Deletion of *Late Cornified Envelope 3B* and *3C* Genes Is Not Associated with Atopic Dermatitis

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Atopic dermatitis (AD) and psoriasis are common skin diseases characterized by cutaneous inflammation and disturbed epidermal differentiation. Genome-wide analyses have shown overlapping susceptibility loci, such as the epidermal differentiation complex on chromosome 1q21. Recently, a deletion on 1q21 (*LCE3C_LCE3B*-del), comprising *LCE3B* and *LCE3C*, two members of the *late cornified envelope* (*LCE*) gene cluster, was found to be associated with psoriasis. Although the mechanistic role of LCE proteins in psoriasis has not been identified, these proteins are putatively involved in skin barrier formation and repair. Considering the potential genetic overlap between the two diseases and the recent finding that mutations in the skin barrier protein filaggrin are associated with AD, we investigated a possible association between *LCE3C_LCE3B*-del and AD. Evaluation of four different cohorts of European ancestry, containing a total of 1075 AD patients and 1658 controls, did not provide evidence for such an association. Subgroup analysis did not reveal an association with concomitant asthma. Our data suggest that the potential roles of skin barrier defects in the pathogenesis of AD and psoriasis are based on distinct genetic causes.

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Abbreviations: AD, atopic dermatitis; CI, confidence interval; EDC, epidermal differentiation complex; FLG, filaggrin; LCE, late cornified envelope; LCE3C_LCE3B-del, the deletion of the LCE3C and LCE3B genes; OR, odds ratio

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INTRODUCTION

Atopic dermatitis (AD) and psoriasis are clinically distinct, chronic inflammatory skin diseases with a strong genetic basis (Leung and Bieber, 2003; Bowcock and Cookson, 2004; Krueger and Bowcock, 2005). Several genome-wide analyses have been performed to discover genetic factors contributing to AD and psoriasis (Cookson, 2004). These analyses have revealed chromosomal regions harboring possible susceptibility loci for both diseases, including chromosomal regions 1q21, 3q21, 17q25, and 20p12. These findings suggested that AD and psoriasis could share some contributing genetic factors, but none of these have been identified to date (Willis-Owen *et al.*, 2007).

Although both diseases are generally regarded as immune-mediated conditions, recent genetic studies have indicated the importance of inherited abnormalities of epidermis-expressed genes as a primary cause (Magert *et al.*, 1999; Chavanas *et al.*, 2000; Cookson, 2004; Palmer *et al.*, 2006; Hollox *et al.*, 2008; De Cid *et al.*, 2009). These findings are in line with the concept of inflammatory epithelial disease, which was coined previously for a number of conditions that were associated with epithelium-expressed genes and immunological mechanisms (Cookson, 2004). The shared locus on chromosome 1q21 overlies the epidermal differentiation complex (EDC), a cluster of genes encoding proteins found in the uppermost layers of the epidermis, which are of great importance for keratinocyte differentiation and skin barrier

maintenance (Mischke *et al.*, 1996). Genes located in the EDC include *loricrin, involucrin, filaggrin* (*FLG*), the *small proline-rich protein* genes, the *S100* genes, and the *late cornified envelope* (*LCE*) genes. Null mutations in the *FLG* gene have been identified as a remarkably strong and widely replicated risk factor for AD and led to a paradigm shift, placing the barrier function of the skin into the center of the pathogenetic concepts (Irvine and McLean, 2006; Palmer *et al.*, 2006; Smith *et al.*, 2006; Weidinger *et al.*, 2006, 2008; Fallon *et al.*, 2009). Interestingly, also the cytokine environment associated with AD (Th2 cytokines) appears to contribute to compromised skin barrier function, as IL-4 and IL-13 suppress *FLG* gene expression (Howell *et al.*, 2007). In contrast to the genetic findings for AD, no associations of *FLG* null alleles with psoriasis could be shown (Huffmeier *et al.*, 2007; Zhao *et al.*, 2007).

