Table 3: Investigated SNPs in genes as represented by significantly differing microsatellites of the individual genotyping step.

Gene	rs#	Allele 01/02	Oligonucleotides (sense/antisense)	RE	TM (°C)	Allele: fragment length (bp)
FLIP	Rs7583529	A/C	GGTGATTATTCGGACCCCA/ AACTACAGATCCCGTGTGGAG	TseI	57	01: 155 02: 103/52
	Rs2041765	T/C	GAACAAGGAGAGAACCTGGAC/ GAGCTGGAAGGCACAGTACA	MboII	56	01: 309 02: 188/121
TNFRSF17	Rs3743591	A/G	ATAAGCAGTTTCTGTTTCAGATGT/ CTCTACAAGAATTCCAGAGCA	BceAI	55	01: 223 02: 147/76
	Rs11570139	C/T	GCCCTGATATTTACACCCTGT/ CAGCCATCTGCAACATGAT	CaiI	54	01: 269 02: 161/108
	Rs373496	T/C	AGGAACTGAAACTCACAATAGC/ CAGCTCATTATCTGTCTGATGTT	AluI	55	01: 247 02: 100/90/54/3
MHC2TA	Rs3087456	G/A	*[1] GTGAAATTAATTTCAGAG**C**TGT/ CTCAGCTTCCCCAAGGAT	BfmI	58	01: 268 02: 231/37

Analyses were performed by using the RFLP method. The table depicts information on the used SNPs as well as RFLP/PCR conditions. *[1] A 5'-tail was added to the mismatch (bold letter) sense primer (5'-CATCGCTGATTCGCACAT-3'). PCR was performed with a third oligonucleotide with the equal sequence as the tail. RE: restriction enzyme; TM: melting temperature (used for annealing in PCR).

erbB3, *TP73*, *TLR9*, *TNFRSF17* and *CARD15* (summarized in Tab. 2).

Individual genotyping

Individual genotyping confirmed significant P values only for the 3 markers *FLIP* ($p = 0.0044$, $p_c > 0.05$, in HWE), *TNFRSF17* ($p = 0.0012$, $p_c < 0.01$, in HWE) and the positive control CARD15 ($p = 0.0050$, $p_c < 0.04$, in HWE). The additional associations for the other markers were rejected (see Tab. 2 and Additional file 1). There were no differences analysing $CARD15^+$ and $CARD15^-$ patients.

SNP genotyping

SNP markers (Tab. 3) were genotyped located in the respective genes in the vicinity of the microsatellites representing *TNFRSF17* and *FLIP*. Thus, SNPs were analyzed spread across the genes representing haplotypes as predisposed by the 'LD Select' method reported before [31]. RFLP analyses did not reveal any association of the selected SNPs, neither by comparing the $CARD15^+$ nor the $CARD15^-$ patients with the control group.

Comparison of TNFRSF17 *microsatellite alleles*

The genotypes of the *TNFRSF17* microsatellite alleles were compared between the patient and control cohorts. Analyses revealed evidence either for a predisposing (allele 3) and a protective allele (2) or linkage between these alleles and the marker alleles, respectively.

Genotypes including allele 2 are overrepresented in the control cohort, whereas those with the apparently predisposing allele 3 are more frequent in the CD cohort, thus confirming the results of individual genotyping (see Fig. 1).

MHC2TA *analyses*

The analyses of the functionally significant polymorphism rs3087456 revealed a marginal association in our CD patients when allele or genotype frequencies were compared between the combined control (WeG and NoG did not differ in allele frequencies) and the patient cohorts (see Tab. 4). Analyses for LD between *TNFRSF17* and *MHC2TA* alleles, however, did not reveal any significant deviations from equilibrium.

Discussion

The pathomechanisms of CD are still not exactly understood, albeit certain *CARD15* variations appear especially frequent in CD patients; thus genetic involvement is proven. These genetic predisposition factors, however, are neither sufficient nor explain they the pathogenesis in all CD patients. In this study we present an association screen mainly for apoptosis and immunity related genes by microsatellite markers as investigated in a 3-step approach.

Our initial analyses revealed 9 significantly different allele distributions of intra- or juxtagenic markers for *FLIP*, *BCL2A1*, *BAG1*, *BPI*, *erbB3*, *TP73*, *TLR9*, *TNFRSF17* and *CARD15* (see Tab. 2). Yet, after correction by Q-value, none of those markers remained significant. On the other hand, a recent study raised the question, whether the correction for multiple comparisons should be applied at all in EAS [26]. For example, in these analyses a previously significantly associated microsatellite (representing the *TNFα* gene), which has been used as a positive control such as *CARD15*, would have been rejected by the correction procedure. Therefore, it remains conceivable that the abovementioned markers represent rather hints for additional predisposing factors/loci with low effect size.