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Research study

Dermatopathology

Association of variation in the *CLDN1* gene with atopic dermatitis in a German case-control cohort

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Abstract

Introduction

Atopic dermatitis is a chronic inflammatory skin disorder caused by complex interaction of genetic and environmental factors. Mutations in the gene encoding filaggrin, a major structural protein in the epidermis, constitute the most significant known risk factor for Atopic dermatitis development so far, implying an impaired skin barrier function as a major pathogenic mechanism in Atopic dermatitis development. However, a reduced skin barrier function has been demonstrated in Atopic dermatitis patients irrespective of *Filaggrin* genotype, suggesting that other factors may also modulate skin barrier integrity. It was recently shown the expression of Claudin-1, a major tight junction protein in the granular layer, was reduced in the skin of Atopic dermatitis patients and that variation in the *Claudin-1* gene was associated with Atopic dermatitis in two small American populations. For replication of these findings, we investigated the role of *Claudin-1* variation in an independent German case-control cohort.

Materials and Methods

Six single nucleotide polymorphisms (SNPs) in the *Claudin-1* gene, including rs16865373 that had shown association with Atopic dermatitis in the European American cohort, were genotyped in 451 unrelated Atopic dermatitis patients and 375 non-atopic controls by means of restriction enzyme digestion. Allele, genotype and haplotype frequencies were subsequently compared between cases and controls.

Results

In this analysis, none of the investigated Single nucleotide polymorphisms or haplotypes in the *Claudin-1* gene showed a significant association with Atopic dermatitis.

Conclusion

Our results do not support a substantial role for *Claudin-1* variation in Atopic dermatitis pathogenesis. Yet, additional studies in independent cohorts are needed to confirm these results.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that presumably arises from complex interaction between genetic and environmental factors[1]. Additional to immune-mediated mechanisms, the importance of an intact skin barrier in the protection against AD has been highlighted by several findings over the recent years[2]. Most importantly, mutations in the gene encoding filaggrin (FLG), a major structural protein in the stratum corneum of the epidermis, have been identified as the most significant known risk factor for AD development so far[3]. The *FLG* gene is located in the epidermal differentiation complex (EDC) on chromosome 1q21 and mutations in *FLG* have consistently been associated with early-onset persistent AD in many populations[4]. However, *FLG* mutations explain ~30% of AD risk[3] and impaired skin barrier function has also been observed in patients who do not harbour *FLG* mutations[5], suggesting that additional genetic factors involved in skin integrity and barrier function may contribute to AD risk.

Tight junctions in the granular layer of the epidermis have been postulated to build a second mechanism of defence against exogenous substances and transepidermal water loss below the strateum corneum[6]. It was recently demonstrated that the expression of claudin-1, a major tight junction protein, was reduced in the skin of patients with AD[7]. Further, single nucleotide polymorphisms (SNPs) in the gene encoding claudin-1 (*CLDN1,* located on chromosome 3q28) were associated with AD in North American cohorts of both European and African American origin[7]. These results strongly implicated tight junction defects in the pathogenesis of AD; however, the analysed cohorts were very small (156 and 152 cases, respectively) and replication has not been reported yet. In order to further investigate the role of *CLDN1* variation for AD pathogenesis, we evaluated six SNPs in the *CLDN1* gene, including rs16865373 that showed association in the European American cohort, in a German AD case-control sample.

Materials and Methods

Subjects

The case-control cohort comprised 451 unrelated patients with AD who were recruited by a consultant specialist for AD (Q.P., Gladbeck, Germany). The diagnosis was based on the criteria developed by Hanifin

