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The *SPINK* gene family and celiac disease susceptibility

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Abstract The gene family of serine protease inhibitors of the Kazal type (*SPINK*) are functional and positional candidate genes for celiac disease (CD). Our aim was to assess the gut mucosal gene expression and genetic association of *SPINK1*, *-2*, *-4*, and *-5* in the Dutch CD population. Gene expression was determined for all four *SPINK* genes by quantitative reverse-transcription polymerase chain reaction in duodenal biopsy samples from untreated (n=15) and diet-treated patients (n=31) and controls (n=16). Genetic association of the four *SPINK* genes was tested within a total of 18 haplotype tagging SNPs, one coding SNP, 310 patients, and 180 controls. The *SPINK4* study cohort was further expanded to include 479 CD cases and 540 controls. *SPINK4* DNA sequence analysis was performed on six members of a multigeneration CD family to detect possible point mutations or deletions. *SPINK4* showed differential gene expression, which was at its highest in untreated patients and dropped sharply upon commencement of a gluten-free diet. Genetic association tests for all four *SPINK* genes were negative, including *SPINK4* in the extended case/control cohort. No *SPINK4* mutations or deletions were observed in the multigeneration CD family with linkage to chromosome 9p21-13 nor was the coding SNP disease-specific. *SPINK4* exhibits CD pathology-related differential gene expression, likely derived from altered goblet cell activity. All of the four *SPINK* genes tested do not contribute to the genetic risk for CD in the Dutch population.

Keywords *SPINK* genes · Celiac disease · Quantitative reverse-transcription polymerase chain reaction · Genetic association

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Introduction

Celiac disease (CD) is a chronic inflammatory condition of the small intestine because of an immunological intolerance for the food protein gluten. Patients have to adhere to a life-long diet devoid of gluten to prevent the detrimental effects of a prolonged nutrient and mineral deficiency (Green and Jabri 2003). Susceptibility for CD is predominantly determined by genetic factors, and the complex inheritance patterns suggest the interaction of multiple genes (van Heel et al. 2005). It is well established that the adaptive immune

response to gluten plays a pivotal role in the pathogenesis of CD. Th1 activation of $CD4^+$ T cells follows gluten-peptide presentation by DQ2 or DQ8 molecules expressed on antigen-presenting cells (Sollid 2002). The *HLA-DQA* and *-DQB* gene variants coding for these molecules are the major genetic determinants for CD susceptibility (Koning et al. 2005). Recently, the importance of innate immunity in CD pathogenesis was also underscored by the observation of induced IL15 expression and NKT cell chemotaxis through the MICA and NKG2D molecules (Hue et al. 2004; Meresse et al. 2004). However, no genetic contribution of the cognate genes has been demonstrated. The notion of crosstalk between the adaptive and innate immune systems is not limited to CD and gets much attention in studies of the inflammatory process (Hoebe et al. 2004). This raises the question whether some aspects of innate immunity may contribute to the genetic susceptibility for CD. The innate immune system uses a wide array of defense mechanisms against the invasion of pathogens. These encompass the expression of pattern recognition receptors, release of antimicrobial molecules, and preservation of epithelial barrier and tissue integrity by, e.g., serine protease inhibitors (Kimbrell and Beutler 2001).

One branch of the family of serine protease inhibitors is that of the Kazal type (*SPINK*) that originally consisted of four members in humans (*SPINK1*, *SPINK2*, *SPINK4*, and *SPINK5*). Recently, as part of a cluster of *SPINK* genes on chromosome 5q32 that already included *SPINK1* and *SPINK5*, five new *SPINK*(-like) members were identified that were located more distally: *SPINK5L2*, *SPINK6*, *SPINK5L3*, *SPINK7*, and *SPINK9*, respectively (NCBI Map Viewer, build 36.1). However, these new members lack functional annotation and were therefore not included in this study. *SPINK* family members 1, 2, and 4 have a comparable size and structure coded for by 4 exons with a single Kazal type serine protease inhibitor domain. *SPINK5*, in contrast, contains 33 exons that encode 15 inhibitory domains. All four *SPINK* members are thought to be involved in the protection against proteolytic degradation of epithelial and mucosal tissues, although their major site of expression may differ. *SPINK1* is expressed in the pancreas and the gastrointestinal tract, and mutations in this gene are reported in various forms of pancreatitis (Pfutzer and Whitcomb 2001). *SPINK2* (located on 4q12) is expressed in the testis, epididymis, and seminal vesicle, where its antimicrobial function may be involved in protection of fertility (Rockett et al. 2004). *SPINK4* was originally isolated from pig intestine (Agerberth et al. 1989) and is abundantly expressed in human and porcine goblet cells in the crypts of Lieberkühn but was also found in monocytes and in the central nervous system (Metsis et al. 1992; Norberg et al. 2003). *SPINK5* is expressed in the thymus, vaginal epithelium, Bartolin's glands, oral mucosa, tonsils, and the parathyroid glands (Magert et al. 1999). Mutations in *SPINK5* are responsible for the Netherton syndrome, a lethal skin disorder characterized by ichthyosis, hair shaft defects, atopy, skin barrier defects, and recurrent bacterial infections (Bitoun et al. 2002). Mouse models of the Netherton syndrome have shown enhanced proteolysis of desmoglein 1 and filaggrin in *SPINK5* mutants (Descargues et al. 2005; Hewett et al. 2005). Moreover, *SPINK5* has also been associated with asthma and atopic dermatitis (Blumenthal 2005).

