Table 2: P values for microsatellite markers located intragenically or in the immediate vicinity of represented genes after the initial step and individual genotyping.

	p values				
gene (as represented by the respective marker)	**after analysis with pooled DNA**	**after summation of alleles beneath 5%**	**after analyses of each single allele (most significant allele)**	**after individual genotyping[1] (p^c value)**	**after correction by Q-value of pooled data**
FLIP	0.2871	0.1936	0.0100	0.0044 ($p^c > 0.05$; c = 9)	n.s.
BCL2A1	0.0948	0.0948	0.0275	n.s.	n.s.
BAG1	0.2541	0.2541	0.0163	n.s.	n.s.
BPI	0.0011	0.0011	0.0031	n.s.	n.s.
erbB3	0.0760	0.0932	0.0100	n.s.	n.s.
TP73	0.5928	0.3535	0.0302	n.s.	n.s.
TLR9	0.3004	0.3004	0.0300	n.s.	n.s.
TNFRSF17	0.0012	0.0014	0.0014	0.0012 ($p^c < 0.01$; c = 6)	n.s.
CARD15	0.0083	0.0247	0.0054	0.0050 ($p^c < 0.04$; c = 7)	n.s.

P values were generated using three different procedures as described in the methods' section. Briefly, data were analysed by means of contingency tables, initially comparing allele distributions represented by the AIF (after analyses with pooled DNA), then after summation of alleles < 5% in order to focus on the major alleles and, finally, after comparison of each single allele between the control and patient cohorts. For analysing the results of the individual genotyping χ^2 testing was utilised.

[1]Genotyping was performed with the same individuals used in the pooling procedure, and, when remaining significant, further individuals were added to the analyses (FLIP: CD = 134, controls = 150; *TNFRSF17*: CD = 147, controls = 135; *CARD15*: CD = 144, controls = 165).

level was set at p = 0.05. In order to focus the statistics on major alleles, all minor alleles with a frequency of less than 0.05 were summarized to a virtual allele. Subsequently, a second statistical analysis by means of contingency tables was undertaken. A third step for statistical testing each allele individually was accomplished (and the summation of all other marker alleles), whereby the respective value of the patient group was compared with those of the controls and subsequent χ^2 analyses. Despite of evidence that correction for multiple comparisons might eliminate 'real positive' results [26], Q value correction was performed with a cut off of 5% for the initial screening procedure [29].

Nevertheless, for selecting markers for further investigations, non-corrected P values were simply ranked according to their evidence for association including all performed statistical procedures.

Individual genotyping

Markers with significantly different allele distributions between patients and controls were controlled by genotyping individual DNA samples of patients and controls in order to exclude false-positive results due to pooling artefacts. Individual genotyping was performed by capillary gel electrophoresis by using the BeckmanCoulter CEQ8000 genetic analysis system (Beckman Coulter, Germany). Results were analysed by comparing each microsatellite allele frequency from the CD cohort with the corresponding allele frequency of the control group by χ^2 testing and corrected by the number of marker specific alleles according to Bonferroni (see Tab. 2 and URL: http://www.ruhr-uni-bochum.de/mhg/marker_information_SENW.pdf). Hardy-Weinberg equilibrium (HWE) was tested using the Genepop program http://wbiomed.curtin.edu.au/genepop.

SNP genotyping

SNPs in genes as represented by significantly associated markers after individual genotyping were investigated by analysis of restriction fragment length polymorphisms (RFLP; see Tab. 3). As the marker representing the *TNFRSF17* gene is located in ~1 MBp distance to the *MHC class II transactivator* (*MHC2TA*) gene, a functional variation (rs3087456, [30]) of *MHC2TA* was genotyped by RFLP analyses in 147 CD patients and 463 healthy controls from the abovementioned control populations (see Tab. 3). The results were evaluated by means of χ^2 -and HWE testing. Linkage disequilibrium (LD) between the marker alleles and the polymorphism was calculated by the Genepop program.

Results

Initial step

Microsatellites representing 245 genes involved in apoptosis regulation (see Tab. 1) were investigated by using EAS. None of the markers presented with significant intra-subgroup differences confirming the homogeneity of the pools. The statistical evaluation of the microsatellite frequencies in the CD patient and the control cohorts revealed 9 significantly different allele distributions of intra- or juxtagenic markers for *FLIP*, *BCL2A1*, *BAG1*, *BPI*,