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and Rajka[8]. Mean age of the AD patients was 19 ± 15 years (median 11 years). 375 control samples were collected in the same private practice. The control subjects had no self-reported allergies or allergic symptoms and no first degree relatives with allergic diseases. They all underwent clinical examination in order to exclude symptoms of AD, asthma or allergic rhinitis. The controls were adults of at least 40 years of age with a mean age of 62 ± 11 years (median 63 years). All patients and controls were Germans of European ancestry. More details concerning the patient and control groups were described elsewhere[9]. 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[10]. A total of six SNPs in the *CLDN1* gene were selected for genotyping, representing the haplotypic block structures according to HapMap (www.hapmap.org). For genotyping, we used polymerase chain reaction (PCR) followed by restriction enzyme digestion. PCR analysis was performed in a total volume of 10 µl, containing 40 ng DNA, 200 mmol of each dNTP, 1.5–3 mmol $MgCl_2$, 5 pmol of each primer and 0.5 U Taq-DNA-polymerase (Genecraft, Münster, Germany). Thermal cycling was performed at the RoboCycler and Biometra *T* cycler (Stratagene, Heidelberg, Germany and Biometra GmbH, Göttingen, Germany, respectively). After two initial cycles at 6°C and 3°C above the annealing temperature, 28–32 cycles of 95°C (30 sec), annealing temperature (30 sec) and 72°C (30 sec) were run. The PCR product was subsequently digested with the respective restriction enzyme. The fragments were then separated on 2.5–3.5% agarose gels in 1xTBE buffer (30–60 min, 200 V) and visualised with ethidium bromide (0.5% [w/v]). Additional information for each SNP (e.g. primer sequences, PCR conditions) is summarised in Table 1.

Statistics

Comparison of genotype and allele frequencies between AD patients and controls was performed according to the $\chi 2$ method; $p < 0.05$ was considered to be significant. Bonferroni correction for multiple testing was performed. Deviations from Hardy-Weinberg equilibrium (HWE) were evaluated using the deFinetti program (www.ihg.gsf.de/cgi-bin/hw/hwa1.pl). Haplotype frequencies were estimated and tested for haplotypic association using Haploview 4.0[11]. Power analyses were performed with the Genetic Power Calculator (www.pngu.mhg.harvard.edu/~purcell/gpc/cc2.html).

Results

The genotypic distributions for all six SNPs were in accordance with HWE in patients and controls. We did not observe a significant association with AD for any of the investigated SNPs (Table 2). Haplotype analyses did not reveal significant association results either (Table 3).

Power estimates ranged between 99 (assuming a genotypic relative risk of two for heterozygotes and four for homozygotes and D' = 0.9 between marker and causal variation) and 64% power considering less strong genetic effects (with genotype relative risks of 1.5/2.25 and D' = 0.8).

Discussion

To our knowledge, this is the first attempt to replicate association of AD with variation in the tight junction protein claudin-1 that was recently described in two small American populations[7]. However, in our German AD case-control cohort, we did not find association with any of the six SNPs in the *CLDN1* gene chosen to represent the most important haploblocks. We also included rs16865373 which had shown association in the European American cohort[7] but could not replicate this finding. Thus, our results do not support a substantial role of this gene for AD pathogenesis.

Besides *FLG* loss-of-function mutations that constitute a major risk factor for AD beyond dispute[3], variations in other skin barrier genes additional to claudin-1 have been implicated in AD pathogenesis recently. For example, a large meta-analysis of GWAS data, including 5,606 AD patients and 20,565 controls from 16 population-based studies, identified genome-wide

Table 1 Primers, PCR conditions and restriction enzymes used for the evaluation of polymorphisms in the *CLDN1* gene

Polymorphism	Primer sequences	Restriction enzyme	Annealing temperature (°C)
rs3732925	F: TGACTCAAATCATCCAGGAGTTCA R: CACAAGCAAGGCGTAGGTAATG	MslI	56
rs3774017	F: CAGGACTTCTTTACACCAAAACCA R: TTAGAAACCTCCACCTGCCAA	TaaI	57
rs746286	F: AGATCCAGTTAGTGCAGGGAGG R: TTCTTTGTATTATGTTGCGCAGG	MboI	56
rs17429833	F: AACTCTCCGCCTTCTGCACCT R: CACTCACTGCTCAGATTCAGCAA	BsaHI	56
rs6781278	F: AGAATCGAAGCATGAAAGAGTCCT R: AATGGCTCAAACCCTAGTGCTG	ScaI	56
rs16865373	F: TTCTGGAAATTCTGTGATTGCTCC R: TGGTTTCTGATTTCTCCAAAGTATCTG	MmcI	58

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Table 2 Genotype and allele frequencies of *CLDN1* polymorphisms in patients with atopic dermatitis (AD) compared to people with no known history of AD (controls)