Interestingly, both *SPINK1* and *SPINK5* are located on chromosome 5q32. This region contains the CELIAC2 susceptibility locus that emerged repeatedly in linkage studies (Babron et al. 2003). Despite the fact that this region is rich in candidate cytokine genes and intense mapping efforts were made, no closely associated genes were identified (Ryan et al. 2005). Likewise, *SPINK4* is located on chromosome 9p13.3 and resides within a linkage region (9p21-13) where we previously identified a novel CD locus that segregated within a four-generation Dutch family (van Belzen et al. 2004). Taken together, the role of *SPINK* genes in epithelial and mucosal protection and the important genetic locations of *SPINK1*, *SPINK4*, and *SPINK5* prompted us to subject the four conventional members of the *SPINK* family to gene expression and genetic association analyses to ascertain their possible role in CD pathogenesis.

Materials and methods

Patient material

Duodenal biopsy samples were collected by endoscopy as part of a routine CD diagnostic procedure or to monitor the response to a gluten-free diet in previously diagnosed patients. All patients were classified using the Marsh nomenclature according to the UEGW criteria (Report of a working group of the United European Gastroenterology Week in Amsterdam 2001). Two biopsy samples taken in parallel to those used for histological examination were pooled and used for determination of gene expression. In total, 62 individuals were examined with quantitative reverse transcription polymerase chain reaction (qRT-PCR), of which 16 were normal controls, and 46 were CD patients (Fig. 1). The patient group consisted of 15 untreated cases with villous atrophy (MIII) and 31 patients treated with a gluten-free diet who were in various stages of mucosal recovery: MII (crypt hyperplasia; $n=11$), MI (lymphocyte infiltration; $n=8$), and M0 (complete remission; $n=12$). The biopsies of all participating patients were reevaluated by an experienced pathologist, and only CD patients with a proven original Marsh III lesion were

