

Evaluation of Single Nucleotide Variants in Ethiopian Patients with Atopic Dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disorder characterized by pruritus and dryness of the skin in typical locations. The incidence rate of AD varies worldwide. In Europe the prevalence is up to 20% in children, while reports in Ethiopia vary between 1% and 2% in rural areas and 19% in urban areas (1, 2).

The major risk gene for AD in Europeans is the filaggrin (*FLG*) gene (1q21), where a number of null-mutations as well as variations in gene dose are associated with disease susceptibility (2). *FLG* mutations have also been identified in the Asian population, although these mutations seem to be more population- or family-specific (2). Little is known about AD susceptibility genes in the African population. Our previous studies indicate a low prevalence of *FLG* null-mutations in Ethiopian cases of AD (1). This is in line with studies performed in the South African AmaXhosa population, suggesting that, in the African population, genes other than *FLG* may be involved in the development of AD (3).

In searching for risk genes for AD in the Ethiopian population, we previously performed whole-exome sequencing (WES) of 22 Ethiopian patients with AD/ichthyosis vulgaris in whom several genetic polymorphisms were detected (1).

This study aimed to elucidate whether some of the single-nucleotide variants (SNVs) identified by Taylan et al. are associated with AD susceptibility in our Ethiopian AD cohort, consisting of 184 cases of AD and 186 healthy controls (Table I) including the 22 previously sequenced individuals (1).

METHODS

All study participants gave written or oral informed consent, which complies with the principles of the Declaration of Helsinki. This study was approved by the Ethics Review Board of the University of Gondar, and the regional ethics committee, Karolinska Institutet, Sweden.

All patients were diagnosed with AD according to the UK Working Party's diagnostic criteria (2) and answered questionnaires regarding other atopic manifestations (1).

The samples were obtained from ALERT Dermatology Hospital, Tikur Anbessa Hospital and Gondar University Hospital. We chose to genotype gene variants in: (i) epidermal differentiation complex (EDC) genes; cornulin (*CRNN*) (4) and hornerin (*HRNR*) (5); (ii) non-EDC genes that had either previously been associated with AD or candidate genes with a known function in the skin; (iii) gene variants that more than one individual was carrying in the WES. Based on this strategy we genotyped 38 SNVs using TaqMan (Applied Biosystems Inc., Foster City, CA, USA) in the following 10 genes; transglutaminase (TGM) 1, 3 and 5 (6), desmoglein (*DSG*) 1 and 3–4 (7), a variant in C11orf30 encoding for the EMSY protein (8), *SPINK5* (11), hornerin (*HRNR*) (5) and cornulin (*CRNN*) (4). Primers/probes were obtained from Thermo-Fisher Scientific Inc., (Foster City, CA, USA). Genotyping indicated that 28 SNVs were non-polymorphic or did not detect minor allele homozygotes in our material (Table SI¹). For the remaining 10 SNVs (Table II and Table SI¹), association with AD was calculated using χ^2 tests on both genotype and allele frequencies. Fisher's exact test was performed in instances where the counts were lower than 5. R program v.2.12.2 was used to calculate logistic-regression using generalized linear modelling between patients and controls. SNV frequencies were checked to follow Hardy-Weinberg equilibrium.

RESULTS

The genotyping success and concordance rates for SNVs showing 3 genotypes was above 90%. Furthermore, all successfully genotyped SNVs (showing all 3 genotypes) correlated with the previous whole exome-sequence results.

In our WES study, 12 SNVs were identified in the *HRNR* gene, which is a component of the epidermal cornified cell envelope thought to share similar features with filaggrin and demonstrated to have reduced expression in the epidermis of patients with AD (5). In the *HRNR* gene, only 3 SNVs demonstrated 3 genotypes and were analysed further. Two SNVs were analysed in *CRNN*, which is a marker of late epidermal differentiation and down-regulated in AD skin (4). However, these SNVs were non-polymorphic in our study material and discarded from further studies. In addition, SNVs were genotyped in *DSG1-4* and *TGM1*, 3 and 5, all of which have important roles in forming the epidermis and are associated with altered expression in AD skin (6, 7). One SNV located in the *c30orf11* region, which has been significantly associated with AD in previous studies, was also analysed (8). In the *SPINK5* gene, 7 SNVs were genotyped. *SPINK5* (5q32) encodes for the

Table I. Ethiopian cases and controls

	Cases (n = 184)	Controls (n = 186)
Female, %	46	51
Age, median; range	17; 2 months–45 years	12; 5–60 years
Early age of onset (<2 years old), %	68	n.a.
Moderate AD (SCORAD 15–40), %	30	n.a.
Severe AD (SCORAD >40), %	70	n.a.

