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INVESTIGATIVE REPORT

A Promoter Polymorphism of the Vitamin D Metabolism Gene *Cyp24a1* is Associated with Severe Atopic Dermatitis in Adults

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Atopic dermatitis (AD) is a chronic inflammatory skin disease in which genetic and environmental factors result in impaired epidermal barrier functioning and an altered immune response. Vitamin D influences these 2 pathomechanisms, and beneficial results have been suggested in AD. The aim of this study was to investigate the potential roles of the 2 essential vitamin D metabolizing enzymes. The frequencies of 6 common polymorphisms in the genes encoding the vitamin D synthesizing enzyme *Cyp27b1* or the inactivating enzyme *Cyp24a1* were assessed in 281 patients with AD and 278 healthy donors in a case-control setting. The *Cyp24a1* rs2248359-major C allele was significantly over-represented in patients with AD compared with controls, which was more pronounced in patients with severe AD. In addition, haplotypes of the *Cyp24a1* and *Cyp27b1* genes were associated with AD. These data support that vitamin D mediates beneficial functions in AD and suggest that future studies on the impact of vitamin D on AD should consider the individual genotypes of the vitamin D metabolizing enzymes. *Key words: vitamin D; atopic dermatitis; metabolism; cyp24a1; cyp27b1.*

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Atopic dermatitis (AD) is a chronic inflammatory skin disease, in which genetic and environmental factors result in impaired epidermal barrier functioning and an altered immune response. Vitamin D influences these 2 mechanisms, and a beneficial impact of vitamin D supplementation in AD has been suggested, e.g. by our findings that a defined vitamin D receptor (VDR) haplotype is more frequent in adult patients with severe AD (1), the beneficial action of a synthetic vitamin D receptor agonist in a pre-clinical model (2), and the association of vitamin D deficiency with AD severity (3) and a recent clinical pilot trial in children with winter-related AD (4).

Most vitamin D functions are mediated by the nuclear VDR following binding of its natural ligand calcitriol (chem. 1,25$(OH)_2$D) and regulation of target gene transcription. In keratinocytes, different genes associated with the epidermal barrier function are induced by VDRs, such as filaggrin, involucrin, loricrin and epidermal transglutaminase (2). VDR also impacts the antigen presentation and T-cell differentiation, resulting in a tolerogenic rather than an inflammatory phenotype. It is notable that bioactive calcitriol is metabolized endogenously in keratinocytes and lymphocytes by enzymes encoded by the genes *Cyp27b1* (calcitriol-synthesis from 25-hydroxyvitamin D) and *Cyp24a1* (calcitriol-inactivation), respectively. Alterations in these 2 genes may impact on VDR activity, e.g. through prolonged or reduced signalling. In agreement with this, single nucleotide polymorphisms (SNPs) in the *Cyp27b1* gene were identified in autoimmune diseases and *Cyp24a1* SNPs were linked to allergic asthma. The aim of the present study was to examine the frequencies of *Cyp27b1* and *Cyp24a1* SNPs in adults with AD and non-atopic individuals.

PATIENTS AND METHODS

For details of patient characteristics and methods, see Appendix S1[1] and Table I. The study procedures were approved by the local ethics committee and performed in accordance with ethical standards on human experimentation and with the Declaration

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Table I. *Demographic characteristics of patients with atopic dermatitis (AD) and controls*

		Patients with AD		
	Controls *n* = 278	All *n* = 281	Mild/moderate (SCORAD 9–40) *n* = 126	Severe (SCORAD > 40) *n* = 155
Male, *n* (%)	98 (35.3)	121 (43.1)	45 (35.7)	76 (49.0)
Female, *n* (%)	180 (64.7)	160 (56.9)	81 (64.3)	79 (51.0)
Age, years, median (IQR)	35 (31–41)	37 (27–48)	33 (26–43)	41 (29–53)
SCORAD, mean ± SD	–	46.8 ± 18.4	27.6 ± 8.1	58.8 ± 11.5

SCORAD: scoring atopic dermatitis; IQR: interquartile range; SD: standard deviation.