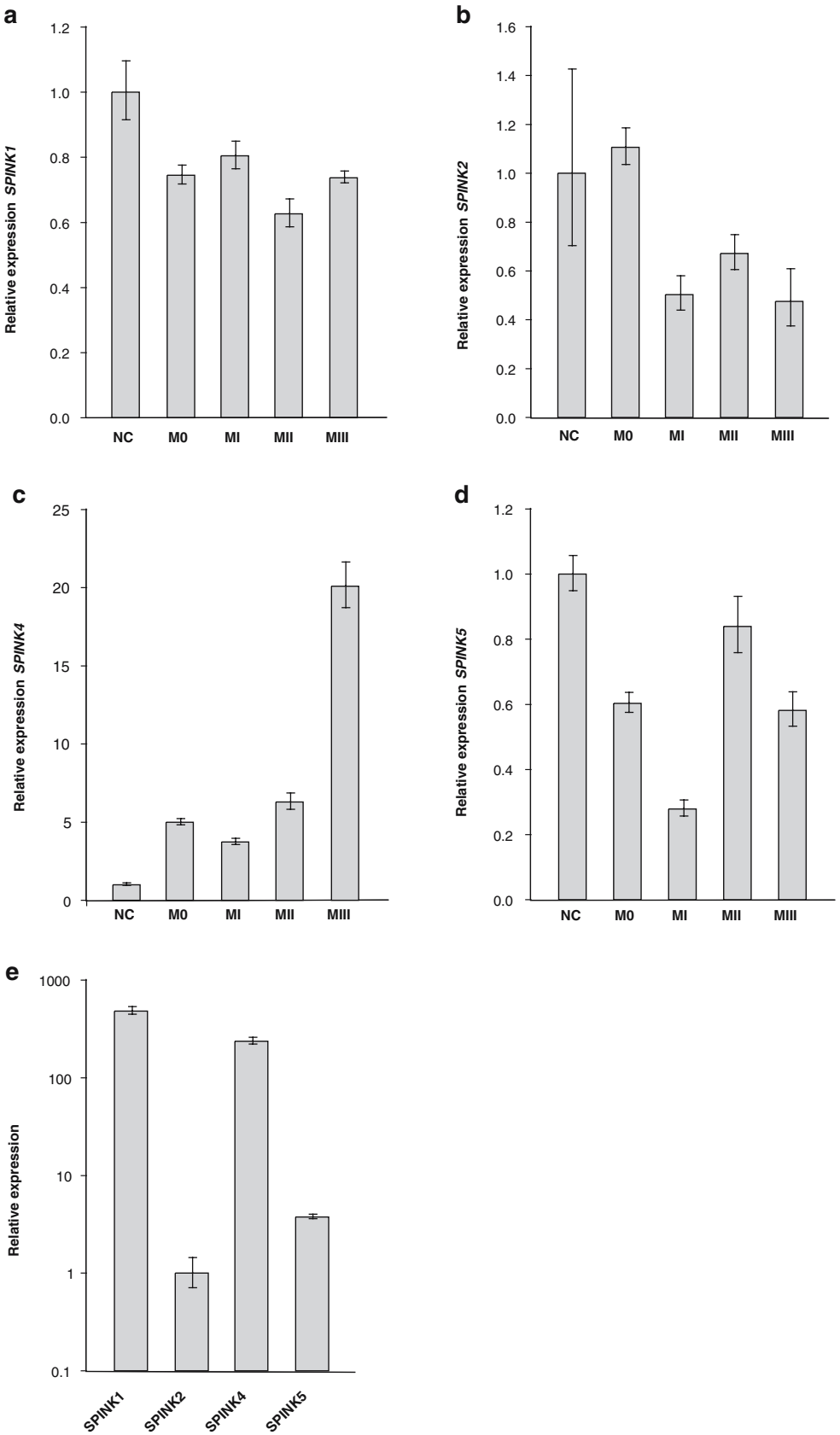

Fig. 1 Results of qRT-PCR of *SPINK* genes in normal controls (*NC*) and CD patients, either untreated (*MIII*) or on a gluten-free diet (*MII–M0*). The Marsh stages refer to the pathological conditions of the mucosa, characterized by atrophy of the villi (*MIII*); hyperplastic crypts between the villi (*MIII–MII*); and enhanced lymphocyte infiltration (*MIII–MII–MI*). Stage *M0* indicates complete remission comparable to controls. The genes tested were as follows: *SPINK1* (**a**); *SPINK2* (**b**); *SPINK4* (**c**); and *SPINK5* (**d**). Measurements were made in triplicate, on pools of separately prepared cDNA samples. Expression data were normalized to the normal control pool.(**e**) Relative expression of all four *SPINK* genes with respect to *SPINK2* in the healthy duodenal mucosa. Note the logarithmic scale here. The *GUSB* gene was used as an endogenous control in all tests. *Error bars* indicate standard deviations

Table 1 Allelic distribution of *SPINK* haplotype tagging SNPs in a Dutch CD case-control cohort