AD: atopic dermatitis; SCORAD: SCORing Atopic Dermatitis; n.a.: not applicable.

¹<https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3051>

Table II. Minor allele frequencies (MAF) in our cases-control material and for the African population in Exac-and GnomAD databases

SNV name	Gene name	Minor Allele	MAF AD patients (n = 184)	MAF controls (n = 186)	Exac African	GnomAD African	p-value
HRNR2 (HRNR:NM_001009931:exon3:c.C2686T:p.R896C)	HRNR	G	0.05	0.07	–	–	0.52
Rs111789738	HRNR	T	0.096	0.086	0.024	0.022	0.88
Rs77898999	HRNR	G	0.023	0.02	0.016	0.015	0.34
SPINK5_6 (SPINK5:NM_001127698:exon7:c.G532A:p.E178K,SPINK5:NM_001127699:exon7:c.G532A:p.E178K,SPINK5:NM_006846:exon7:c.G532A:p.E178K)	SPINK5	G	0.062	0.07	–	–	0.92
Rs2303063	SPINK5	G	0.34	0.4	0.24	0.24	0.07
Rs2303067	SPINK5	G	0.35	0.34	0.24	0.24	0.24
Rs3188691	SPINK5	A	0.063	0.068	0.025	0.023	0.27
Rs114998364	TGM3	G	0.062	0.051	0.031	0.028	0.57
Rs36040686	DSG4	C	0.077	0.065	0.046	0.033	0.63
Rs140750904	DSG4	G	0.039	0.023	0	0.00012	0.54

p-values for the whole Ethiopian cases-control cohort for all successfully genotyped single-nucleotide variants (SNVs). AD: atopic dermatitis.

skin barrier protein *Lympho-epithelial Kazal-type-related inhibitor* (LEKTI), which plays a major role in controlling the proteolytic processing of profilaggrin into FLG monomers. Loss-of-function mutations of *SPINK5* cause the rare autosomal recessive disorder Netherton syndrome characterized by congenital ichthyosis as well as manifestations overlapping with AD. SNVs in this gene have previously shown modest association with AD in European and Asian populations (9). When association was calculated only between patients with severe AD and controls, significant association was observed for both rs230363 ($p=0.023$) and rs2303067 ($p=0.025$) in *SPINK5*. These findings are in line with previous studies in European and Asian populations (9). This prompted us to perform immunohistochemistry with the *SPINK5* antibody (Novus Biologicals, Abingdon Oxon, UK), on skin biopsies of Ethiopian patients with AD. Similar staining signals were indicated in a rs230363 homozygous wild-type carrier a homozygous minor allele-carrier and a heterozygous carrier (Fig. S1¹). However, we did not find any association when logistic regression was calculated. Association was also tested in all SNVs for age at onset (ages <2, 5 and 8 years, respectively) vs. controls and for AD severity measured by SCORing Atopic Dermatitis (SCORAD).

DISCUSSION

In our study, most SNVs had low minor allele frequencies (MAF) corresponding to frequencies in the African population in the online databases ExAc and GnomAD (Table II and Table SI¹) and, therefore, since our cohort is small, we may be limited to identify all 3 genotypes for many of the SNVs. Although it may be concluded that, due to low MAF, none of the variants would have a large effect on risk of AD. On the other hand, since most of the genes analysed are known to have altered expression in patients with AD compared with healthy controls, a dose-dependent or change of expression of the genes in the Ethiopian population that lead to AD cannot be ruled out.

Bearing in mind that *FLG* is not associated with AD in the Ethiopian population and no other major disease-susceptibility genes have been identified so far these re-

sults indicate that more genetic studies need to be carried out in order to understand the genetic background of AD in the Ethiopian population.

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The authors have no conflicts of interest to declare.

