
41	f	47	Dermal melanocytic nevus	V600E	wt	wt
42	f	45	Dermal melanocytic nevus	V600E	wt	wt
43	f	13	Dermal melanocytic nevus	wt	wt	wt
44	f	52	Dermal melanocytic nevus	V600E	wt	wt
45	m	46	Dermal melanocytic nevus	wt	wt	wt
46	f	40	Dermal melanocytic nevus	wt	wt	wt

m, male; f, female; wt, wild-type; na, not available.

to exclude false-negative results. Furthermore, we screened 46 melanocytic nevi (9 lentiginous, 3 junctional, 11 compound, and 23 dermal nevi) for the *BRAF* hotspot mutation, which could be detected in 26/46 (57%) of the nevi. Interestingly, the V600E mutation was present in only 17% of the lentiginous/junctional nevi, whereas 55% of the compound nevi and 78% of the dermal nevi revealed this hotspot mutation (Figure 1b). The difference in the *BRAF* V600E mutation frequency between LS and melanocytic nevi was highly significant ($P<0.001$; Fisher's exact test). Because most of the patients in the investigated cohort originated from southeastern Germany, we cannot exclude the possibility that a different genetic population would show varying results.

Because solar lentigos and seborrheic keratoses display *FGFR3* and *PIK3CA* mutations (Hafner *et al.*, 2007, 2009), we also analyzed these genes in LS lesions and melanocytic nevi. *FGFR3* mutations were analyzed using a multiplex SNaPshot (Applied Biosystems, Foster City, CA) assay covering 11 point mutations (R248C, S249C, G372C, S373C, Y375C, G382R, A393E, K652E, K652M, K652Q, and K652T) that had previously been described in skin tumors (Hafner *et al.*, 2006). For the detection of four *PIK3CA* hotspot mutations (E542K, E545K, E545G, and H1047R), a recently developed *PIK3CA* SNaPshot assay was used (Hurst *et al.*, 2009). None of the 58 LS lesions or the 46 melanocytic nevi displayed an *FGFR3* or a *PIK3CA* point mutation (Table 1).

Our results indicate that the *BRAF* V600E hotspot mutation is not involved in the pathogenesis of LS. In contrast, 33% of PUVA (psoralen plus UVA)