				Cases (n=310)				Controls (n=180)					
			Allele	Allele counts		Allele frequency (%)		Allele counts		Allele frequency (%)			
Gene name	SNP i.d.	Position*	Minor/major	Minor	Major	Minor	Major	Minor	Major	Minor	Major	χ^2	P value
SPINK1	rs10515593	147,178,993	A/G	119	449	21	79	76	268	22.1	77.9	0.166	0.6834
SPINK1	rs3777125	147,184,010	C/G	228	336	40.4	59.6	150	200	42.9	57.1	0.527	0.4681
SPINK1	rs4705204	147,195,313	C/A	125	429	22.6	77.4	60	276	17.9	82.1	2.813	0.0935
SPINK1	rs891992	147,205,707	G/A	77	483	13.8	86.2	47	303	13.4	86.6	0.019	0.8906
SPINK2	rs10015630	57,520,070	A/G	216	328	39.7	60.3	136	216	38.6	61.4	0.102	0.7489
SPINK2	rs781542	57,528,232	G/A	220	390	36.1	63.9	123	221	35.8	64.2	0.009	0.9237
SPINK4	rs563353	33,206,428	G/C	120	472	20.3	79.7	67	279	19.4	80.6	0.112	0.7375
SPINK4	rs563512	33,206,484	G/A	50	556	8.3	91.7	36	310	10.4	89.6	1.243	0.2648
SPINK4	rs891671	33,222,271	G/T	70	540	11.5	88.5	52	304	14.6	85.4	1.998	0.1575
SPINK4	rs706107**	33,230,225	A/G	117	499	19	81	81	269	23.1	76.9	2.358	0.1246
SPINK4	rs706109	33,230,668	A/G	118	486	19.5	80.5	82	276	22.9	77.1	1.549	0.2133
SPINK4	rs706115	33,243,605	G/C	77	537	12.5	87.5	52	308	14.4	85.6	0.716	0.3975
SPINK5	rs3756688	147,422,972	G/A	232	380	37.9	62.1	135	221	37.9	62.1	0.000	0.9968
SPINK5	rs4472254	147,433,830	A/C	240	376	39	61	142	214	39.9	60.1	0.081	0.7757
SPINK5	rs4519913	147,452,004	G/A	279	331	45.7	54.3	168	192	46.7	53.3	0.079	0.7792
SPINK5	rs1422987	147,466,552	T/C	49	559	8.1	91.9	27	329	7.6	92.4	0.070	0.7917
SPINK5	rs3815740	147,471,386	G/A	38	574	6.2	93.8	21	337	5.9	94.1	0.047	0.8291
SPINK5	rs2052532	147,476,500	G/A	200	414	32.6	67.4	117	241	32.7	67.3	0.001	0.9723
SPINK5	rs3764930	147,485,309	G/A	199	413	32.5	67.5	117	243	32.5	67.5	0.000	0.9958

*Basepair position according to NCBI build 35.1
**Coding SNP, nonsynonymous change (Val7Ile)

included in this study. The genetic association study on all four *SPINK* genes was initially conducted on a cohort of 310 independent CD patients and 180 independent age- and sex-matched controls, all of which were from Dutch Caucasian decent. In a second stage, exclusively focused on all *SPINK4* SNPs, we added 360 controls to a total of 540. In case of three *SPINK4* variants with suggestive *P* values, the power of the study was further enhanced by adding 169 extra CD cases to a total of 479. In parallel, we also examined the *SPINK4* gene in a previously described four-generation Dutch CD family (van Belzen et al. 2004). Six family members and a CEPH control were subjected to DNA sequence analysis of the *SPINK4* coding regions and splice sites boundaries. Additionally, we performed *SPINK4* SNP haplotype analysis in this family. All patients and family members that volunteered for this study signed an informed consent. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Expression study

The isolation of total RNA from biopsy samples and the analysis of gene expression by real-time qRT-PCR on an ABI Prism 7900HT was performed as described before (Wapenaar et al. 2004). We used the commercially available Assay-on-Demand test for *SPINK1* (Hs00162154_m1), *SPINK2* (Hs00221653_m1), *SPINK4* (Hs00205508_m1), *SPINK5* (Hs00199260), and the endogenous control gene GUSB (PDAR 4326320E; Applied Biosystems, Foster City, CA). All samples were tested in triplicate on pooled cDNA samples representing each Marsh class. The results were confirmed with cDNA from individual samples tested in duplicate. Relative levels of gene expression were obtained using the SDS2.1 software (Applied Biosystems).

Genetic association and data analysis

Haplotype tagging SNPs were selected for *SPINK1*, *SPINK2*, *SPINK4*, and *SPINK5* based on HapMap (Phase I) data using Haploview (Barrett et al. 2005). For each haploblock containing SNPs in high linkage disequilibrium, one or more representative SNPs were selected that should capture the genetic variation within that block. For the four *SPINK* genes tested, this resulted in a set of 18 haplotype tagging SNPs and one coding SNP (see Table 1 and Fig. 2). SNP assays were obtained from Applied Biosystems and analyzed on an ABI Prism 7900HT. Hardy–Weinberg Equilibrium (HWE)