REFERENCES

1. Taylan F, Nilsson D, Asad S, Lieden A, Wahlgren CF, Winge MC, et al. Whole-exome sequencing of Ethiopian patients with ichthyosis vulgaris and atopic dermatitis. *J Allergy Clin Immunol* 2015; 136: 507–509.
2. Brown SJ, Kroboth K, Sandilands A, Campbell LE, Pohler E, Kezic S, et al. Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. *J Invest Dermatol* 2012; 132: 98–104.
3. Thawer-Esmail F, Jakasa I, Todd G, Wen Y, Brown SJ, Kroboth K, et al. South African amaXhosa patients with atopic dermatitis have decreased levels of filaggrin breakdown products but no loss-of-function mutations in filaggrin. *J Allergy Clin Immunol* 2014; 133: 280–282.
4. Lieden A, Ekelund E, Kuo IC, Kockum I, Huang CH, Mallbris L, et al. Cornulin, a marker of late epidermal differentiation, is down-regulated in eczema. *Allergy* 2009; 64: 304–311.
5. Henry J, Hsu CY, Haftek M, Nachat R, de Koning HD, Gardinal-Galera I, et al. Hornerin is a component of the epidermal cornified cell envelopes. *FASEB J* 2011; 25: 1567–1576.
6. Lieden A, Winge MC, Saaf A, Kockum I, Ekelund E, Rodriguez E, et al. Genetic variation in the epidermal transglutaminase genes is not associated with atopic dermatitis. *PLoS One* 2012; 7: e49694.
7. Samuelov L, Sarig O, Harmon RM, Rapaport D, Ishida-Yamamoto A, Isakov O, et al. Desmoglein 1 deficiency results in severe dermatitis, multiple allergies and metabolic wasting. *Nature Genetics* 2013; 45: 1244–1248.
8. O'Regan GM, Campbell LE, Cordell HJ, Irvine AD, McLean WH, Brown SJ. Chromosome 11q13.5 variant associated with childhood eczema: an effect supplementary to filaggrin mutations. *J Allergy Clin Immunol* 2010; 125: 170–174.
9. Dezman K, Korosec P, Rupnik H, Rijavec M. *SPINK5* is associated with early-onset and *CHI3L1* with late-onset atopic dermatitis. *Int J Immunogenetics* 2017; 44: 212–218.

Table S1. Genomic position/reference for all genotyped single-nucleotide variants (SNVs)