Although *FLG* mutations account for 13% of the population attributable risk in AD (O'Regan *et al.*, 2008), they only partially explain the linkage signal of AD to the EDC (Morar *et al.*, 2007). In addition, in a recent large-scale genome-wide association study, evidence for additional AD risk factors in the EDC, apart from prevalent *FLG* mutations, was obtained (Esparza-Gordillo *et al.*, 2009).

Apart from single-nucleotide polymorphisms (SNPs), DNA copy number variation represents a considerable source of human genetic diversity (Redon *et al.*, 2006; Korbel *et al.*, 2007; Kidd *et al.*, 2008). De Cid *et al.* (2009) discovered that the deletion of two genes of the *LCE* gene family, *LCE3B* and *LCE3C* (annotated as *LCE3C_LCE3B*-del), located in the EDC, was significantly associated with psoriasis in individuals of European ancestry. This finding has recently been replicated by an independent study in German psoriasis patients (Huffmeier *et al.*, 2009). In addition, in an independent genome-wide association study of a Chinese cohort, association with SNPs in a strong linkage disequilibrium with the deletion were identified as risk factors for psoriasis (Zhang *et al.*, 2009). LCE proteins are incorporated in the cornified envelope during epidermal differentiation, as shown by Marshall *et al.* (2001). The work by De Cid *et al.* (2009) suggests that one of the psoriasis-associated genes, *LCE3C,* is involved in repair of skin barrier function, as its expression is only induced upon epidermal activation.

These observations together with the role of skin barrier maintenance in both diseases prompted us to investigate a possible genetic association between the *LCE3C_LCE3B*-del allele and AD. Our analysis of four European case–control cohorts, a total of over 2500 samples, however, did not support such an association.

RESULTS

Analysis of association of *LCE3C_LCE3B*-del with AD

To investigate a possible association of AD with the deletion of the *LCE3B* and *LCE3C* genes, we genotyped four European case–control cohorts, consisting of adult cases from The Netherlands and Germany and children with AD from Ireland and Italy (see Table 1 and Supplementary Information for cohort summaries and description). The Dutch, German, and Irish cohort were genotyped by using the direct

Table 1. Cohort characteristics

Population	Phenotype	N (%)	Ratio	Mean age (SD)	% Male
The Netherlands	Control	386		48 (16)	56
	AD	295	1.3	34 (9.3)	33
	AD + asthma	172 (58)	2.2	34 (8.8)	30
German replicate	Control	248		39 (13)	36
	AD	258	1.0	38 (16)	38
	AD + asthma	83 (32)	3.0	36 (15)	36
Irish replicate	Control	578		36 (3.5)	30
	AD	314	2.1	4.0 (3.8)	64
	AD + asthma	98 (31)	5.9	7.8 (4.3)	64
Italian replicate	Control	446		42 (15)	62
	AD	208	1.5	8.9 (6.2)	60
	AD + asthma	38 (18)	11	13 (6.5)	79

Atopic dermatitis (AD) + asthma is a subgroup of the AD cohort. (%) Is the percentage of the total AD cohort, which is present in the AD + asthma cohort. Ratio, control/case ratio.

PCR-based method described by De Cid *et al.* (2009). The Italian cohort was genotyped by analyzing SNP rs4112788, which can be used as a proxy for the deletion as it is in strong linkage disequilibrium with the deletion ($r^2 = 0.928$ and $D' = 0.988$) (De Cid *et al.*, 2009). In the different control groups, frequencies of the *LCE3C_LCE3B*-del allele varied between 59 and 66%. This heterogeneity of the control groups in different ethnic backgrounds was already known from our previous study (De Cid *et al.*, 2009). In the Dutch cohort, we found a significantly higher frequency of the *LCE3C_LCE3B*-del allele in AD patients (66%) compared with healthy controls (59%) (Supplementary Table 1). Further analysis were performed by using logistic regression models, in which the wt/wt genotype (homozygous for the undeleted *LCE3C_LCE3B* allele) was used as reference category. For the Dutch cohort, these analyses showed only for the del/del genotype (homozygous for the *LCE3C_LCE3B* deletion) a significant association with AD ($P = 0.022$), corresponding odds ratio (OR) of 1.71 (95% confidence interval (95% CI): 1.08–2.70). For the del/wt genotype (heterozygous for the *LCE3C_LCE3B* deletion) no association with AD was found ($P = 0.420$) (Table 2). In none of the other cohorts a significant association with AD and the del/del genotype was found (Table 2). For the del/wt genotype a significant association was found in the German cohort ($P = 0.018$), which was in the opposite direction to the Dutch cohort (OR 0.52, 95% CI: 0.31–0.90). When all cohorts were combined to increase statistical power (Table 2, combined power is 1.0), no significant association was detected for either of the two genotypes ($P = 0.665$ and 0.945). It has to be noted, however, that for the del/del analysis significant heterogeneity was found between the cohorts.

