

Variation in genes of the epidermal differentiation complex in German atopic dermatitis patients

S. Stemmler,* M. Nothnagel,† Q. Parwez,‡ E. Petrasch-Parwez,§ J. T. Epplen* & S. Hoffjan*

Summary

The filaggrin (*FLG*) gene is one of the most widely replicated susceptibility genes for atopic dermatitis (AD) so far. Yet, *FLG* mutations cannot fully account for the original linkage peak on chromosome 1q21, a region comprising the so-called epidermal differentiation complex (EDC). Since the EDC contains numerous genes relevant for epidermal differentiation, we sought to evaluate variation in other genes located in this region in a German AD case-control cohort. Thirty-two single nucleotide polymorphisms (SNPs) in 21 genes across the EDC were genotyped in 402 unrelated AD patients and 325 non-atopic controls by means of restriction enzyme digestion or TaqMan assays. Allele and genotype frequencies were tested for differences between patients and controls by logistic regression. Haplotype frequencies were evaluated using the FAMHAP software. Except for the already known association with *FLG*, we did not identify any additional significant associations of EDC genes with AD.

Thus, in this German cohort, there is no evidence that additional genes in the EDC region apart from *FLG* contribute substantially to AD pathogenesis.

Introduction

Atopic dermatitis (AD) is a chronic pruritic skin disease resulting from a complex interaction between genetic and environmental factors (Wuthrich *et al.*, 2007). Recent findings have suggested that an impaired function of the epidermal barrier may play a crucial role in the development of AD. A genome-wide linkage study in the British population revealed linkage to a region on chromosome 1q21 named the epidermal differentiation complex (EDC) (Cookson *et al.*, 2001), which comprises a number of genes relevant for epidermal differentiation (Mischke *et al.*,

1996). Furthermore, microarray analysis of atopic skin lesions revealed altered expression of some genes located within the EDC compared to control samples (Sugiura *et al.*, 2005). Subsequently, loss-of-function mutations in the filaggrin gene (*FLG*) were identified (R501X and 2282del4) that showed strong association with AD in numerous European cohorts (Barker *et al.*, 2006; Palmer *et al.*, 2006; Weidinger *et al.*, 2006, 2008; Morar *et al.*, 2007; Brown *et al.*, 2008; Ekelund *et al.*, 2008; Henderson *et al.*, 2008), including our own German AD case-control cohort (Stemmler *et al.*, 2006). Additionally, unique *FLG* variation was identified in a Japanese cohort and equally associated with AD (Nomura *et al.*, 2007; Enomoto *et al.*, 2008). Thus, *FLG* represents one of the most widely replicated susceptibility genes for AD to date.

Yet, when the original linkage peak on chromosome 1q21 was reinvestigated, taking the *FLG* mutations into account, the LOD score fell only from 3.57 to 2.03 (Morar *et al.*, 2007). This finding indicated that additional genetic variation at this locus influencing AD may be present (Morar *et al.*, 2007). We, therefore, investigated variation within various genes across the EDC in a German case-control cohort in order to detect additional associations apart from the *FLG* gene. Thirty-two single nucleotide polymorphisms (SNPs) in 21 genes were genotyped in 402 unrelated AD patients as well as 325 non-atopic controls and subsequently these SNPs were evaluated for an association with AD.

Materials and methods

Subjects

The German AD case-control cohort has been described in detail before (Macaluso *et al.*, 2007). In brief, 402 unrelated patients with AD were recruited by a consultant specialist for AD (Q.P., Gladbeck, Germany), including 238 children and 164 adults. Mean age of the AD patients was 18 ± 17 years (median 11 years). The AD diagnosis was based on the criteria developed by Hanifin and Rajka (1980). Three hundred and twenty-five control samples from adults of at least 40 years of age were collected in the same private practice as the AD patients. The control subjects underwent clinical examination in order to exclude symptoms of AD, asthma or allergic rhinitis, had no self-reported allergies or allergic symptoms and no first-degree