SNV name	Gene name	Chr	Genomic Position	Reference	Type of mutation	Alleles	Genotyping result	MAF case	MAF cont	Exac All	MAF African	Exac	GnomAD All	GnomAD African
SPINK5_5	SPINK5	5	14744998	SPINK5:NM_001127698:exon3:c.C194T;p.T65M,SPINK5:NM_001127699:exon3:c.C194T;p.T65M,SPINK5:NM_006846:exon3:c.C194T;p.T65M	Missense	C/T	Non-polymorphic			0.0002	0			
SPINK5_6	SPINK5	5	147469114	SPINK5:NM_001127698:exon7:c.G532A;p.E178K,SPINK5:NM_006846:exon7:c.G532A;p.E178K,SPINK5:NM_001127699:exon7:c.G532A;p.E178K,SPINK5:NM_006846:exon13:c.G1103A;p.S368N	Missense	G/A	OK	0.062	0.07					
rs2303063	SPINK5	5	147480027	SPINK5:NM_001127698:exon14:c.A1258C;p.S368N	Missense	G/A	OK	0.34	0.4	0.5	0.24	0.24	0.5	0.24
rs2303067	SPINK5	5	147480955	SPINK5:NM_001127698:exon15:c.G1322A;p.R442E	Missense	G/A	OK	0.34	0.34	0.5	0.24	0.24	0.5	0.24
rs34393923	SPINK5	5	147481363	SPINK5:NM_001127699:exon15:c.G1322A;p.R442E,SPINK5:NM_006846:exon15:c.G1322A;p.R442E,SPINK5:NM_006846:exon15:c.G1322A;p.R442E,SPINK5:NM_006846:exon15:c.G1322A;p.R442E	Missense	G/A	Non-polymorphic			0.0022	0.024	0.024	0.0023	0.024
rs111662216	SPINK5	5	147496004-06	SPINK5:NM_001127698:exon22:c.2087_2089del;p.696_697del,SPINK5:NM_001127699:exon22:c.2087_2089del;p.696_697del,SPINK5:NM_006846:exon22:c.2087_2089del;p.696_697del	Non-frameshift /GTG deletion	G/T		0.003	0.004	0.0021	0.024			
rs3188691	SPINK5	5	147506583	SPINK5:NM_006846:exon30:c.A2905G;p.K969E,SPINK5:NM_01127698:exon31:c.A2995G;p.K999E	Missense	A/G	OK	0.063	0.068	0.003	0.025	0.0032	0.0032	0.023
rs145120458	CRNN	1	152383382	CRNN:NM_016190:exon3:c.G176A;p.R59H	Missense	G/A	Non-polymorphic			0.002	0.0057	0.002	0.002	0.0058
rs35492900	CRNN	1	152382885	CRNN:NM_016190:exon3:c.G673C;p.E225Q	Missense	G/C	Non-polymorphic			0.00079	0.008	0.0008	0.0008	0.0078
EMSY	c30orf11	11	76256857	C11orf30:NM_020193:exon20:c.T3290C;p.I1097T	Missense	T/C	Two-cluster	0.071	0.054					
rs2229462	TGM1	14	24731015	TGM1:NM_000359:exon3:c.G394A;p.D132N	Missense	G/A	Two-cluster	0.065	0.056	0.00081	0.0089	0.0007	0.0007	0.0083
rs114998364	TGM3	20	2321094	TGM3:NM_003245:exon13:c.G1949A;p.G650E	Missense	G/A	OK	0.063	0.051	0.0027	0.031	0.0026	0.0026	0.028
rs80058195	TGM5	15	43525791	TGM5:NM_004245:exon11:c.T1724C;p.V575A,TGM5:NM_004245:exon11:c.T1724C;p.V575A,TGM5:NM_004245:exon11:c.T1724C;p.V575A,TGM5:NM_004245:exon11:c.T1724C;p.V575A	Missense	T/C	Two-cluster	0.044	0.044	0.0028	0.032	0.0029	0.0029	0.032
rs140779290	TGM5	15	43545103	TGM5:NM_004245:exon5:c.A470G;p.N157S,TGM5:NM_004245:exon5:c.A470G;p.N157S,TGM5:NM_004245:exon5:c.A470G;p.N157S,TGM5:NM_004245:exon5:c.A470G;p.N157S	Missense	A/G	Two-cluster	0.057	0.061	8.246e-06	0	0.000018	0.000018	0.00004
rs138507857	TGM5	15	43552428	TGM5:NM_001631:exon3:c.C258G;p.S86R	Missense	C/G	Two-cluster	0.05	0.06	0.00024	0	0.00022	0.00022	0.00004
rs16961655	DSG1	18	28916495	DSG1:NM_001942:exon9:c.C1184G;p.T395S	Missense	C/G	Non-polymorphic			0.0025	0.027	0.0026	0.0026	0.027