Table 2. Frequencies of the different LCE3B_LCE3C genotypes and analysis of the association with AD

| Population | Phenotype | Samples | LCE3C_LCE3B | | | del/wt | | del/del | | Power |
			del/del	wt/del	wt/wt	OR 95% CI	*P*-value	OR 95% CI	*P*-value	
The Netherlands	Control	386	141 (0.36)	176 (0.46)	69 (0.18)					
N=681	AD	295	136 (0.46)	120 (0.41)	39 (0.13)	1.21 (0.76–1.90)	0.420	1.71 (1.08–2.70)	0.022	0.92
German replicate	Control	248	90 (0.36)	131 (0.53)	27 (0.11)					
N=506	AD	258	99 (0.38)	114 (0.44)	45 (0.17)	0.52 (0.31–0.90)	0.018	0.66 (0.38–1.15)	0.143	0.82
Irish replicate	Control	578	256 (0.44)	250 (0.43)	72 (0.13)					
N=892	AD	314	118 (0.38)	148 (0.47)	48 (0.15)	0.89 (0.58–1.35)	0.578	0.69 (0.45–1.06)	0.089	0.96
Italian replicate	Control	446	156 (0.35)	211 (0.47)	79 (0.18)					
N=654	AD	208	71 (0.34)	106 (0.51)	31 (0.15)	1.28 (0.80–2.06)	0.309	1.16 (0.70–1.92)	0.562	0.87
All*	Control	1,658	643 (0.39)	768 (0.46)	247 (0.15)					
N=2,733	AD	1,075	424 (0.39)	488 (0.46)	163 (0.15)	0.95 (0.76–1.20)	0.665	0.99 (0.78–1.26)	0.945**	1.0

OR 95% CI, odds ratio and 95% confidence interval for del/wt and del/del *LCE3C_LCE3B* genotypes and atopic dermatitis (AD) obtained by logistic regression using wt/wt as a reference; *Values presented in the table are adjusted by population. For All, del/del **significant heterogenity was found.

Table 3. Frequencies of the different LCE3B_LCE3C genotypes and analysis of the association with AD + asthma

| Population | Phenotype | Samples | LCE3C_LCE3B | | | del/wt | | del/del | | Power |
			del/del	wt/del	wt/wt	OR 95% CI	*P*-value	OR 95% CI	*P*-value	
The Netherlands	Control	386	141 (0.36)	176 (0.46)	69 (0.18)					
N=558	AD + asthma	172	77 (0.45)	73 (0.42)	22 (0.13)	1.30 (0.75–2.26)	0.350	1.71 (0.98–2.98)	0.057	0.80
German replicate	Control	248	90 (0.36)	131 (0.53)	27 (0.11)					
N=331	AD + asthma	83	32 (0.39)	36 (0.43)	15 (0.18)	0.50 (0.24–1.03)	0.059	0.64 (0.30–1.35)	0.243	0.50
Irish replicate	Control	578	256 (0.44)	250 (0.43)	72 (0.13)					
N=676	AD + asthma	98	42 (0.43)	47 (0.48)	9 (0.09)	1.50 (0.70–3.22)	0.292	1.31 (0.61–2.82)	0.487	0.64
Italian replicate	Control	446	156 (0.35)	211 (0.47)	79 (0.18)					
N=484	AD + asthma	38	17 (0.45)	18 (0.47)	3 (0.08)	2.25 (0.64–7.84)	0.204	2.87 (0.82–10.1)	0.100	0.30
All*	Control	1,658	643 (0.39)	768 (0.46)	247 (0.15)					
N=2,049	AD + asthma	391	168 (0.43)	174 (0.44)	49 (0.13)	1.16 (0.81–1.66)	0.416	1.35 (0.94–1.94)	0.103	1.0

OR 95% CI, odds ratio and 95% confidence interval for del/wt and del/del *LCE3C_LCE3B* genotypes and atopic dermatitis (AD) + asthma obtained by logistic regression using wt/wt as a reference; *Values presented in the table are adjusted by population.