* Department of Human Genetics, Ruhr University, Bochum, Germany, † Institute of Medical Informatics and Statistics (IMIS), University of Kiel, Kiel, Germany, ‡ Private medical practice, Gladbeck, Germany, and § Department of Neuroanatomy and Molecular Brain Research, Ruhr University Bochum, Germany

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Correspondence: Susanne Stemmler, MD, Department of Human Genetics, Ruhr University, Universitätsstrasse 150, 44801 Bochum, Germany, Tel: 0234/32-25762; Fax: 0234/32-14196; E-mail: susanne.stemmler@rub.de

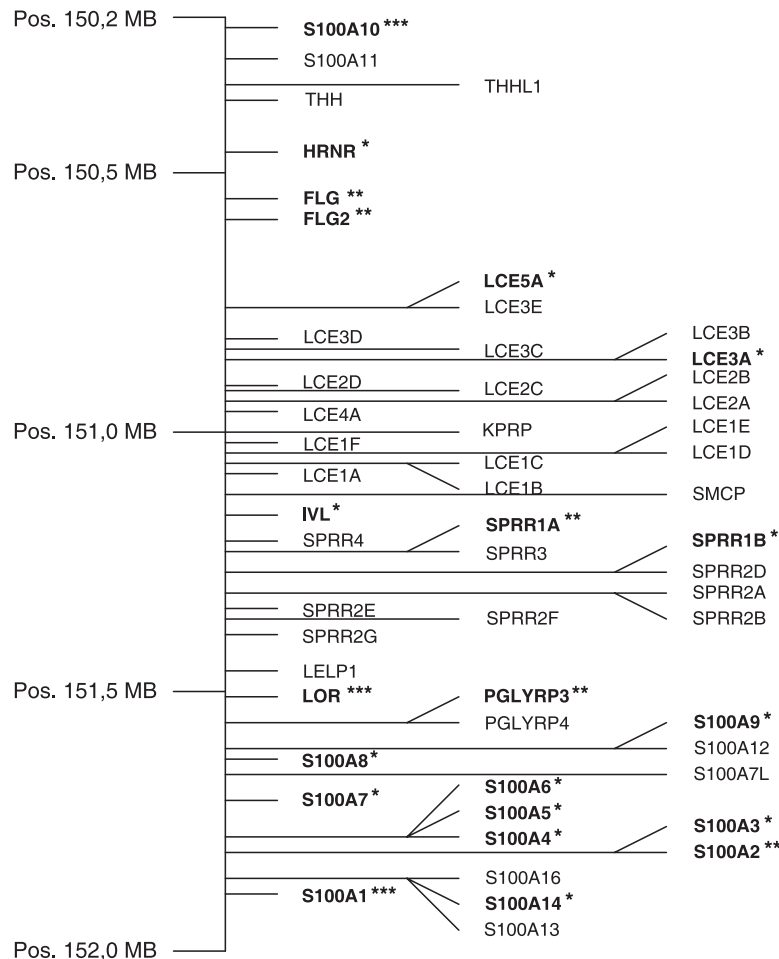


Figure 1. Illustration of the epidermal differentiation complex and location of the known genes. The genes that were investigated in this study are printed in bold letters. Asterisks indicate numbers of single nucleotide polymorphisms in each gene genotyped.

relatives with allergic diseases. Mean age of the controls was 62 ± 11 years (median 63 years). All patients and controls were Germans of European ancestry. Informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Ruhr University Bochum, and the Declaration of Helsinki protocols were followed.

Genotyping

DNA of AD patients and controls was extracted according to a standard method (Miller *et al.*, 1988). Thirty-two SNPs in 21 genes across the EDC were chosen from public databases (SNPper (www.snpper.org), HapMap, 2003) according to their location and frequency (Fig. 1). More specifically, genes that showed evidence for differential expression (e.g. *S100A7*, *S100A8*, *LOR* and *FLG* (Sugiura *et al.*, 2005)) were analysed first. The other genes were selected in order to cover all relevant regions of the EDC. We focused on coding variation and 5'- or 3'-polymorphisms with putative functional relevance, whenever available. Only SNPs with a minor allele frequency of ≥ 0.05 were chosen. Evaluation of the most

frequent *FLG* mutations, R501X and 2282del4, in this cohort had already been presented before (Stemmler *et al.*, 2006). These two variations were included in the analysis again in order to compare the results with the newly investigated SNPs. After this original report, the cohort sizes had slightly increased (Stemmler *et al.*, 2006). Genotyping was performed by restriction enzyme digestion or TaqMan Assay (Applied Biosystems, Foster City, CA, USA). Primer sequences, polymerase chain reaction (PCR) conditions and restriction enzymes are summarized in Table S1, Supporting Information.