rs112401974	DSG1	18	28934485	DSG1:NM_001942:exon15:c.C2326T;p.P776S	Missense	C/T	Non-polymorphic			3.295e-05	0.00038	0.00039	0.00039	0.00045
DSG1_1	DSG1	18	28935224	DSG1:NM_001942:exon15:c.A3065T;p.H1022L	Missense	A/G	Non-polymorphic							
DSG1_3	DSG1	18	28935262	DSG1:NM_001942:exon15:c.G3103A;p.A1035T	Missense	G/A	Non-polymorphic							
rs200219673	DSG4	18	28966732	DSG4:NM_001134453:exon3:c.G166A;p.A56T,DSG4:NM_001134453:exon3:c.G166A;p.A56T,DSG4:NM_001134453:exon3:c.G166A;p.A56T,DSG4:NM_001134453:exon3:c.G166A;p.A56T	Missense	G/A	Two-cluster	0.006	0.0025	9.076e-05	0.00028	0.000052	0.000052	0.00026
rs36040686	DSG4	18	28968349	S79L,DSG4:NM_177986:exon4:c.C236T;p.S79L	Missense	C/T	Two-cluster	0.077	0.065	0.042	0.046	0.0041	0.0041	0.0033
rs143110911	DSG4	18	28968862	DSG4:NM_001134453:exon5:c.G398A;p.R133Q,DSG4:NM_177986:exon5:c.G398A;p.R133Q	Missense	G/A	Non-polymorphic			9.082e-05	9.718e-05	0.000079	0.000079	0.00024
rs140750904	DSG4	18	28972253	DSG4:NM_001134453:exon8:c.G955A;p.D319N,DSG4:NM_177986:exon8:c.G955A;p.D319N	Missense	G/A	OK	0.039	0.023	0.0006	0	0.00051	0.00051	0.00012
rs35378785	DSG4	18	28979427	DSG4:NM_001134453:exon9:c.G1198A;p.G400R,DSG4:NM_177986:exon9:c.G1198A;p.G400R	Missense	G/A	Two-cluster	0.024	0.013	0.0043	0.0046	0.043	0.043	0.0033
rs16961975	DSG3	18	29046606	DSG3:NM_001944:exon11:c.G1525A;p.V509M	Missense	G/A	Two-cluster	0.012	0.018	0.027	0.034	0.024	0.024	0.032
DSG3_2	DSG3	18	29055892	DSG3:NM_001944:exon16:c.T2669A;p.V890D	Missense	T/A	Two-cluster	0.01	0.018					
HRNR3	HRNR	1	152190027	HRNR:NM_001009931:exon3:c.C4078T;p.P1360S	Missense	G/A	Fail-manufacture							
HRNR2	HRNR	1	152191419	HRNR:NM_001009931:exon3:c.C2686T;p.R896C	Missense	G/A	OK	0.048	0.07					
rs111789738	HRNR	1	152191698	HRNR:NM_001009931:exon3:c.A2407G;p.S803G	Missense	T/C	OK	0.096	0.086	0.0021	0.024	0.0021	0.0021	0.022
rs77898999	HRNR	1	152191712	HRNR:NM_001009931:exon3:c.C2393A;p.T798K	Missense	G/T	OK	0.023	0.019	0.0014	0.016	0.0014	0.0014	0.015
HRNR1	HRNR	1	152191822	HRNR:NM_001009931:exon3:c.A2283C;p.Q761H	Missense	T/G	Fail-manufacture							
HRNR5	HRNR	1	152192541	HRNR:NM_001009931:exon3:c.T1564A;p.S522T	Missense	A/T	Non-polymorphic							
HRNR4	HRNR	1	152192605	HRNR:NM_001009931:exon3:c.G1500C;p.Q500H	Missense	C/G	Two-cluster	0.0057	0.007		0	0.00045	0.00045	0.000041
rs142030922	HRNR	1	152192882	HRNR:NM_001009931:exon3:c.G1223A;p.R408H	Missense	C/T	Fail-manufacture							
rs148892565	HRNR	1	152193177	HRNR:NM_001009931:exon3:c.G928A;p.V310I	Missense	C/T	Non-polymorphic			0.00018	0.00038	0.00018	0.00018	0.00026
rs187389918	HRNR	1	152193390	HRNR:NM_001009931:exon3:c.G715C;p.G239R	Missense	C/G	Two-cluster	0.022	0.034	0.00019681	0.0008	0.00019	0.00019	0.00024
rs148822876	HRNR	1	152193720	HRNR:NM_001009931:exon3:c.C385T;p.H129Y	Missense	G/A	Non-polymorphic			3.296e-05	0.00019	0.00005	0.00005	0.00041
rs139222671	HRNR	1	152193780	HRNR:NM_001009931:exon3:c.C325A;p.Q109K	Missense	G/T	Two-cluster	0.036	0.023	6.59E-05	0	0.00072	0	0