Analysis of association of *LCE3C_LCE3B*-del with AD + asthma or serum IgE

Subgroup analysis of the different AD cohorts revealed that the Dutch cohort, which showed a significant association, contained a higher percentage of patients with concomitant asthma (58 versus 18–32% in the other cohorts). In this cohort, we found a significantly higher frequency of the *LCE3C_LCE3B*-del allele in the subgroup of AD + asthma patients (66%) compared with healthy controls (59%) (Supplementary Table 2). In contrast, logistic regression models, again using wt/wt as reference, showed no association for the del/wt genotype with AD + asthma ($P = 0.350$, OR 1.30, 95% CI: 0.75–2.26, Table 3). For the del/del genotype an association of borderline significance was found for AD + asthma ($P = 0.057$, OR 1.71, 95% CI: 0.98–2.98) (Table 3). Stratification for asthma in the other cohorts,

however, did not reveal significant associations between AD + asthma and *LCE3C_LCE3B*-del (Table 3). For the del/wt genotype an association of borderline significance for *LCE3C_LCE3B*-del and AD + asthma was found in the German cohort ($P = 0.059$), which was in the opposite direction to the Dutch cohort (OR 0.50, 95% CI: 0.24–1.03). Combining all AD + asthma data from the four cohorts, to increase statistical power (see Table 3 combined power is 1.0), revealed no significant association of asthma + AD and the *LCE3B_LCE3C*-del allele ($n = 2049$, $P = 0.416$ and 0.103 for the del/wt and del/del genotypes, respectively, Table 3).

As a region on 1q21 in close proximity to the *LCE* cluster was previously reported to be associated with total serum IgE levels (Sharma *et al.*, 2007), we further examined the association of the *LCE3C_LCE3B*-del allele with total serum

IgE levels in our Dutch cohort. As expected, the cases (mean total IgE 115 kU l^{-1}) had significantly higher total IgE serum levels than the controls (mean total IgE 20.7 kU l^{-1}, $P<0.001$). However, analysis of variance did not show differences in IgE serum levels for the different genotypes within the patient groups separately and overall ($P=0.351$, data not shown).

DISCUSSION

Our analysis of the association of *LCE3C_LCE3B*-del and AD showed varying results in the different cohorts. A significant positive association was found in the Dutch cohort, whereas a weak association in the opposite direction was found for the del/wt genotype in the German cohort. The apparent association found in the Dutch cohort might be due to population heterogeneity, or could be a false-positive finding resulting from small cohort sizes. Because of the high linkage disequilibrium between SNP rs4112788 and LCE3B_LCE3C-del ($r^2=0.928$ and $D'=0.988$), although admittedly not absolute, we decided to include both the *LCE3C_LCE3B*-del and the rs4112788 results (Italian data set) in our overall analysis. On the basis of the combined results, we conclude that there is no evidence for association of *LCE3C_LCE3B*-del and AD in individuals of European ancestry. These results underline the importance of replication studies. Similarly, we did not detect an association of *LCE3C_LCE3B*-del and AD+asthma. It has to be noted, however, that the power to detect an effect for AD+asthma was smaller than that for AD alone. For the AD+asthma phenotypes, the power of the individual cohorts is rather small (Table 3); however, the power of combined analysis both in AD and AD+asthma is 1.0. It should be noted that for a part of the Dutch control cohort, the Irish and Italian control cohorts no information about asthma status was available, which may limit the interpretation of our analysis.