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Table II. *Single nucleotide polymorphism frequencies in the atopic dermatitis (AD) patient and healthy control groups*

	Controls *n* (%)	AD–group *n* (%)	Odds ratio (95% CI)	*p*-value	Severe AD[b] *n* (%)	Odds ratio (95% CI)	*p*-value
Cyp27b1[a]							
rs703842							
CC	125 (45.1)	127 (45.5)	–	–	67 (43.5)	–	–
CT	119 (43.0)	122 (43.7)	1.0 (0.7–1.4)	1.0	73 (47.4)	0.9 (0.6–1.3)	0.60
TT	33 (11.9)	30 (10.8)	1.1 (0.6–1.9)	0.8	14 (9.1	1.3 (0.6–2.5)	0.62
n	277	279			154		
Hardy–Weinberg equilibrium	0.85	1.0			0.65		
Cyp27b1[a]							
rs10877012							
CC	139 (50.2)	132 (47.3)	–	–	70 (45.5)	–	–
CA	108 (39.0)	118 (42.3)	0.9 (0.6–1.2)	0.49	71 (46.1)	0.8 (0.5–1.2)	0.25
AA	30 (10.8)	29 (10.4)	1.0 (0.6–1.7)	0.92	13 (8.4)	1.2 (0.6–2.4)	0.81
n	277	279			154		
Hardy–Weinberg equilibrium	0.44	0.94			0.70		
Cyp27b1[a]							
rs3782130							
CC	125 (45.1)	127 (45.5)	–	–	67 (43.5)	–	–
CG	119 (43.0)	122 (43.7)	1.0 (0.7–1.4)	1.00	73 (47.4)	0.9 (0.6–1.3)	0.60
GG	33 (11.9)	30 (10.8)	1.1 (0.6–1.9)	0.81	14 (9.1)	1.3 (0.6–2.5)	0.62
n	277	279			154		
Hardy–Weinberg equilibrium	0.85	1.00			0.65		
Cyp27b1[a]							
rs4646536							
TT	123 (44.4)	128 (45.9)	–	–	68 (44.2)	–	–
TC	126 (45.5)	121 (43.4)	1.1 (0.8–1.5)	0.72	72 (46.8)	1.0 (0.6–1.5)	1.00
CC	28 (10.1)	30 (10.8)	1.0 (0.6–1.7)	1.00	14 (9.1)	1.1 (0.6–2.2	0.92
n	277	279			154		
Hardy–Weinberg equilibrium	0.88	0.98			0.71		
Cyp24a1[a]							
rs2248359							
CC	132 (47.5)	133 (49.4)	–	–	82 (55.8)	–	–
CT	112 (40.3)	120 (44.6)	0.9 (0.7–1.3)	0.8	59 (40.1)	1.2 (0.8–1.4	0.44
TT	34 (12.2)	16 (5.9)	**2.1 (1.1–4.1)**	**0.03**	6 (4.1)	**3.5 (1.4–8.8)**	**0.008**
n	278	269			147		
Hardy–Weinberg equilibrium	0.41	0.26			0.51		
Cyp24a1[a]							
rs2296241							
GG	78 (28.1)	58 (21.6)	–	–	35 (23.8)	–	–
GA	123 (44.2)	141 (52.4)	**0.6 (0.4–1.0)**	**0.05**	77 (52.4)	0.7 (0.4–1.2	0.23
AA	77 (27.7)	70 (26.0)	0.8 (0.5–1.3)	0.47	35 (23.8)	1.0 (0.6–1.7)	0.92
n	278	269			147		
Hardy–Weinberg equilibrium	0.16	0.71			0.85		

[a]Analysis for some DNAs failed; [b]SCORAD>40.
95% CI: 95% confidence interval.
Values in **bold** are statistically significant.

of Helsinki 1975, 1983 revision. Both cohorts were genotyped for 6 SNPs in genes encoding *Cyp27b1* and *Cyp24a1* using real-time-PCR with subsequent melting curve analysis. The haplotype sequences were analysed *in silico*, as described previously (1). Serum concentrations of 25(OH)D were measured (by enzyme-immunoassay (EIA), IDS Systems, Hamburg, Germany).