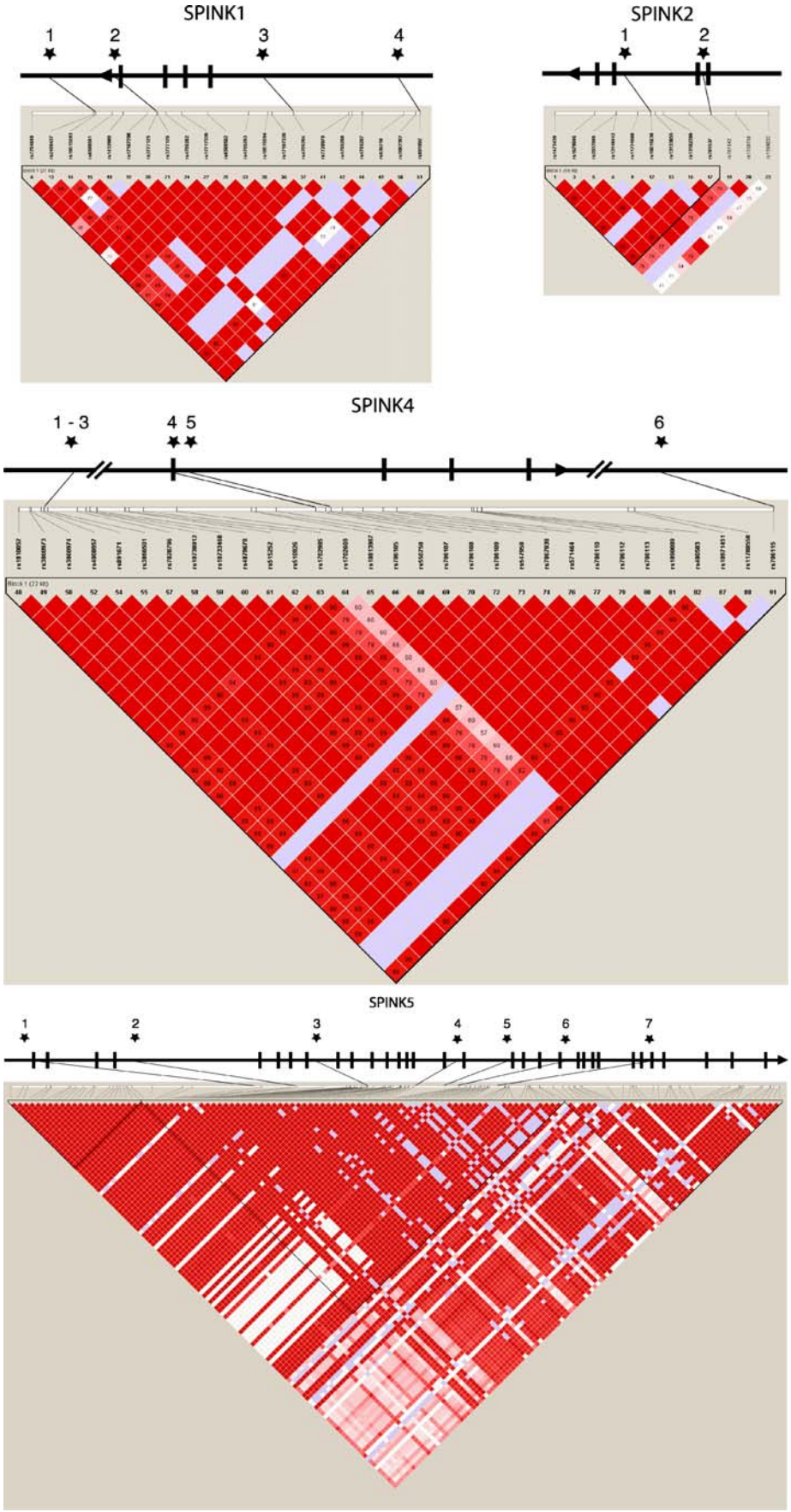

Fig. 2 Genomic organization of the four *SPINK* genes. The *upper horizontal line* indicates exon locations (*vertical bars*) and SNP positions (*numbered asterisks*). The SNPs are numbered for each gene consecutively as they appear in Table 1. *SPINK4* SNP no. *4* represents the nonsynonymous (Val7Ile) coding SNP rs706107. The *arrow points* indicate the orientation of transcription. The *lower portion* of the figure shows the pairwise linkage-disequilibrium structure between indicated SNPs given by *D′* statistics based on the European population in the HapMap database (Phase II). *Darker red* intensities indicate higher *D′* values (*numbers* indicate D' value, whereas SNP pairs without number have a *D′*=1)

