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.

Accepted Aug 25, 2015; Epub ahead of print Aug 28, 2015

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PATIENTS AND METHODS
For details of patient characteristics and methods, see Appendix S1 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

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

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>All</th>
<th>Mild/moderate (SCORAD 9–40)</th>
<th>Severe (SCORAD&gt;40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>98 (35.3)</td>
<td>121 (43.1)</td>
<td>45 (35.7)</td>
<td>76 (49.0)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>180 (64.7)</td>
<td>160 (56.9)</td>
<td>81 (64.3)</td>
<td>79 (51.0)</td>
</tr>
<tr>
<td>Age, years, median (IQR)</td>
<td>35 (31–41)</td>
<td>37 (27–48)</td>
<td>33 (26–43)</td>
<td>41 (29–53)</td>
</tr>
<tr>
<td>SCORAD, mean ± SD</td>
<td>46.8 ± 18.4</td>
<td>27.6 ± 8.1</td>
<td></td>
<td>58.8 ± 11.5</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Controls n (%)</th>
<th>AD–group n (%)</th>
<th>Odds ratio (95% CI)</th>
<th>p–value</th>
<th>Severe AD* n (%)</th>
<th>Odds ratio (95% CI)</th>
<th>p–value</th>
</tr>
</thead>
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<tr>
<td><strong>Cyp27b1</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>125 (45.1)</td>
<td>127 (45.5)</td>
<td>–</td>
<td>–</td>
<td>67 (43.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CT</td>
<td>119 (43.0)</td>
<td>122 (43.7)</td>
<td>1.0 (0.7–1.4)</td>
<td>1.0</td>
<td>73 (47.4)</td>
<td>0.9 (0.6–1.3)</td>
<td>0.60</td>
</tr>
<tr>
<td>TT</td>
<td>33 (11.9)</td>
<td>30 (10.8)</td>
<td>1.1 (0.6–1.9)</td>
<td>0.8</td>
<td>14 (9.1)</td>
<td>1.3 (0.6–2.5)</td>
<td>0.62</td>
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<tr>
<td>n</td>
<td>277</td>
<td>279</td>
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<tr>
<td>Hardy–Weinberg equilibrium</td>
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<td><strong>Cyp27b1</strong></td>
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<tr>
<td>CC</td>
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<td>–</td>
<td>–</td>
<td>70 (45.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CA</td>
<td>108 (39.0)</td>
<td>118 (42.3)</td>
<td>0.9 (0.6–1.2)</td>
<td>0.49</td>
<td>71 (46.1)</td>
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<tr>
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<td>67 (43.5)</td>
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<td>1.00</td>
<td>73 (47.4)</td>
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<td>1.3 (0.6–2.5)</td>
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<td>Hardy–Weinberg equilibrium</td>
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<td>TT</td>
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<td>128 (45.9)</td>
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<td>68 (44.2)</td>
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<tr>
<td>TC</td>
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<td>0.72</td>
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<td>14 (9.1)</td>
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<tr>
<td>CC</td>
<td>132 (47.5)</td>
<td>133 (49.4)</td>
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<td>–</td>
<td>82 (55.8)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>CT</td>
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<td>0.8</td>
<td>59 (40.1)</td>
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<td>0.44</td>
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<td>16 (5.9)</td>
<td><strong>2.1 (1.1–4.1)</strong></td>
<td><strong>0.03</strong></td>
<td>6 (4.1)</td>
<td><strong>3.5 (1.4–8.8)</strong></td>
<td><strong>0.008</strong></td>
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<tr>
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<td>269</td>
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<tr>
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<td>0.26</td>
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<td>0.51</td>
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</tr>
<tr>
<td>GG</td>
<td>78 (28.1)</td>
<td>58 (21.6)</td>
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<td>35 (23.8)</td>
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<td>–</td>
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<tr>
<td>GA</td>
<td>123 (44.2)</td>
<td>141 (52.4)</td>
<td><strong>0.6 (0.4–1.0)</strong></td>
<td><strong>0.05</strong></td>
<td>77 (52.4)</td>
<td>0.7 (0.4–1.2)</td>
<td>0.23</td>
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<tr>
<td>AA</td>
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<td>70 (26.0)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.47</td>
<td>35 (23.8)</td>
<td>1.0 (0.6–1.7)</td>
<td>0.92</td>
</tr>
<tr>
<td>n</td>
<td>278</td>
<td>269</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardy–Weinberg equilibrium</td>
<td>0.16</td>
<td>0.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Analysis for some DNAs failed; aSCORAD>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 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†). 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†), suggesting functional relevance (5).

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

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CYP-SNP in atopic dermatitis

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

<table>
<thead>
<tr>
<th>Number</th>
<th>Cyp27b1 rs703842</th>
<th>Cyp27b1 rs10877012</th>
<th>Cyp27b1 rs3782130</th>
<th>Cyp27b1 rs4646536</th>
<th>Control (%) (n=277)</th>
<th>AD (%) (n=279)</th>
<th>p*</th>
<th>p corr</th>
<th>SCORAD &gt; 40</th>
<th>Severe AD* (%) (n=154)</th>
<th>p*</th>
<th>p corr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>-</td>
<td>63.7</td>
<td>67.2</td>
<td>0.222</td>
<td>0.567</td>
<td>66.9</td>
<td>0.438</td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>A</td>
<td>G</td>
<td>C</td>
<td>27.1</td>
<td>31.3</td>
<td>0.116</td>
<td>0.465</td>
<td>31.1</td>
<td>0.201</td>
<td>0.546</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>T</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>3.4</td>
<td>0.9</td>
<td>0.004</td>
<td>0.009</td>
<td>1.0</td>
<td>0.029</td>
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<td>A</td>
<td>G</td>
<td>T</td>
<td>1.6</td>
<td>-</td>
<td>0.3</td>
<td>0.087</td>
<td>0.380</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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). 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).

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). 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; 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 over-representation 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>25(OH)D concentration (nmol/l)</th>
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</thead>
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<td></td>
<td></td>
<td>Wt (n)</td>
<td>Het (n)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>Cyp27b1</td>
<td>rs703842</td>
<td>CC (62)</td>
<td>CT (68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.7 ± 19.2</td>
<td>37.0 ± 16.8</td>
</tr>
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<td>rs10877012</td>
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<td>CA (66)</td>
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<td>37.2 ± 14.1</td>
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<td>37.2 ± 19.5</td>
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<td>CT (66)</td>
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<td>37.1 ± 19.2</td>
<td>36.6 ± 16.7</td>
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<td>rs2248359</td>
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<td>CT (43)</td>
</tr>
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<td>35.9 ± 16.8</td>
<td>38.5 ± 18.3</td>
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<tr>
<td></td>
<td>rs2296241</td>
<td>CC (68)</td>
<td>GA (68)</td>
</tr>
<tr>
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<td></td>
<td>36.8 ± 19.5</td>
<td>37.7 ± 16.4</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGEMENTS

The authors would like to thank Sabine Dölle for clinical support and Fränzi Creutzburg, Diana Wöllner and Dennis Ernst for excellent technical assistance.

JH, LH, RRS, MW and GH have no conflicts of interest to declare. This work was supported by a research grant to MW from the “Investitionsbank Berlin”, from the Deutsche Forschungsgemeinschaft (DFG, SFB650-TP5 and TRR130-P19) to MW and GH and by the Charité – Universitätsmedizin Berlin.

REFERENCES