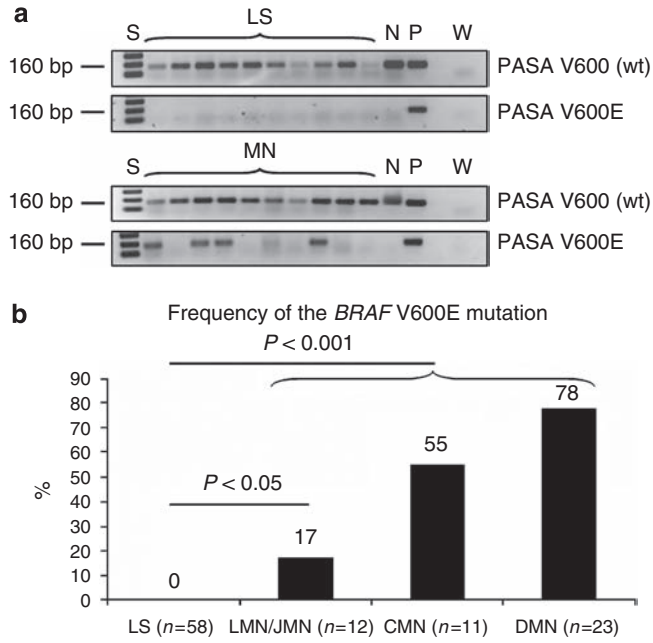

Figure 1. BRAF V600E mutation analysis. (**a**) Analysis of the *BRAF* V600E hotspot mutation using PCR amplification of specific alleles (PASA). We analyzed lentigo simplex (LS) and melanocytic nevi (MN). For each lesion, two PCRs were performed; one using a wild-type (wt) primer for codon 600 (top), the other V600E mutation–specific primers (bottom). The PCR products were visualized on an agarose gel (160 bp). Because the mutations are heterozygous, each sample displayed the *BRAF* wt allele. An additional band in the second PCR, with the V600E-specific primer, indicates a V600E mutation. The LS did not show any BRAF mutations, in contrast to some MN. (**b**) The frequency of the BRAF V600E mutation increased continuously from LS to dermal melanocytic nevus. The difference between LS lesions and lentiginous/junctional melanocytic nevi ($P<0.05$), as well as that between LS and all melanocytic nevus types together ($P<0.001$), was significant (Fisher's exact test). CMN, compound melanocytic nevus; DMN, dermal melanocytic nevus; JMN, junctional melanocytic nevus; LMN, lentiginous melanocytic nevus; N, negative control (cell lines HZT or RT4); P, positive control (cell line HT29); S, standard; W, H_2O control.

lentigines showed this mutation in a previous report (Lassacher *et al.*, 2006). Melanocytic nevi harbor *BRAF* mutations very frequently, with a reported range of 20–80% (Thomas, 2006). In our study, the *BRAF* V600E mutation was present in 57% of the melanocytic nevi. *BRAF* mutations are thought to stimulate the proliferation of melanocytes in nevi and melanomas. It has been proposed that a further proliferation of the melanocytes in LS may result in the formation of melanocytic cell nests and subsequently in a lentiginous or junctional melanocytic nevus. In this case, LS would represent a precursor lesion of melanocytic nevi (Ackerman and Ragaz, 1984). However, we found no *BRAF* V600E mutations in LS, indicating that this benign lesion does not share a common genetic background with melanocytic nevus. This finding could be explained in two ways: (i) LS is not a precursor lesion of melanocytic nevus and instead represents an independent benign skin tumor or (ii) LS is a precursor lesion of melanocytic nevus, but the *BRAF* V600E mutations occur late in this lentigo–nevus sequence. The latter would be congruent with the observed trend in this study that the frequency of *BRAF* V600E mutations increased from lentiginous/junctional nevi to compound and dermal nevi, implying that *BRAF* mutations occur during the proposed progression from junctional to compound and dermal nevus rather than from LS to junctional nevus. However, the differences between the types of melanocytic nevi were not statistically significant, which may be attributable to the small sampling size of the melanocytic nevus subgroups.

LS must also be discriminated from solar lentigo. We analyzed a possible common pathogenetic background of both lesions. Although solar lentigos display *FGFR3* and *PIK3CA* mutations and therefore share a common genetic basis with seborrheic keratoses, the LS in our study did not show any of these mutations. There has been a report of an H1047R *PIK3CA* mutation in one LS sample, which was included in a large mutation profiling study of various tumors (Thomas *et al.*, 2007). This H1047R mutation was also covered by the *PIK3CA* SNaPshot assay used in our study, but we could not detect any *PIK3CA* mutations in LS. Our results suggest that *PIK3CA* hotspot mutations do not play a major role in the pathogenesis of LS. The lack of *FGFR3* and *PIK3CA* mutations in LS implies that this lesion has a different genetic background from that of solar lentigo and seborrheic keratosis.

Therefore, we have shown that the absence of *BRAF*, *FGFR3*, and *PIK3CA* mutations can clearly differentiate LS from melanocytic nevi and solar lentigos. The results furthermore indicate that LS has a distinct yet unknown genetic basis, which does not necessarily exclude the proposed lentigo–nevus sequence.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Christian Hafner[1], Robert Stoehr[2], Johanna M.M. van Oers[3], Ellen C. Zwarthoff[3], Ferdinand Hofstaedter[4], Christoph Klein[5], Michael Landthaler[1], Arndt Hartmann[2] and Thomas Vogt[1]

[1]Department of Dermatology, University of Regensburg, Regensburg, Germany; [2]Institute of Pathology, University of Erlangen-Nürnberg, Erlangen, Germany; [3]Department of Pathology, Josephine Nefkens Institute, Erasmus MC, Rotterdam, The Netherlands; [4]Institute of Pathology, University of Regensburg, Regensburg, Germany and [5]Division of Oncogenomics, Institute of Pathology, University of Regensburg, Regensburg, Germany
E-mail: christian.hafner@klinik.uniregensburg.de

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