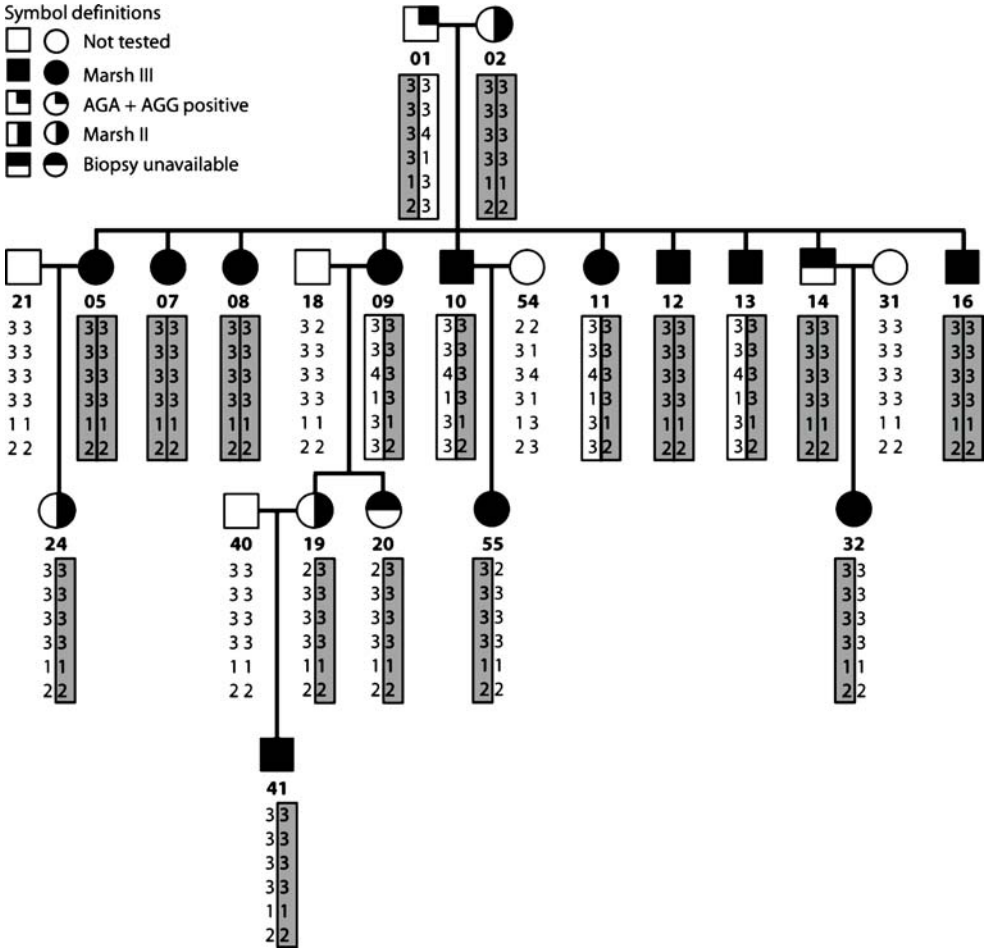

Fig. 3 Pedigree of the Dutch multigeneration CD family. Only affected descendents are depicted (10 out of 13 siblings in the second generation were affected). The grandparental *SPINK4* haplotypes that are *boxed* and *shaded* are identical to the grandmaternal at-risk haplotype (noninformative). The SNPs are ordered (*top-to-bottom*) as they appear in Table 1. Genotype numbers *1*, *2*, *3*, and *4* refer to A, C, G, and T alleles, respectively. Sequence analysis was performed on family members *02*, *08*, *21*, *31*, *32*, and *41*. Family member index numbers are indicated in *bold*

was evaluated separately in cases and controls for all SNPs tested. Allele frequencies were compared between cases and controls, and *P* values were obtained by χ^2 analysis.

DNA sequence analysis

DNA sequence analysis was performed on *SPINK4* in six members of a four-generation Dutch CD family and one CEPH control (family, 1,331; individual, 2). Of these six family members, four were affected (index 02, 08, 32, and 41) and carried the disease-linked haplotype, and two were nonaffected (index 21 and 31) without this haplotype (Fig. 3). All coding sequences of the *SPINK4* gene were PCR-amplified, including the intron–exon boundaries (for primers and protocols, see supplementary data Table 1). PCR products were examined on a 2% agarose gel and purified with a Millipore Vacuum Manifold (Billerica, MA). Samples were prepared with the BigDye terminator cycle sequencing ready kit (Applied Biosystems) according to the manufacturer's protocol. PCR and sequencing amplification were performed on a GeneAmp PCR system 9700 (Perkin Elmer, Foster City, CA). Sequences were run on an ABI Prism 3730 analyzer (Applied Biosystems). Analysis and sequence alignment was carried out with Sequence Navigator (Applied Biosystems) and Vector NTI (InforMax, Massachusetts) software packages.

