Appendix S1

SUPPLEMENTARY MATERIAL AND METHODS

Genotyping

Genomic DNA was isolated from whole blood using a MasterPure DNA Purification Kit for Blood Version II (Epicentre) according to the manufacturer's instruction. SNPs in the investigated genes were identified using the National Center for Biotechnology Information (NCBI) and HapMap databases and SNP in *LELP1* (rs7534334), *HRNR* (rs11204937, rs877776), and *FLG* loss-of-function mutations (S3247X, R2447X, R501X, 2282del4) were genotyped by PCR-RFLP. The 506-bp PCR product of *LELP1* rs7534334 (primers: 5'-CCTCCACCATGTACAACGCT, 5'-TTGCATTAAACCCATGCAGCC) was digested with restriction enzyme *MwoI*. The 213-bp PCR product of *HRNR* rs11204937 (primers: 5'-CTTCAGTTCGTTAGCCAGATGTATTGT, 5'-GCAGTAATCTTTGCCAATGATTTTA) was digested with restriction enzyme *RsaI*. The 619-bp PCR product of *HRNR* rs877776 (primers: 5'-ATGAAGGAGGCGGAGAAGAAA, 5'-TCCTTGATCCAACAACGATTCCT) was digested with restriction enzyme *HpyCH*4III. The 141-bp PCR product of *FLG* R501X (primers: 5'-GTCCAGACCTATTTACCGATT, 5'-GATCGCACCACGAGCAG) was digested with restriction enzyme *NlaIII*. The 198-bp PCR product of *FLG*-2282del4 (primers: 5'-TCCCGCCACCAGCTCC, 5'-GTGGCTCTGCTGATGGTGA) was digested with restriction enzyme *DraIII*. *FLG* mutation (S3247X) was genotyped by fluorescently labelled probes (primers: 5'-GAAGACTCTGAGAGGTGGTC, 5'-GTCTCTGCGTGACGAGTG, probe sequence: A': CTGTTCAGGAGCAGTAAAGGCAC - 3'-fluorescein A": CTGTTCAGGAGCAGTCAAGGCAC-3'-fluorescein B: 5'-LC640 - CTCCAGACACCCCAGGTCCCAT -3'-phosphorylated). *FLG* mutation (R2447X) was genotyped by fluorescently labelled probes (primers: 5'- TCTTGGGACGTTGAGTGC, 5'-TTCCTCTACCAGGTGAGCA, probe sequence: A': CTGTCTCATGCCTGCTTGTGGT - 3'-fluorescein A'': CTGTCTCGTGCCTGCTTGTGGT - 3'-fluorescein B: 5'-LC640 - ATCCTTGTCTTCCTCCAGTGCTGG -3'-phosphorylated).

Statistical analysis

Statistical analyses were conducted using Excel 2003 (Microsoft Corp., Redmond, WA, USA), STATISTICA (Version 8.0: StatSoft, Tulsa, OK, USA). Distribution of the genotypes for the investigated gene polymorphisms was consistent with a Hardy-Weinberg equilibrium in the patients with AD and the control group. The Shapiro-Wilk W test was used for normality check. Mann–Whitney *U* test was used for comparisons of 2 groups. For multiple testing, Kruskal-Wallis analysis of variance (ANOVA) with Dunn's *post-hoc* test was used. A logistic regression model was used to calculate the odds ratio (OR) and 95% confidence intervals (95% CI). Estimation of linkage disequilibrium between pairs of SNP was performed with the expectation-maximization algorithm, using Arlequin v 3.1 software (25). $p < 0.05$ was regarded as statistically significant. Qualitative data were compared according to the number of cases in each compared category and/or their expected values, using the Pearson's χ^2 test, with Yates correction, or Fisher's exact test.