Statistics

Linkage disequilibrium (LD) coefficients were estimated using HAPLOVIEW 4.0 (Barrett *et al.*, 2005). HAPLOVIEW was also used for LD visualization. Single-marker analyses were carried out in the R statistical environment, version 2.6.2 (R_Development_Core_Team, 2008). Deviations from Hardy-Weinberg equilibrium (HWE) were tested using an exact test (Wigginton *et al.*, 2005), as implemented in the R-hwde package. Allele and genotype frequencies were tested for differences between patients

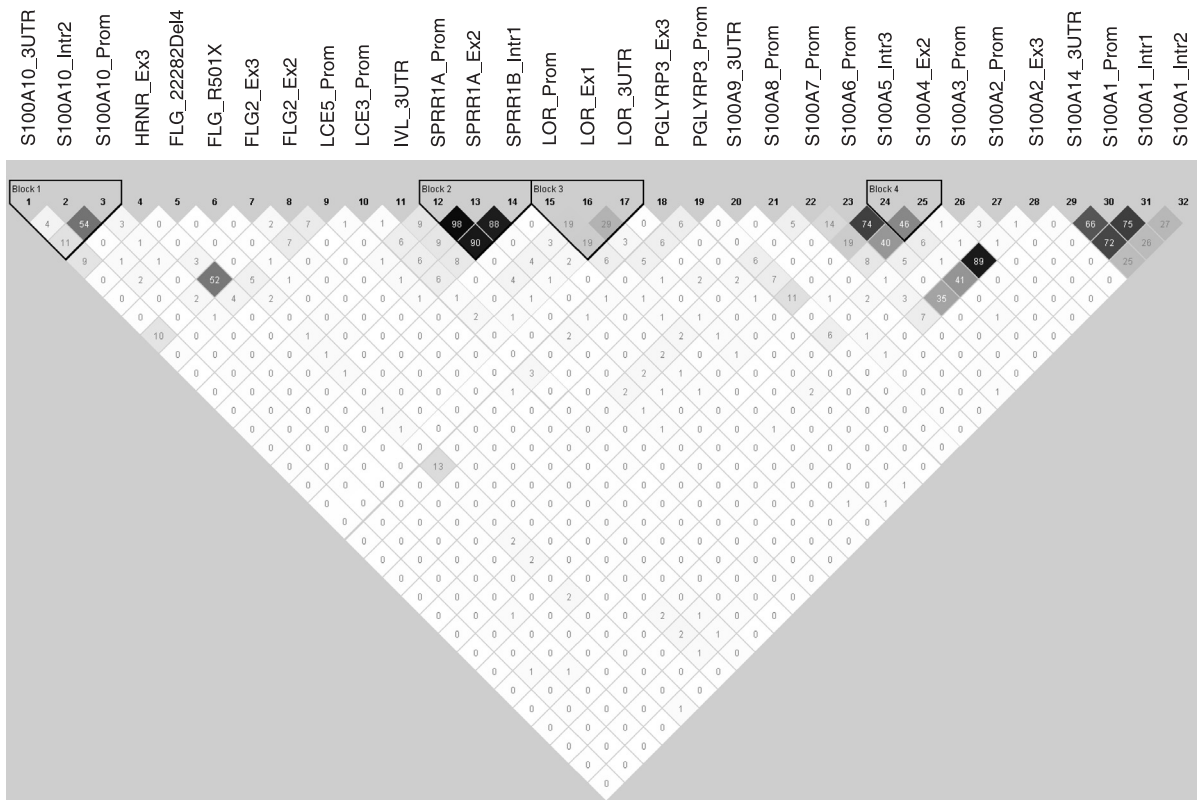


Figure 2. Linkage disequilibrium structure (r^2) of epidermal differentiation complex variation in controls.