RESULTS

Significant over-representation of the *Cyp24a1*-SNP rs2248359 major C allele genotypes were found in patients with AD compared with healthy controls (odds ratio (OR) 2.10; 95% confidence interval (95% CI) 1.1–4.1, $p = 0.03$, Table II). In patients stratified according to severity, this over-representation was even more pronounced (OR 3.5 (1.4–8.8). The *Cyp24a1* SNP rs2296241 was neither associated with AD as such nor after stratification according to severity (Table II). The linkage disequilibrium (LD) was average to high between both *Cyp24a1* SNPs (D'=70; maximum 100=linked, Fig. S1[1]). The haplotype rs2248359T, rs2296241A (*Cyp24a1*-TA) was more frequent in healthy individuals ($p = 0.005$–0.044) and, conversely, the haplotype *Cyp24a1*-CA in patients with severe AD or AD ($p = 0.003$–0.012), respectively (Table III, with or without correction for multiple comparisons). It is notable that both *Cyp24a1*-SNPs are located in evolutionarily conserved regions of the human and murine genome (Fig. S2[1]), suggesting functional relevance (5).

Regarding the *Cyp27b1*-polymorphisms, no significant differences were observed in genotype distribution

Table III. *Haplotypes of Cyp27b1 and Cyp24a1 genotypes of atopic dermatitis (AD) and healthy controls*

Number	*Cyp27b1* rs703842	*Cyp27b1* rs10877012	*Cyp27b1* rs3782130	*Cyp27b1* rs4646536	Control (%) (n=277)	AD (%) (n=279)	p^b	$p_{corr}{}^c$	Severe AD[a] (%) (n=154)	p^b	$p_{corr}{}^c$
1	C	C	C	T	63.7	67.2	0.222	0.567	66.9	0.438	0.965
2	T	A	G	C	27.1	31.3	0.116	0.465	31.1	0.201	0.546
3	**T**	**C**	**G**	**C**	3.4	0.9	**0.004**	**0.009**	1.0	**0.029**	**0.098**
4	T	A	G	T	1.6				0.3	0.087	0.380
			Cyp24a1 rs2248359	*Cyp24a1* rs2296241	(n=278)	(n=269)			(n=147)		
5			C	G	46.4	42.8	0.229	0.576	45.7	0.822	0.995
6			**T**	**A**	28.6	23.3	**0.044**	0.143	19.9	**0.005**	**0.021**
7			**C**	**A**	21.2	29.0	**0.003**	**0.012**	30.1	**0.003**	**0.015**
8			T	G	3.8	5.0	0.328	0.725	4.3	0.649	0.964

[a]SCORAD>40; [b]p-value 2×2 χ^2-test; [c]p_{corr}=permutated p-value.
Values in **bold** are statistically significant.

between both groups ($p>0.05$; Table II). The *Cyp27b1* haplotypes were tightly genetically linked (D'=94–97, Fig. S1[1]). The rare haplotype TCGC (rs703842T, rs10877012C, rs3782130G, rs4646536C) was found to be protective for AD in a small subpopulation of healthy individuals ($p=0.004$). One of 4 investigated Cyp27b1-SNP, rs4646536, was evolutionarily conserved (Fig. S3[1]).

In addition, serum 25(OH)D concentrations among 98 patients with AD (38.1±19.0 nmol/l) and 45 control subjects (36.4±16.4 nmol/l) were comparable between the groups ($p=0.77$, Fig. S4[1]). The 25(OH)D levels were not associated with any SNP or haplotype investigated in this study ($p=0.382$–0.977) (see Appendix S1[1]; Table IV).