Fail-manufacture: single-nucleotide polymorphisms (snps) for which primer/probes failed to be manufactured by the vendor. Snps marked non-polymorphic, indicate snps which were seen to be non-polymorphic in our Ethiopian case-control material. Two-cluste: snps for which no homozygotes were detected for the minor allele. OK: snps for which all 3 genotypes were observed. All snps marked two-cluster and OK were seen to follow H-W.

Table SII. Genomic position/reference for all successfully genotyped single-nucleotide variants (SNVs)

SNV name	Gene name	Chr	Genomic position	Reference	Alleles
HRNR2	<i>HRNR</i>	1	152191419	HRNR:NM_001009931:exon3:c.C2686T:p.R896C	G/A
rs111789738	<i>HRNR</i>	1	152191698	HRNR:NM_001009931:exon3:c.A2407G:p.S803G	T/C
rs77898999	<i>HRNR</i>	1	152191712	HRNR:NM_001009931:exon3:c.C2393A:p.T798K	G/T
SPINK5_6	<i>SPINK5</i>	5	147469114	SPINK5:NM_001127698:exon7:c.G532A:p.E178K	G/A
rs2303063	<i>SPINK5</i>	5	147480027	SPINK5:NM_001127698:exon13:c.G1103A:p.S368N	G/A
rs2303067	<i>SPINK5</i>	5	147480955	SPINK5:NM_001127698:exon14:c.A1258G:p.K420E	A/G
rs3188691	<i>SPINK5</i>	5	147506583	SPINK5:NM_006846:exon30:c.A2905G:p.K969E,SPINK5:NM_001127698:exon31:c.A2995G:p.K999E	A/G
rs114998364	<i>TGM3</i>	20	2321094	TGM3:NM_003245:exon13:c.G1949A:p.G650E	G/A
rs36040686	<i>DSG4</i>	18	28968349	DSG4:NM_001134453:exon4:c.C236T:p.S79L,DSG4:NM_177986:exon4:c.C236T:p.S79L	C/T
rs140750904	<i>DSG4</i>	18	28972253	DSG4:NM_001134453:exon8:c.G955A:p.D319N,DSG4:NM_177986:exon8:c.G955A:p.D319N	G/A

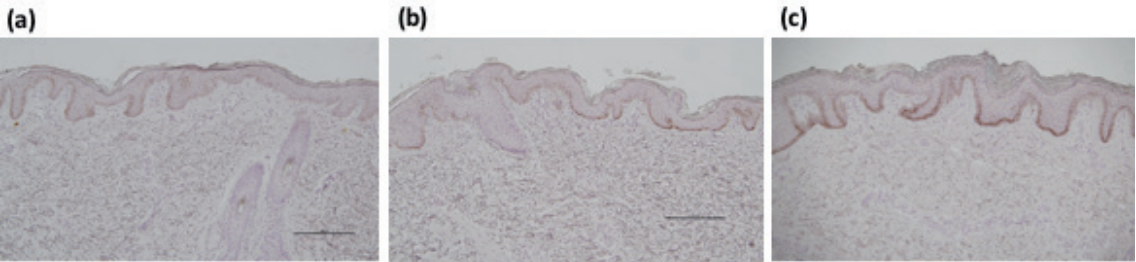
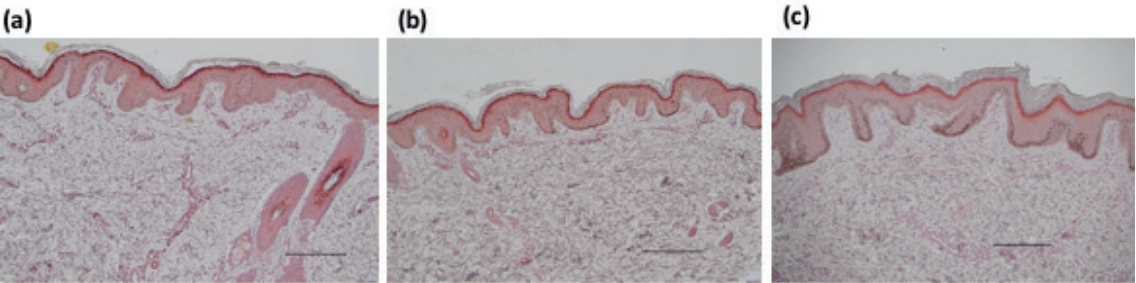
Negative staining (blank)**SPINK5 staining**

Fig. S1. Immunohistochemical staining of skin for SPINK5 expression in 3 Ethiopian patients with atopic dermatitis (AD). Figure indicates similar *SPINK5* expression in the stratum corneum in: (a) rs230363 homozygous wild-type carrier; (b) rs230363 homozygous minor allele-carrier; and (c) rs230363 heterozygous carrier. The negative control indicates no *SPINK5* expression in all 3 samples. Magnification $\times 10$.