and controls by logistic regression, using the glm implementation in R. Significance was assessed by a likelihood-ratio test. We assumed each SNP to act under a dominant, a multiplicative and a recessive genetic model, respectively. A χ^2 test was used to compare the combined allele frequencies of the two known *FLG* null mutations (R501X and 2282del4) in cases and controls. Haplotype frequencies were estimated, and haplotypic association was tested using the FAMHAP software which employs an EM-based algorithm (Becker & Knapp, 2004). 95% confidence intervals for the haplotype frequency estimates were estimated using non-parametric bootstrapping with 10 000 replications. Power analyses were performed with the Genetic Power Calculator (The Vanderbilt Medical Center Power and Sample Size Calculation (www.mc.vanderbilt.edu/prevmed/ps)).

Results

Thirty-two SNPs in 21 genes across the EDC were evaluated for an association with AD. Only S100A7_Prom showed a deviation from HWE in the controls at the 0.01 level ($P = 0.008$). Given the multiplicity of tests, this result is likely falsely positive. Genotyping errors were excluded by random re-evaluation. Regions of high LD were mainly found for SNPs within genes but also for closely related genes such as S100A4-7, while most SNPs were uncorrelated (Fig. 2). Based on this LD structure, we found that the

number of independent hypotheses of genetic association exceeded at least 20 and therefore applied a Bonferroni correction factor of 20, yielding a corrected significance level of 0.0025. While we initially found some nominally significant associations with AD in our cohort, none of the associations remained significant after correction for multiple testing (Table 1). In accordance with our original report (Stemmler *et al.*, 2006), we went on to analyse the combined allele frequencies of the two known *FLG* null mutations (R501X and 2282del4) in all AD patients as well as the subgroups of patients with age of onset in childhood (< 18 years) and age of onset before 2 years. These analyses confirmed significant associations of the combined allele frequencies of all three groups compared to non-atopic controls ($P = 0.0064$, $P = 0.00018$ and $P = 0.000026$, respectively; Table 2). Slightly different P -values are explained by the slightly increased cohort sizes since the original report (Stemmler *et al.*, 2006). The four other SNPs that showed nominally significant associations with AD in any of the tested models ($0.01 \leq P \leq 0.05$ for LOR_Prom, PGLYRP_Ex3, S100A8_Prom and S100A2_Ex3) were equally reinvestigated in the group of AD patients with age of onset before 2 years, but no additional associations were observed (data not shown).

Additionally, we assessed the power of our study setting under a multiplicative genetic model. Assuming a genotype relative risk of 2 for heterozygotes and 4 for homozygotes and $D' = 0.9$ between marker and causal variation, we

Table 1. Nominal *P*-values obtained from single-marker association analysis. Each single nucleotide polymorphism was considered to act under a dominant, a multiplicative, and a recessive model, respectively. Nominally significant *P*-values are set in bold. None of these markers remained significant after correction for multiple testing