Results

SPINK gene expression in the CD mucosa

The expression of all four conventional members of the human *SPINK* family was determined by real-time qRT-PCR on duodenal biopsy-derived cDNA pools from normal controls and CD patients, either untreated or in various stages of remission on a gluten-free diet. The results shown in Fig. 1 indicate that only *SPINK4* (Fig. 1c) is differentially expressed, and that its transcriptional activity, which is at its highest in Marsh III (20-fold compared to controls), decreases sharply (fourfold) when patients improve and make a transition to Marsh II. To preclude that the results for *SPINK4* might be biased by fortuitous differences in

Table 2 Allelic distribution of three selected *SPINK4* SNPs in the extended Dutch CD case-control cohort

				Cases (n=479)				Controls (n=540)					
			Allele	Allele counts		Allele frequency (%)		Allele counts		Allele frequency (%)			
Gene name	SNP i.d.	Position*	Minor/major	Minor	Major	Minor	Major	Minor	Major	Minor	Major	χ^2	P value
SPINK4	rs891671	33,222,271	G/T	126	814	13.4	86.6	158	904	14.9	85.1	0.889	0.3457
SPINK4	rs706107**	33,230,225	A/G	205	737	21.8	78.2	242	814	22.9	77.1	0.382	0.5365
SPINK4	rs706109	33,230,668	A/G	212	724	22.6	77.4	242	816	22.9	77.1	0.014	0.9053

*Basepair position according to NCBI build 35.1
**Coding SNP, nonsynonymous change (Val7Ile)

individual expression levels within the generated pools, we also examined the control and case samples each separately. This did not change the observed drop in *SPINK4* expression during tissue recovery (see supplementary Fig. 1). Likewise, we performed the same analysis for the other three *SPINK* genes without affecting the profile already observed in the pools (results not shown). We also examined the relative expression of the four *SPINK* genes with respect to each other in the normal intestinal mucosa. This showed that both *SPINK1* and *SPINK4* have the highest expression, which is respectively 480-fold and 240-fold higher compared to *SPINK2*, whereas *SPINK5* is in the same order of magnitude (fivefold) as *SPINK2* (Fig. 1e). In conclusion, only the *SPINK4* gene appears to be differentially regulated in the intestinal mucosa during recovery from the gluten-evoked CD lesion. This observation prompted us to examine whether *SPINK4*, or any of the other *SPINK* genes, could also be causally related to the CD pathogenesis.

Genetic association analysis of *SPINK* genes

We designed a haplotype tagging SNP strategy to capture all genetic variation in *SPINK1*, *-2*, *-4*, and *-5*. An overview of these four *SPINK* genes with their genomic organization, linkage-disequilibrium structure, and the position of the haplotype tagging SNPs used is depicted in Fig. 2. Initially, these haplotype tagging SNPs were tested in 310 CD cases and 180 controls (Table 1) and showed no significant association for any of the haplotype tagging SNPs in the four SPINK genes. Despite the initial negative result, we decided to pursue *SPINK4* further because it is expressed in goblet cells (Metsis et al. 1992), displayed a CD pathology-related differential expression in the intestinal mucosa, and mapped within a CD linkage region (van Belzen et al. 2004). Initially, we expanded the control group with 360 samples to a total of 540 for all *SPINK4* SNPs tested. As a result, the Val7Ile coding variant rs706107 and its flanking haplotype tagging SNPs rs891671 and rs706109 yielded suggestive but nonsignificant P values of 0.0595, 0.0510, and 0.1122, respectively (data not shown). To increase the power of the study even further, we subsequently added 169 CD cases to a total of 479. The effect on the P values of the three SNPs tested was such that they dropped below the significance threshold (see Table 2). From this, we conclude that the four *SPINK* genes tested do not contribute to the genetic susceptibility in the Dutch CD population.

SPINK4 sequence analysis in a multigeneration family

We have previously described a four-generation CD family with an extraordinary high incidence of affected individuals (see Fig. 3). The disease segregated with a grandmaternal haplotype on chromosome 9p21-13 (van Belzen et al. 2004), a region that encompasses *SPINK4*. The apparent dominant inheritance pattern could be caused by a mutation that is rare in the general CD population but present with a high phenotypic penetration in this specific family. To assess if any functional variants of the *SPINK4* gene were present in this family, we sequenced all its exons and intron–exon boundaries in six family members. However, we did not observe mutations in any of the samples tested (results not shown). Neither was the exon 1 coding SNP rs706107 specific for affected individuals as all seven individuals tested (including the CEPH control) carried the most frequent GG genotype (Fig. 3). To exclude the possibility of deletions in *SPINK4* to be misinterpreted from the sequence data as homozygous genotypes, we also performed segregation analysis of the grandparental *SPINK4* haplotypes within the entire family but observed no suspect inheritance pattern (Fig. 3). In conclusion, we have found no evidence that *SPINK4* is a candidate gene for the chromosome 9p21-13 CD locus in the Dutch population in general or in the multigeneration Dutch CD family specifically.

