Appendix S1.

PATIENTS AND METHODS

Study population

Individuals with AD (study group, $n = 281$) were recruited from the Department of Dermatology, Venereology and Allergy, Charité – Universitätsmedizin Berlin, Germany. AD was diagnosed by an experienced dermatologist according to published criteria (S1) with a mild–moderate (9–40) or severe (>40) phenotype, as defined by SCORAD (SCORingAtopicDermatitis) (S1). All patients with AD expressed elevated serum immunoglobulin E (IgE) concentrations. As a control group, 278 healthy, unrelated individuals, matched for age and sex before analysis were recruited by the Klinik für Dermatologie and the Institut für Mikrobiologie und Hygiene, Charité – Universitätsmedizin Berlin, Germany. Exclusion criteria for the control group were a positive individual or family history of AD, allergic asthma and allergic rhinoconjunctivitis. Table I summarizes the clinical data of both groups. All participants were adults (18–65 years) of Caucasian ethnicity. The study procedures followed were in accordance with the Declaration of Helsinki 1975, revised in 1983, and was approved by the ethics board of the Charité – Universitätsmedizin Berlin. Written informed consent was obtained from all participants.

Genotype assessment

DNA was isolated from ethylenediaminetetraacetic (EDTA) whole blood samples using the QiAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). *Cyp27b1* and *Cyp24a1*-SNPs were determined by real-time PCR with subsequent melting curve analysis employing a LightCycler480 (Roche Applied Science, Mannheim, Germany). Melting peaks were as follows: *Cyp27b1* rs703842. wt: 54°C and mut: 63°C; *Cyp27b1* rs10877012. wt: 54°C and mut: 59°C; *Cyp27b1* rs3782130. wt: 51°C and mut: 60; *Cyp27b1* rs4646536. wt: 53°C and mut: 63°C; *Cyp24a1* rs2248359. wt: 52°C and mut: 59°C; *Cyp24a1* rs2296241. wt: 50°C and mut: 57°C. Negative template controls were included in each reaction. The identified genotypes were validated from PCR products applying Sanger sequencing (data not shown). Details regarding the primer sequences and PCR conditions are shown in Table SI.

25(OH)D measurement

A single measurement of 25(OH)D serum concentration was obtained from 98 patients with AD and 45 healthy controls by using an enzyme linked immunosorbent assay (ELISA) (Immunodiagnostics Systems GmbH, Frankfurt/M, Germany). All sera were collected between October and March (low ultraviolet (UV) radiation). Serum 25(OH)D concentrations >75 nmol/l were regarded as sufficiency, 50–75 nmol/l as insufficiency and <50 nmol/l as deficiency (S2) (Table IV).

In silico *analysis*

The *Cyp27b1* and *Cyp24a1* genes were analysed as described previously in (S3) using UCSC genome browser (http://genome.ucsc.edu/) to identify the sequence of the human and mouse *Cyp27b1* and *Cyp24a1* gene including the flanking 5'-5000 bp upstream and 3'-1000 bp downstream. Evolutionary conservation was determined using mVISTA (http://genome.lbl.gov/vista/mvista/submit.shtml).

Statistical methods

Genotype frequences were compared between non-atopic controls and patients with AD as such or stratified according disease severity. The allele frequencies were compared using 2×2 χ^2-test and 3×2 Cochrane-Armitage trend test. Correction for multiple testing was performed by permutation analysis (10^6 permutations; indicated as p_{corr}, Haploview software 4.2, www.broadinstitute.org and (S4)). Whitney-Mann *U* test and Kruskal–Wallis test were used for independent groups (XLSTAT; www.xlstat.com). $p < 0.05$ was considered statistically

Table SI. *Primer sequences and PCR conditions for genotyping* Cyp27b1 *and* Cyp24a1

	rs703842		rs10877012
	Annealing: 55°C, elongation time 13 s		Annealing: 56°C, elongation time 16 s
Cyp27b1	Melting point major=54°C; minor=63°C		Melting point major=54°C; minor=59°C
rs703842 F	CAgCCTgCCTggTCACT	rs10877012 F	ACTggCACAgAgCCTgATAg
rs703842 R	CCCACCTTCTTTAAgAgAgTACTgT	rs10877012 R	CgTATgCCTgTAgTgCCTTg
rs703842 S	TgCTCTTTTCTCTAATCCCTggg	rs10877012 S	AAAATAAAATAATCTCCCACAgTTTACC
Anc rs703842	gACgAggTCCAgCTAAggTAgAgTAAgCAg	Anc rs10877012	CTCTgCTCCTTACTCCCTCATTCCCC
	rs3782130		rs4646536
	Annealing: 58°C, elongation time 24 s		Annealing: 56°C, elongation time 15 s
	Melting point major=51°C; minor=60°C		Melting point major=53°C; minor=63°C
rs3782130 F	ggCCCTCTCTCTgTTgCCTAg	rs4646536 F	gggTTTggAAgTTggAAACAAT
rs3782130 R	CAACACTTTgggAggCTAAgg	rs4646536 R	AgTAgAAAgggTgCATAggCTTTAC
rs3782130 S	TTTTTTgTAgAgACAgTTCCACTATgT	rs4646536 S	gCCTCATCTTgCTgTCTCCA
Anc rs3782130	gCCCAggCTggTCTCAgACTC	Anc rs4646536	TTTgTgCTTTgCAACCTAgACTgTACCC
	rs2248359		rs2296241
	Annealing: 58°C, elongation time 11 s		Annealing: 54°C, elongation time 11 s
Cyp24a1	Melting point major=52°C; minor=59°C		Melting point major=50°C; minor=57°C
rs2248359 F	CCAACTTCCgTTCCTgAATTAgC	rs2296241 F	gTTTTCCTTCAACggCTTTAA
rs2248359 R	ACCACCgCCCCCAgTAC	rs2296241 R	AACgTggCCTCTTTCATCA
rs2248359 S	gCCAgCCgCgATAACTCT	rs2296241 S	CCCATAAAATCAgCCAAgACC
Anc rs2248359	AAATTgTgTATgTTTgTCACTggACCTgCC	Anc rs2296241	TCAAAgAAAACAACCgCAAAAgACAC

All oligonucleotides in 5'-3' format. All PCR protocols included an initial 4 min 95°C denaturation step, 40 cycles (5 s 95°C, 20 s annealing temperature as indicated and elongation time as indicated by 72°C) followed by melting curve analysis. Melting point of the major and minor allele transcripts are provided in °C. F: forward sense; R: reverse primer; S: Sensor; 3'-FITC-labelled sensor probe; Anc: Anchor, 5'-LC640(Cy5)-labelled probe.

significant. The single SNP analysis, Hardy-Weinberg calculations, haplotype analysis and linkage analysis were performed by using the Haploview software.

SUPPLEMENTARY REFERENCES

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