Gene	Polymorphism	Model		
		Dominant	Multiplicative	Recessive
<i>S100A10</i>	3'UTR	0.381	0.251	0.182
<i>S100A10</i>	Intron 2	0.935	0.871	0.614
<i>S100A10</i>	Promoter	0.751	0.959	0.767
<i>HRNR</i>	Exon 3	0.516	0.593	0.952
<i>FLG</i>	2282del4	0.027	0.013	0.029
<i>FLG</i>	R501X	0.123	0.188	0.880
<i>FLG2</i>	Exon 3	0.916	0.908	0.942
<i>FLG2</i>	Exon 2	0.818	0.755	0.658
<i>LCE5</i>	Promoter	0.051	0.069	0.442
<i>LCE3</i>	Promoter	0.999	0.499	0.360
<i>IVL</i>	3'UTR	0.290	0.351	0.987
<i>SPRR1A</i>	Promoter	0.769	0.283	0.116
<i>SPRR1A</i>	Exon 2	0.070	0.214	0.726
<i>SPRR1B</i>	Intron 1	0.146	0.186	0.455
<i>LOR</i>	Promoter	0.411	0.145	0.048
<i>LOR</i>	Exon 1	0.688	0.588	0.283
<i>LOR</i>	3'UTR	0.673	0.333	0.181
<i>PGLYRP3</i>	Exon 3	0.032	0.074	0.616
<i>PGLYRP3</i>	Promoter	0.483	0.680	0.513
<i>S100A9</i>	3'UTR	0.627	0.900	0.218
<i>S100A8</i>	Promoter	0.645	0.335	0.038
<i>S100A7</i>	Promoter	0.304	0.159	0.125
<i>S100A6</i>	Promoter	0.373	0.366	0.687
<i>S100A5</i>	Intron 3	0.397	0.435	0.911
<i>S100A4</i>	Exon 2	0.111	0.231	0.059
<i>S100A3</i>	Promoter	0.477	0.368	0.351
<i>S100A2</i>	Promoter	0.618	0.470	0.373
<i>S100A2</i>	Exon 3	0.087	0.233	0.029
<i>S100A14</i>	3'UTR	0.169	0.461	0.905
<i>S100A1</i>	Promoter	0.579	0.961	0.629
<i>S100A1</i>	Intron 1	0.128	0.186	0.495
<i>S100A1</i>	Intron 2	0.127	0.080	0.169

would have 99% power to detect a potential effect of a variant with a minor allele frequency of 0.1. Considering less strong genetic effects, with genotype relative risks of 1.5/2.25 and $D' = 0.8$, would still yield 61% power.

Table 2. Combined allele frequencies for *FLG* mutations R501X and 2282del4 in all patients with atopic dermatitis (AD), patients with age of onset in childhood and age of onset before 2 years compared to non-atopic controls

Genotype	Allele	All AD patients	Onset of AD in childhood	Onset of AD before 2 years	Non-atopic controls
AA		339 (84.5%)	246 (81.2%)	189 (79.4%)	293 (90.4%)
Aa		55 (13.7%)	50 (16.5%)	42 (17.6%)	30 (9.3%)
aa		7 (1.7%)	7 (2.3%)	7 (2.9%)	1 (0.3%)
<i>n</i>		401	303	238	324
<i>P</i> vs. controls		0.053	0.0047*	0.0021*	—
	A	733 (91.4%)	542 (89.4%)	420 (88.2%)	616 (95.1%)
	a	69 (8.6%)	64 (10.6%)	56 (11.8%)	32 (4.9%)
	<i>n</i>	802	606	476	648
<i>P</i> vs. controls		0.0064*	0.00018*	0.000026*	—

* Significantly different ($P < 0.05$).

A, wild-type allele; a, mutated allele (either R501X or 2282del4 mutation).

Haplotype analyses did not reveal any significant associations. For the *LOR* and *FLG* genes, nominal global *P*-values below 0.05 were observed ($P = 0.02$ and $P = 0.01$, respectively), but these results did not withstand Bonferroni correction (Table 3).

Discussion

The *FLG* gene is one of the most widely replicated susceptibility genes for AD so far. For the combined genotype (R501X or 2282del4), a meta-analysis recently found an overall odds ratio (OR) of 4.09 from case-control studies and an OR of 2.06 from family studies (Baurecht *et al.*, 2007). On the other hand, very little is known to date about variation in EDC genes apart from *FLG*, even though *FLG* mutations could not fully account for the original linkage peak on chromosome 1q21 (Morar *et al.*, 2007). *PGLYRP3* variation for example was associated with psoriasis (Sun *et al.*, 2006), but not yet evaluated for AD. Several S100 proteins have shown overexpression in AD (Sugiura *et al.*, 2005; Glaser *et al.*, 2008; Merryman-Simpson *et al.*, 2008) but association studies for these genes have not been published yet. We, therefore, investigated variation in several additional EDC genes in this present study in order to more thoroughly cover the whole EDC region.