Polymorphism	Genotypes	Alleles	AD patients Numbers inside the brackets are percentage values (%)	Controls Numbers inside the brackets are percentage values (%)	*p*-value	p_c-value*
rs3732925	T/T T/C C/C		417 (93.1) 31 (6.9) 0 (0.0)	296 (94.3) 18 (5.7) 0 (0.0)	0.51	n.s.
		T C	865 (96.5) 31 (3.5)	610 (97.1) 18 (2.9)	0.52	n.s.
rs3774017	G/G G/A A/A		1 (0.2) 38 (8.2) 423 (91.6)	2 (0.6) 18 (5.7) 298 (92.5)	0.18	n.s.
		A G	40 (4.3) 884 (95.7)	22 (3.5) 614 (96.5)	0.39	n.s.
rs746286	A/A A/G G/G		241 (53.4) 173 (38.4) 37 (8.2)	160 (50.1) 135 (42.6) 22 (6.9)	0.46	n.s.
		A G	655 (72.6) 247 (27.4)	455 (71.8) 179 (28.2)	0.71	n.s.
rs17429833	C/C C/G G/G		9 (2.5) 48 (13.4) 300 (84.0)	6 (2.0) 36 (12.3) 251 (85.7)	0.87	n.s.
		C G	66 (9.2) 648 (90.8)	48 (8.2) 538 (91.8)	0.50	n.s.
rs6781278	G/G G/A A/A		131 (28.2) 235 (50.5) 99 (21.3)	97 (30.2) 152 (47.4) 72 (22.4)	0.68	n.s.
		T C	497 (53.4) 433 (46.6)	346 (53.9) 296 (46.1)	0.86	n.s.
rs16865373	C/C C/T T/T		432 (94.1) 27 (5.9) 0 (0.0)	308 (93.6) 21 (6.4) 0 (0.0)	0.77	n.s.
		C T	891 (97.1) 27 (2.9)	637 (96.8) 21 (3.2)	0.78	n.s.

**p*-value after Bonferroni correction
n.s. = not significant

Table 3 Frequencies of CLDN1 haplotypes in patients with atopic dermatitis (AD) compared to people with no known history of AD (controls)

Haplotype	Frequency in AD patients	Frequency in controls	*p*-value
121221	0.425	0.416	0.748
122211	0.267	0.270	0.919
121211	0.148	0.158	0.618
121111	0.094	0.067	0.090
211221	0.025	0.029	0.721
121212	0.022	0.030	0.399

significant association with polymorphisms near the *OVOL1* and *ACTL9* genes, both involved in epidermal proliferation and differentiation[12]. Furthermore, a 24-bp deletion in the gene encoding small proline-rich protein 3 (*SPRR3*), located within the EDC, was shown to be associated with AD in cohorts from Germany, Poland and the Czech Republic[13]. Thus, complex genetic modifications in epidermal barrier functions may underlie AD pathogenesis. However,

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the latter association results also still await replication in independent cohorts. For *CLDN1,* we could not confirm the reported association with AD.

We are aware of the fact that the results presented here require replication in additional independent cohorts and that the statistical power is only moderate due to the comparatively small cohort sizes. Still, the same cohort has successfully been used to detect other associations with AD before, including association with *FLG* mutations[14]. In addition, power calculations revealed between 64 and 99% power to detect a significant association, dependent on the assumed genotypic relative risk. Therefore, we believe that we could not have missed a substantial effect of *CLDN1* variation in the given cohort. Still, comprehensive evaluation of the tested as well as additional *CLDN1* SNPs in independent populations is needed in order to elucidate the role of this gene for AD pathogenesis and open the way for novel therapeutic straegies[15,16].

Conclusion

We did not find association of *CLDN1* SNPs with AD, suggesting that variation in other epidermal genes may contribute to the observed epidermal barrier dysfunction. Novel therapeutic strategies are already under scrutiny that aim at strengthening the epidermal barrier in order to modulate the disease course and the acquisition of additional allergic disorders. Therefore, a better understanding of the genetic factors involved in skin integrity and barrier function is warranted in order to establish the basis for a more personalised therapy in the future.

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Abbreviations list

AD, Atopic dermatitis; *CLDN1*, *Claudin-1;* EDC, Epidermal differentiation complex; FLG, Filaggrin; HWE, Hardy-Weinberg equilibrium; n.s., Not significant; PCR, Polymerase chain reaction; SNPs, Single nucleotide polymorphisms; TBE, Tris-Borate-EDTA (pH 8.3).

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