the innate immune system is supposed to present a crucial step in the pathogenesis of CD [5]. This fact has been confirmed genetically by several CD associations of genes such as CD14, TLR4 and in some instances the interaction of their variations with CARD15 [6,7]. In regard to the polarized T helper (Th) response, the adaptive immune system appears affected in CD as well [8-10]. Moreover, several studies implicated a role of programmed cell death in CD [11-15]. Apoptosis mediates 'self-tolerance', the elimination of autoreactive immune compartments. In addition, the thoroughly controlled termination of a physiological immune response is due to the process of programmed cell death. In CD mucosal T cells show less susceptibility to apoptosis [16]. In this context TNFα protein exerts multiple physiological effects, and anti-TNFα therapeutic strategies (*e.g.* infliximab) are effective in (maintaining) remission of CD [17]. In several studies it has been revealed that treatment of CD patients with infliximab leads to an activation of T cells rendering them susceptible for apoptosis [18,19]. Interestingly, the effect of this treatment may not be due to neutralisation of soluble TNFα (and its binding to the TNFRs), but rather it may be caused by its affinity to membrane-bound TNFα putatively changing the ratio of anti- and pro-apoptotic mediators towards induction of apoptosis [18,20]. Although the mechanisms of the causal role of T cells responses in CD remain to be determined in detail, there is substantial clinical evidence that suggests a role for uncontrolled activated T lymphocytes in the pathogenic process of CD [21-24]. Nevertheless, it is uncertain, whether a genetic basis for a decreased activation/apoptosis of T lymphocytes in CD patients exists, and whether increased anti-apoptotic markers, found in T cells of these patients are due to the mucosal inflammation, secondarily [18].

In such a complex situation we used extended association screening (EAS) with markers representing 245 apoptosis- and (innate) immunity-related genes. The majority of the investigated markers have been successfully utilized in respective studies before [25,26]. Our population based linkage mapping comprises a 3-stage analysis with pooled DNA in the initial phase and subsequently individual genotyping. In order to confirm such results, several tagging SNPs of the adjacent gene represented by the marker were analysed. Here, we investigated the role of distinct biological pathways for the susceptibility of CD.

Materials and methods

Patients

One hundred and fifty eight well-characterized patients with a clinical, endoscopical and histological diagnosis of CD were included. This patient cohort has been reported before [27,28]. All patients were of German origin and the diagnosis of CD was adjusted according to the diagnostic criteria of the European Community Workshop on Inflammatory Bowel Diseases (IBD). As controls a group of healthy northern German (NoG) and western German (WeG) origin were analysed. In the initial step a group of ~100 NoG individuals were used. In order to exclude population stratification, genotyping of chosen SNPs was performed in 180–460 NoG and WeG individuals.

Pooling of DNA

The DNA concentration from each individual of the patient and control cohorts was quantified by spectrophotometry, carried out four times, and then diluted accordingly to 100 ng/μl. In a second step the DNA was diluted to a concentration of 65 ng/μl and once more measured by spectrophotometry. Finally, DNA diluted to 50 ng/μl was adjusted to a final amount of 1000 ng for each individual in a pool of 50 persons. In the initial stage, marker analyses were performed with two patient and two control subpools, respectively.

Tailed primer PCR

Tailed primer PCR was performed as described before [25]: An 18 bp-tail was added to each sense oligonucleotide. PCR reaction included three oligonulceotides, two of which were target specific. The third one consists of the same sequence as the abovementioned tail that was additionally fluorescence-labelled.

Microsatellite markers

Intragenic microsatellite or markers located in the immediate vicinity (<50 kb) of the specific gene were included. Information on the oligonucleotide sequences and location of markers are given at the website (Additional file 1; see also Tab. 1). As reported before, only markers with equal "intra-subgroup" allele distributions with ≥ 2 alleles were considered in subsequent analyses [25]. Significantly associated markers were genotyped individually in order to exclude false-positive results due to possible pooling artefacts. All in all, 245 microsatellite markers representing distinct genes were analysed on an ABI377 slab-gel system (Applied Biosystems, Darmstadt, Germany).

Statistics for initial comparisons of allele frequencies

Raw data from ABI377 profiles were analysed by the Genotyper software (ABI) producing a marker specific allele image profile (AIP) which includes different heights of peaks reflecting the allele frequencies. In order to test differences of the AIPs between CD patients and the controls, all peak heights were summarized for each pool and set to 100 %. The total allele count for each distinct allele was then estimated. Thereupon, the AIPs of the case and control pools were compared statistically by means of contingency tables. Hence, P values are nominal and approximate, because estimated rather than observed counts were used for allele frequencies. The significance