Discussion

Chronic inflammatory conditions and autoimmune disorders are typically characterized by a deregulated adaptive and innate immune system. The innate defense consists of multiple components that include physical barriers, antimicrobial molecules, pattern recognition receptors, circulating phagocytes, and the complement system (Hoebe et al. 2004). A breach of the epithelial barrier and loss of microbial containment is often the first of a series of events that trigger or sustain chronic inflammatory diseases (Tlaskalova-Hogenova et al. 2004) as described, e.g., in Crohn's disease, atopic eczema, asthma, and psoriasis (Schreiber et al. 2005). In CD, the gut–lumen separation is undermined by dietary gluten that evokes a combined innate and adaptive immune response (Londei et al. 2005). It is the joined action of gluten peptides, environmental factors, and genetic determinants that precipitates this enteropathy. The human leukocyte antigen locus is the major genetic contribution to the adaptive Th1 reaction (Koning et al. 2005). Recently, we identified *MYO9B* as a susceptibility gene in the Dutch population that possibly has an effect on epithelial barrier integrity (Monsuur et al. 2005). Several other studies have underscored the involvement of innate immunity in CD, however, without identification of underlying causative gene variants (Londei et al. 2005). Interestingly, it was also reported that the epithelial glycocalyx and the bacterial composition in the CD gut is distinct (Forsberg et al. 2004; Tjellstrom et al. 2005).

In search of genes that may have a primary contribution to CD pathogenesis, we focused our attention to the *SPINK* family of serine protease inhibitors that play an important role in tissue preservation through the containment of uncontrolled proteolysis and bacterial growth. In this study, we demonstrated differential gene expression of mucosal *SPINK4* in CD. Crypt hyperplasia is a feature of the Marsh III and Marsh II stages of CD, and the concomitant increase in the number of goblet cells may contribute to the increased *SPINK4* expression. However, the observed sharp decrease in gene expression sets in during the MIII/MII transition, whereas crypt normalization is observed only later at the MII/MI recovery phase. This suggests that *SPINK4* downregulation sets in soon after commencement of the gluten-free diet. This *SPINK4* differential expression probably reflects altered goblet cell activity, but its functional significance and regulatory mechanism in CD pathology remains to be established.

The combination of functional relevance and mapping to CD linkage intervals pointed to the *SPINK* family members as attractive functional and positional candidate genes. We have chosen a robust strategy for genetic association testing based on haplotype tagging SNPs and linkage-disequilibrium structure of the *SPINK* loci applied to a considerably sized Dutch case-control cohort. With our study design, we had 75% power to confirm association with *SPINK1*, *-2*, and *-5* (relative risk 2.0; allele frequency 0.1–0.45; 95% confidence interval), whereas this was even 95% (RR 2.0) and 80% (RR 1.6) for *SPINK4*. These power estimates reflect a Type I error rate of 0.05, which is appropriate for testing a previously reported result. Initial detection of a new genetic association would require much more stringent criteria to assure reproducibility, and power would be correspondingly less.

In parallel, we examined the extended Dutch CD family for variants and deletions in *SPINK4*. We hypothesized that a specific *SPINK4* mutation, although rare in the general population, could have a dramatic impact on mucus composition, bacterial containment, and gluten sensitivity, thereby explaining the apparent dominant and high penetration inheritance pattern in our extended CD family. With both approaches, we were not able to establish a genetic involvement of the *SPINK* genes tested. However, we cannot completely rule out the possibility of a rare noncoding mutation in *SPINK4* (outside the splice donor and acceptor regions) that might specifically segregate in this atypical CD family, characterized by an exceptional high prevalence of affected members.

Despite this negative result in the Dutch CD population, we cannot formally rule out the possibility of genetic contribution of *SPINK* genes to CD in other European populations like the Italian in whom, unlike the Dutch (van Belzen et al. 2003), chromosome 5q linkage was established (Greco et al. 1998; Percopo et al. 2003). Genuine population heterogeneity has been reported before, e.g., between *CARD15*/*NOD2* and Crohn's disease (Lesage et al. 2002; Croucher et al. 2003) and between *SPINK5* and asthma (Blumenthal 2005; Jongepier et al. 2005). The new *SPINK* members on chromosome 5q (*SPINK5L2*, *SPINK6*, *SPINK5L3*, *SPINK7*, and *SPINK9*) were not part of this study. Currently, no functional annotation is available for these genes that are located near *SPINK1* and *SPINK5* in a chromosomal region that appears to have been subjected to gene duplication during evolution. Therefore, we cannot exclude their possible involvement in CD or any other inflammatory disorder.

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