Previous studies on *FLG* and *LCE3C_LCE3B*-del have indicated that polymorphisms and mutations in genes encoding skin barrier proteins make a sizeable contribution to the genetic basis of both AD (estimated population attributable risk of *FLG* is 13% (O'Regan *et al.*, 2008)) and psoriasis (estimated population attributable risk of *LCE3C_LCE3B*-del is 21% (De Cid *et al.*, 2009)). For AD it is very likely that *FLG* mutations directly affect barrier function of normal skin and contribute to percutaneous antigen priming and the subsequent immunological sequelae leading to overt disease (Fallon *et al.*, 2009). In the case of psoriasis, the role of the deleted *LCE* genes is less clear as they are not normally expressed in intact skin, but only induced upon activation. Preliminary findings from our laboratory indicate that this is also true for *LCE3B* and *LCE3C* expression in AD (data not shown). It was recently suggested that other genes of the EDC contribute to AD, and clearly there are many other plausible candidate genes in this region, which may on their own or through interaction with *FLG* lead to barrier dysfunction and AD (Morar *et al.*, 2007). As there was no convincing association between *LCE3C_LCE3B*-del and AD, we did not further investigate a possible interaction with *FLG* null alleles. We conclude that, despite the shared locus on chromosome 1, the psoriasis-associated deletion of *LCE3* genes does not contribute to AD. Our data indicate that the potential roles of skin barrier defects in the pathogenesis of AD and psoriasis are based on distinct genetic causes.

MATERIALS AND METHODS

Study populations

A total of 1075 patients and 1658 controls of European origin from four cohorts was investigated. Basic characteristics of the study cohorts are shown in Table 1, and a detailed description of the study population is given in the Supplementary Information. In all studies informed consent was obtained, and all studies have been approved by the local ethical committees: for the Netherlands, The medical ethics committee of the University Medical Center Groningen and ''Commissie Mensgebonden Onderzoek Arnhem-Nijmegen''; for Germany, ''Bayerische Landesärtzekammer'' Munich and the ethics committee of the University of Bonn; for Italy, the medical ethics committee ''Policlinico Tor Vergata''; and for Ireland, The Research Ethics Committees of Our Lady's Children's Hospital Crumlin (cases) and Trinity College Dublin (Trinity Biobank controls). The investigations were conducted according to the Declaration of Helsinki principles.

Genotyping

The Dutch, German, and Irish cohort were genotyped by using the direct PCR-based method described by De Cid *et al.* (2009). The Italian cohort was genotyped by analyzing SNP rs4112788, which was shown to be in a strong linkage disequilibrium with the deletion ($r^2=0.93$ and $D'=0.99$) (De Cid *et al.*, 2009). Genotyping was successful in 98.1% of probands. We did not observe significant evidence for deviation from Hardy–Weinberg equilibrium in any of the study groups.

Statistical analysis

Descriptive statistics for quantitative values are given as means± standard deviation. The observed genotype frequencies were compared with the expected Hardy–Weinberg distribution by χ^2-test. Logistic regression models were used to assess the genetic effect of the *LCE3C_LCE3B*-del allele on AD risk: data from co-dominant and genotype-specific models were calculated (for results from co-dominant models, see Supplementary Tables 1 and 2). Genotype-specific models were used in the main text of this article, as these models are biologically relevant. The presented data in the main text are derived from models that are unadjusted for age and sex. Unadjusted OR and 95% CI were calculated using homozygosity for the undeleted allele (wt/wt) as a reference category. Overall-values present in the tables are adjusted by population. The sex-adjusted analysis for the Italian and Irish cohorts are present in Supplementary Table 3. To test if the cohorts show heterogeneity, the Cochran–Mantel–Haenszel statistics were performed, for del/del compared with wt/wt genotypes significant differences were found ($P=0.003$). Values for total serum IgE levels were only available from the Dutch cohort. These values were first log-transformed and comparisons were carried out using one-way analysis of variance. The statistical analysis was performed using SPSS software 16.0 (SPSS, Chicago, IL). Power calculations were performed using Stata Software 10.0 (StataCorp LP, College Station, TX), assumptions in the power analysis (OR of 1.7) were derived from our previous study (De Cid *et al.*, 2009).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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