Overall, we did not find evidence that any additional gene in this region apart from *FLG* contributes substantially to AD pathogenesis. Interestingly, even the *FLG* mutations R501X and 2282del4, when analysed separately, did not show an association with AD strong enough to withstand Bonferroni correction (*P*-value for 2282del4: 0.013 for the multiplicative model, *P*-value for R501X: not significant). Yet, the previous analysis of these two mutations in our cohort (Stemmler *et al.*, 2006) had shown comparable results ($P = 0.008$ for 2282del4; *P*-value for R501X: not significant). Evaluation of the combined allele frequencies, on the other hand, confirmed significant associations with AD, especially for the group of patients with age of onset before 2 years, in the present study ($P = 0.000026$). Since the greatest effect for *FLG* mutations was seen in this latter group, we

Table 3. Estimated haplotype frequencies for epidermal differentiation complex genes in which more than one single nucleotide polymorphism was genotyped. 95% confidence intervals (95% CI) for the estimates are given in parentheses

Gene	Haplotype	Frequency in atopic dermatitis patients (95% CI) <i>n</i> = 402	Frequency in controls (95% CI) <i>n</i> = 325	Nominal global <i>P</i> -value	Corrected <i>P</i> -value
<i>S100A10</i>	111	0.577 (0.540–0.613)	0.655 (0.526–0.603)	0.141	NS
	112	0.038 (0.025–0.052)	0.035 (0.022–0.051)		
	122	0.313 (0.280–0.345)	0.301 (0.268–0.336)		
	212	0.071 (0.053–0.090)	0.086 (0.065–0.109)		
<i>FLG</i>	11	0.913 (0.892–0.933)	0.951 (0.935–0.966)	0.026	0.208
	12	0.019 (0.009–0.029)	0.012 (0.003–0.020)		
	21	0.068 (0.049–0.086)	0.039 (0.025–0.054)		
<i>FLG2</i>	11	0.678 (0.648–0.711)	0.684 (0.648–0.718)	0.954	NS
	12	0.150 (0.123–0.174)	0.142 (0.117–0.170)		
	21	0.170 (0.144–0.197)	0.170 (0.142–0.202)		
<i>SPRR1A</i>	11	0.549 (0.515–0.582)	0.577 (0.537–0.612)	0.406	NS
	22	0.449 (0.414–0.481)	0.418 (0.383–0.458)		
<i>LOR</i>	111	—	0.019 (0.009–0.030)	0.013	0.104
	112	0.379 (0.345–0.412)	0.351 (0.315–0.385)		
	121	0.383 (0.348–0.417)	0.361 (0.321–0.402)		
	211	0.234 (0.204–0.264)	0.264 (0.229–0.301)		
<i>PGLYRP3</i>	11	0.545 (0.510–0.578)	0.494 (0.457–0.529)	0.194	NS
	12	0.091 (0.069–0.112)	0.097 (0.073–0.121)		
	21	0.362 (0.330–0.393)	0.406 (0.369–0.443)		
<i>S100A2</i>	11	0.774 (0.744–0.802)	0.775 (0.742–0.807)	0.409	NS
	12	0.049 (0.035–0.066)	0.064 (0.043–0.083)		
	21	0.177 (0.151–0.204)	0.161 (0.129–0.188)		
<i>S100A1</i>	111	0.210 (0.180–0.241)	0.220 (0.185–0.256)	0.063	NS
	112	0.267 (0.233–0.302)	0.236 (0.200–0.272)		
	121	0.023 (0.013–0.034)	0.045 (0.030–0.063)		
	211	0.029 (0.016–0.045)	0.016 (0.007–0.027)		
	221	0.438 (0.402–0.477)	0.463 (0.425–0.504)		
	222	0.022 (0.011–0.034)	0.015 (0.004–0.026)		

1, major allele; 2, minor allele; NS, not significant ($P > 0.05$).

reinvestigated all other SNPs that showed marginal, but not statistically significant associations with AD in any of the tested models for an association with the subtype of AD with age of onset before 2 years. Yet, no additional associations were observed, affirming *FLG* as the only known susceptibility gene in this region to date.