DISCUSSION

The data presented here suggest that altered vitamin D metabolism due to genetic variances impacts on the pathogenesis of AD. We identified significant overrepresentation of the *Cyp24a1* rs2248359 SNP C allele and a haplotype with rs2296241 (No. 7 in Table III) in adults with severe AD compared with healthy controls. These polymorphisms in the promoter region or exon 4, respectively, are located in evolutionarily conserved regions between humans and mice, suggesting a functional relevance, e.g. by conserved transcription factor binding or protein function (5). The identified *Cyp24a1* allele was shown to result in enhanced mRNA expression and calcitriol-inactivation, resulting in decreased VDR activity (6). In agreement, this *Cyp24a1* allele has also been identified in patients with allergic asthma (7, 8), a disease in which epidemiological data suggest beneficial functions of vitamin D-signalling (9). Thus, the *Cyp24a1*-SNP may be involved in the pathogenesis of AD by reducing VDR activity that mediates beneficial functions. The SNP frequencies in the *Cyp27b1*-gene encoding the enzyme synthesizing active calcitriol from its precursor were comparable between the AD and control groups. Of interest, we identified a rare subtype of adult patients with severe AD carrying a defined *Cyp27b1* genotype (number 3 in Table III, 3 AD patients, 9 controls), which is thought to result in a loss of function, as the respective alleles were previously associated with reduced *Cyp27b1* mRNA expression (10, 11), reduced 25(OH)D-activation (12, 13), and the vitamin D-susceptible disease multiple sclerosis (14). However, the relevance of the genotypes identified here in AD is not known. As the expression and function of VDR and vitamin D metabolism are regulated in a cell-specific manner, functional genetic assays should consider the complex spatio-temporal interaction of cells in AD, which has not yet been established, but is an interesting topic for further research.

The present study did not find a significant impact of any *Cyp24a1* or *Cyp27b1* SNP with 25(OH)D serum concentrations. This may be attributed to the low sample size, or more probably, to the low 25(OH)D-concentrations resulting rather from the insufficient UVB exposure during the winter months (15) than from VDR-dependent action of vitamin D metabolizing enzymes. Whether more prominent differences are prevalent during summer, in vitamin D sufficiency, is not known.

In conclusion, this study shows a weak, but significant, association of defined genetic

Table IV. *Serum 25(OH)D concentration in relation to the genotype*

Gene	SNP	Genotype Wt (n)	Het (n)	Hom (n)	25(OH)D concentration (nmol/l) Wt Mean±SD	Het Mean±SD	Mut Mean±SD	p*
Cyp27b1	rs703842	CC (62)	CT (68)	TT (12)	36.7±19.2	37.0±16.8	45.8±20.1	0.697
	rs10877012	CC (64)	CA (66)	AA (12)	37.2±14.1	36.5±18.5	45.8±19.9	0.863
	rs3782130	CC (64)	CG (66)	GG (12)	37.2±19.5	36.5±16.4	45.8±20.1	0.862
	rs4646536	TT (64)	CT (66)	CC (12)	37.1±19.2	36.6±16.7	45.8±20.1	0.896
Cyp24a1	rs2248359	CC (68)	CT (43)	TT (22)	35.9±16.8	38.5±18.3	41.4±20.6	0.382
	rs2296241	GG (30)	GA (68)	AA (35)	36.8±19.5	37.7±16.4	38.3±20.1	0.977

SD: standard deviation; Wt: major allele; Het: heterozygous; Hom: homozygous minor allele; *Kruskal–Wallis test.

variations in vitamin D metabolism with AD in adults. This may represent a polygenic disease background for AD and/or suggests that a subgroup of patients with AD benefits from VDR signalling, as suggested by a recent controlled clinical trial in children with AD (4). To determine whether the findings of the present study are clinically relevant requires both reproduction of the findings in an independent cohort and proof-of-concept in a controlled clinical trial in adults investigating the impact of 25(OH)D on AD, including monitoring of 25(OH)D status and consideration of the individual genotype.

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