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Intestinal barrier gene variants may not explain the increased levels of antigliadin antibodies, suggesting other mechanisms than altered permeability

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ABSTRACT

Various genes may influence intestinal barrier function, including *MAGI2*, *MYO9B*, and *PARD3*, which are associated with celiac disease. Because direct measurement of intestinal permeability is difficult, antibodies against gliadin (AGA) and Baker's yeast (anti-*Saccharomyces cerevisiae* antibodies [ASCA]) can be used as an indirect test. The objective of this study was to investigate whether intestinal permeability, represented by AGA, was correlated with *MAGI2, MYO9B*, and *PARD3*. Analyses were performed in patients with Down syndrome, a population with suspected increased intestinal permeability. Correlations between AGA and ASCA were investigated. Patients with Down syndrome ($n = 126$) were genotyped for six single-nucleotide polymorphisms in *MAGI2* (rs1496770, rs6962966, rs9640699), *MYO9B* (rs1457092, rs2305764), and *PARD3* (rs10763976). An allele dosage association of these risk genes and AGA levels was performed. The correlation between AGA and ASCA was studied. A strong correlation was found between AGA and ASCA ($p < 0.01$). The patient group with one or more risk genotypes had lower mean AGA levels (trend test $p = 0.007$) and consisted of a larger number of patients with normal AGA levels ($p = 9.3 \times 10^{-5}$). Celiac-associated risk genotypes are associated with lower AGA values instead of elevated ones. Thus, other immunologic phenomena play a role in the increased prevalence of elevated AGA in patients with Down syndrome, possibly involving altered induction and/or maintenance of tolerance.

1. Introduction

Independent studies have suggested that alterations in intestinal permeability are involved in the pathogenesis of different autoimmune diseases [1]. Recent animal studies indicate that in type 1 diabetes, celiac disease (CD), and inflammatory bowel disease, increased permeability was present before disease onset but was also required for the development of disease, suggesting that it plays a role in the pathogenesis [1,2]. Tight junctions seal the route between the intestinal epithelial cells, and therefore play a role in regulating intestinal permeability [1]. Therefore, genes encoding tight junction proteins could be highly relevant candidates for autoimmune diseases of the intestine, such as CD and inflammatory bowel disease.

In previous studies, we found several genetic associations in CD patients for candidate tight junction genes at chromosomes 7, 10, and 19 [3,4]. *PARD3* encodes the protein PAR-3 that has a role in regulating epithelial cell polarity and facilitating tight junction formation [5]. Similarly, *MAGI2* encodes the protein MAGI-2 that localizes to the tight junction, where it acts as a scaffold to directly interact with the lipid phosphatase tumor suppressor PTEN (phosphatase and tensin homolog) [6]. *MAGI2* was also found to be associated with ulcerative colitis. Likewise, *MYO9B* [3,4] encodes a single motor protein [7] belonging to the class IX myosin molecules and thought to influence tight junction assembly, which can result in enhanced paracellular permeability [8,9]. These observations suggest that autoimmune diseases such as CD and ulcerative colitis share part of their etiology through tight-junction–mediated intestinal barrier defects [4].

We hypothesized that healthy carriers of these celiac associated tight junction gene variants, might have an increased intes-

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tinal permeability. Although intestinal permeability is difficult to quantify, an indirect test to study intestinal permeability is to measure the level of food antigen antibodies, such as gliadin antibodies (AGA) and Baker's yeast or anti-*Saccharomyces cerevisiae* antibodies (ASCA). These antibodies are produced when one of the above antigens crosses the impaired intestinal barrier and becomes trapped by the intolerant immune system. It has been suggested that mild intestinal permeability alteration can cause elevated AGA antibody levels and correlate well with other permeability tests such as sugar absorption tests [10–12].

Up to 20% of patients with Down syndrome show increased levels of AGA without manifesting CD [13–17]. This may imply that patients with Down syndrome have an enhanced intestinal permeability. In support of this, studies showed that 90% of patients with Down syndrome had reduced xylose absorption [18] and an impaired digestic absorption [19]. Thus, patients with Down syndrome are an interesting group to study a possible correlation between the three gene variants thought to be involved in intestinal permeability and levels of AGA. In this study was investigated whether three genetic variants (*MAGI2*, *MYO9B*, and *PARD3*) were correlated with increased intestinal permeability, represented by AGA. The second aim was to investigate possible correlations between AGA and ASCA.

2. Methods

2.1. Study design and population

We included 126 consecutive children with Down syndrome, aged 19 years or younger and registered at the VU University Medical Centre in the period 2001–2008. The diagnosis of Down syndrome was confirmed by karyotyping in all cases except one. Routine serologic celiac screening was performed, as it is a common condition in Down syndrome. Down syndrome patients with positive tissue transglutaminase antibodies (tTGA) and/or endomysium antibodies (EMA) were excluded. The levels of AGA (immunoglobulin [Ig]A and IgG) and ASCA (IgA and IgG) antibodies were also measured. We did not perform intestinal biopsy in the AGA-positive patients for ethical reasons as the recent literature shows that most CD patients with Down syndrome have been detected among EMA and tTGA-positive Down syndrome patients [20]. This study was approved by the Medical Ethical Committee of the VU University Medical Centre.