It could still be possible that one or more of the other EDC genes may have a weak influence on AD pathogenesis that was not detected in the present study. First, the cohort sizes may have been too small to discover weak or rare genetic factors. Yet, power analysis suggested that the study was sufficiently powered (between 61% and 99% depending on the underlying assumption). Furthermore, the cohort evaluated in this present study has been thoroughly recruited by a single AD expert and, thus, represents a highly controlled sample set, minimizing phenotypic heterogeneity as a potential confounder. We, therefore, believe that we had sufficient power to detect relevant association with any of the investigated EDC genes. Second, we focused on evaluating variation across the whole EDC instead of a more thoroughly cover variation in each specific gene, so that in some of the smaller genes only singular SNPs were genotyped. Furthermore, we only examined a representative fraction of EDC genes but

not all genes located in the EDC. Thus, there is a chance that we may have missed the disease-causing variation in any of them. Third, a weak pathogenic influence may only be detected in connection with other genetic variation or specific environmental factors. Recently, a significant interaction between *FLG* loss-of-function mutations and cat ownership at birth on the development of early-life AD could be demonstrated in two independent birth cohorts (Bisgaard *et al.*, 2008). Harboring a *FLG* mutation increased the risk of AD during the first year of life ($P = 0.005$), with a further increase in risk related to cat exposure at birth among children with a *FLG* mutation ($P < 0.0001$) (Bisgaard *et al.*, 2008). Since we did not have information on environmental factors such as cat or dog presence, we were unable to include this information in the analysis at this point.

In conclusion, our study did not yield evidence that additional genes in the EDC region, apart from *FLG*, contribute substantially to AD pathogenesis. Thorough association analyses including gene/gene as well as gene/environment interactions are warranted in order to clarify the role of EDC variation for AD pathogenesis comprehensively and thus, pave the way for more specific molecular targeting for therapy in the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primer sequences, PCR conditions and restriction enzymes for all investigated SNPs in the EDC region

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Supplementary table 1: Primer sequences, PCR conditions and restriction enzymes for all investigated SNPs

Gene	Location	rs number	Amino acid	Primer	Annealing temperature	Restriction enzyme
<i>S100A10</i>	3'UTR	rs1387835	G/A	F: CTGAATCCAATTTTCATAGCAAAGTG R: AGGAGGAACATTTTGATTGATTAT	56°C	RsaI
<i>S100A10</i>	Intron 2	rs3791153	T/C	F: CATCGGGTTATCTGTGTCTGTTGT R: TTCAGTAGATGACTGACACTGCTCC	56°C	MspI
<i>S100A10</i>	Promoter	rs3007708	A/C	F: CGCCCAATACTTGGAACCTCTTC R: CACCAGTGGAGGAAATCTCGG	56°C	BseNI
<i>HRNR</i>	Exon 3	rs11204937	G/A	F: CTTCAGTTCGTTAGCCAGATGTATTGT R: GCAGTAATCTTTGCCAATGATTTTAGT	54°C	RsaI
<i>FLG</i>	Exon 3	---	delCAGT	F: TCCCGCCACCAGCTCC R: GTGGCTCTGCTGATGGTGA	56°C	DrallI
<i>FLG</i>	Exon 3	---	Arg/Stop	F: CACTGGAGGAAGACAAGGATCG R: CCCTCTTGGGACGCTGAA	54°C	NlaIII
<i>FLG2</i>	Exon 3	rs2282302	Cys/Ser	F: GACAGTGGGAGGCGAAGTCAT R: CAGGTTGACCACATCCAGAGG	56°C	Ddel
<i>FLG2</i>	Exon 2	rs3818831	Leu/Phe	F: CTTGCAAAAAGATGACCGACCT R: CAGAGCTTGTACAGGACTCCAGC	56°C	AluI
<i>LCE5A</i>	Promoter	rs11581947	A/G	F: GCTTTTGCTCCACCCTCAGTAT R: GATCCAAAATAAAATTGTAGGCCCA	56°C	BsuRI
<i>LCE3A</i>	Promoter	rs7527788	C/T	F: AAGTGAACAGCAGCCAGAGG R: ATGATGCACAGCCCAACTATGT	56°C	Hin6I
<i>IVL</i>	3'UTR	rs3820136	C/T	F: ACCTGTGTATGGGTCAATGGC R: CATGGCTGGCAAGTGTGTT	56°C	Eco130I
<i>SPRR1A</i>	Promoter	rs1611753	T/C	F: CAAGGCCTCAACAGGACTCAGAT R: TCATTCTCACCTGTCACACATG	56°C	AluI
<i>SPRR1A</i>	Exon 2	rs1611764	Val/Ile	F: ACATTTGAAGCATGAATTCTCAGC R: GAGTGACCGTTGAAGGGCAG	56°C	HinfI
<i>SPRR1B</i>	Intron 1	rs2070964	T/C	TaqMan-Assay		
<i>LOR</i>	Promoter	rs943967	A/G	F: CACCTCAAGACTCTGGGACCTATT R: TGAAGGTTTTGGCATTCTTGAGAT	56°C	NsiI
<i>LOR</i>	Exon 1	rs6661601	Ser/Gly	F: AAGCATAATGAAGGCTTTCTGCA R: GCTGCTACCGCCCCCTG	56°C	MspA1I
<i>LOR</i>	3'UTR	rs12136773	A/G	F: GCTACTCAGACTCACGGAGACA	56°C	NlaIII

				R: AACATTCAACCCGAGAAAGCTG		
PGLYRP3	Exon 3	rs843971	G/A	F: CATCGCCTTCTTTGGCAATAAGTTA R: TTTCAAGAGAAGGGTGATCATCAGA	56°C	TruI
PGLYRP3	Promoter	rs821422	T/C	F: TGCTTGTAAGTTCCTCATCTGAGC R: GGCAACTCCTGGTTCCTCATAGA	56°C	AvaI
S100A9	3'UTR	rs2070864	A/G	F: TGAAGTCAAGGCTGATGATGAGT R: CATATCAAGGGGTAGAGATAGGC	55°C	Eco0109I
S100A8	Promoter	rs3806232	A/G	F: AGGAATGGATATAGCCCTTCGC R: CGTGTCTGGCTGATGGC	56°C	Bsh1236I
S100A7	Promoter	rs3006433	G/A	F: CAGACCCTGATGTGACCCAAG R: TGCATCCTCCTCAACCCTCAT	56°C	NdeI
S100A6	Promoter	rs3762262	G/A	F: GGAAGGAGTGGAGGTGTTGGT R: TCCTCTGCCAGACCGTGAAT	56°C	MspI
S100A5	Intron 3	rs477339	G/C	F: GGATGCAGTGGCTTCACAGC R: GATCATGCCATTGCACTCCA	56°C	BseDI
S100A4	Exon 2	rs1050923	A/G	F: TGTCCACCTTCCACAAGTACTCG R: AAGACTGCTGCCCTCCTCTGT	56°C	HpyF10VI
S100A3	Promoter	rs1005436	C/T	F: TCTTCAGGGAGAGTCTTGGCC R: CCTTCCTGGAATTGCTCACCTC	56°C	XapI
S100A2	Promoter	rs2071576	G/A	F: CAGGTTGCTTCTCTCTCACACC R: GGACTGGGCATCCTGGACCGG	56°C	BtgI
S100A2	Exon 3	rs1047325	G/A	F: GGGCTGAAGAAGCTGATGGTC R: CAAAGGCATCAACAGTCCTGG	56°C	HincII
S100A14	3'UTR	rs11548102	T/A	F: CTGTGGGCTGGAGATAAACT R: AGACTCCTCCACCCATGAGCT	56°C	MnII
S100A1	Promoter	rs999418	T/C	F: ATTTCCGTGGTCGATTCTGAAG R: TGATTAGACATGGGTATGGTGGC	56°C	MvaI
S100A1	Intron 1	rs985242	C/G	F: CAGAGTTGAAGGACCAAAAGGG R: ACTACAGCCTTCCTTCTGCTGC	56°C	BsuRI
S100A1	Intron 2	rs1046379	C/T	F: TAGGCAACAGAAGCCCTCAGG R: GGAAGAGGGAGGTGTATAGGGAA	54°C	AvaII