2.2. Measurements

2.2.1. Serologic markers

IgA- and IgG-AGA were determined by standard in-house enzyme-linked immunosorbent assay using gliadin extract (Sigma-Aldrich, Zwijndrecht, The Netherlands) as substrate (IgA cut-off value 4 U/ml, IgG cut-off value 21 U/ml). IgA-ASCA and IgG-ASCA were determined using a commercial enzyme-linked immunosorbent assay (Inova Diagnostics, San Diego, CA), according to the manufacturer's instructions (IgA and IgG cut-off 25 U/ml). IgA-AGA was analyzed in 119 patients, IgG-AGA in 119, IgA-ASCA in 74, and IgG-ASCA in 74 patients (supplementary Table 1; http://linkinghub.elsevier.com/retrieve/pii/S0198-8859(10)00 026-1).

2.2.2. Genotyping

A total of 126 children with Down syndrome were genotyped for six single-nucleotide polymorphisms (SNPs) in *MAGI2* (rs1496770, rs6962966, rs9640699), *MYO9B* (rs1457092, rs2305764), and *PARD3* (rs10763976). The genotyping was successful in 123 patients (call rate = 96.2%) for rs1496770, in 126 (100%) for rs6962966, in 126 (100%) for rs9640699, in 124 patients (98.4%) for rs1457092, in 122 (96.8%) for rs2305764, and in 124 patients (98.4%) for rs10763976. Genotype frequency for SNP rs6962966 was not in Hardy–Weinberg equilibrium, [21] and this SNP was excluded from further analysis. Genotyping of all polymorphisms was performed using TaqMan, as described elsewhere [3,4].

2.3. Data analysis

First we studied whether the levels of AGA and ASCA were correlated with each other. We normalized the distribution of the levels of AGA and ASCA by taking their natural logarithms (ln). The correlation analysis was performed using Pearson's correlation test.

Next we checked whether the accumulative load of genetic risk variations was associated with permeability, represented by the levels of AGA. For this analysis we focused on AGA, as it showed the highest sensitivity to alterations in intestinal permeability, *i.e.*, even a mild intestinal permeability alteration can cause elevated levels of AGA [10,11]. Furthermore, the elevation in AGA was most pronounced. Because the study size was limited, and the alterations in the intestinal permeability were determined by the net effect of all the genetic variation involved, we decided to analyze the collective effect of genetic risk variants on permeability. Thus, a genetic risk load was calculated as the sum of the number of times a participant was a carrier of the recessive genotype of the studied risk variants. We first analyzed the association of the genetic risk load to quantitative levels of AGA, and then we studied the association of genetic risk load to qualitative levels of AGA. Patients with no genetic risk factors were considered as the reference group to which other patients with a genetic risk load of at least one were compared. We used the analysis of variance, linear regression, χ^2-test and logistic regression to analyze the quantitative and qualitative data, implemented in SPSS version 15.0.

3. Results

3.1. Serologic study

IgA-AGA was elevated in 17 patients (14%, 10 patients aged <6 years). The mean of IgA-AGA level was 2.7 U/ml (range, 0.3–27.1). IgG-AGA was elevated in 34 patients (29%, 21 patients aged <6 years). The mean of IgG-AGA levels was 23.3 U/ml (range, 0.6–441.0). IgA-ASCA was elevated in two patients (3%, values 25.2 and 25.6 U/ml; cut off 25 U/ml). IgG-ASCA was elevated in four patients (5%, values 28, −28, −41, and −66 U/ml; cut off 25 U/ml). The levels

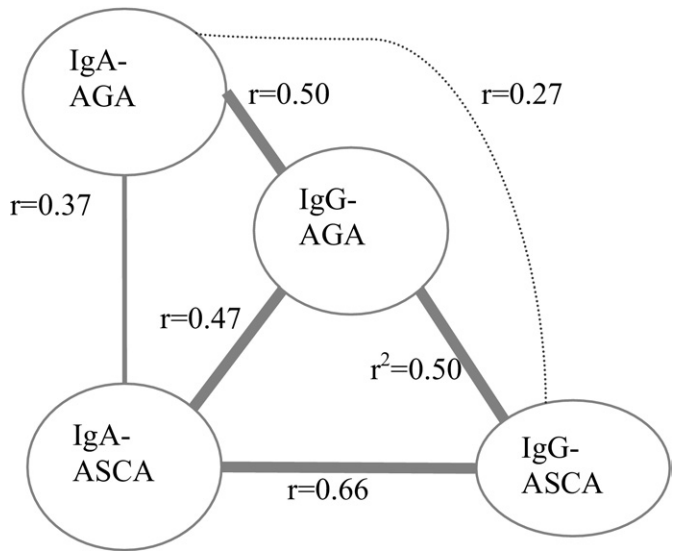

Fig. 1. Correlation between the levels of different serologic food antigen antibodies. r^2: Pearson correlation coefficient, IgA-AGA: IgA antigliadin antibody, IgG-AGA: IgG antigliadin antibody, IgA-ASCA: IgA-anti-*Saccharomyces cerevisiae* antibody, IgG-ASCA: IgG-anti-*Saccharomyces cerevisiae* antibody. ▪▪▪▪▪▪▪ Pearson correlation significant at $p < 0.05$ ▬▬ Pearson correlation significant at $p < 0.01$ ▬▬ Pearson correlation significant at $p < 0.0001$.

of AGA and ASCA antibodies were significantly correlated with each other, suggesting a common pathophysiological pathway for all tested antibodies (Fig. 1, supplementary Tables 1 and 2; http://linkinghub.elsevier.com/retrieve/pii/S0198-8859(10)00 026-1). Our data suggest that there is a strong correlation between IgG-AGA, IgG-ASCA and IgA-ASCA, and to a lesser extent to IgA-AGA.

3.2. Genetic study

There were 24 patients with no genetic risk factor, 57 patients had one recessive risk genotype, 33 patients had two, seven patients had three or more risk genotypes, of whom one patient had a recessive risk genotype for all four studied genetic variants. The

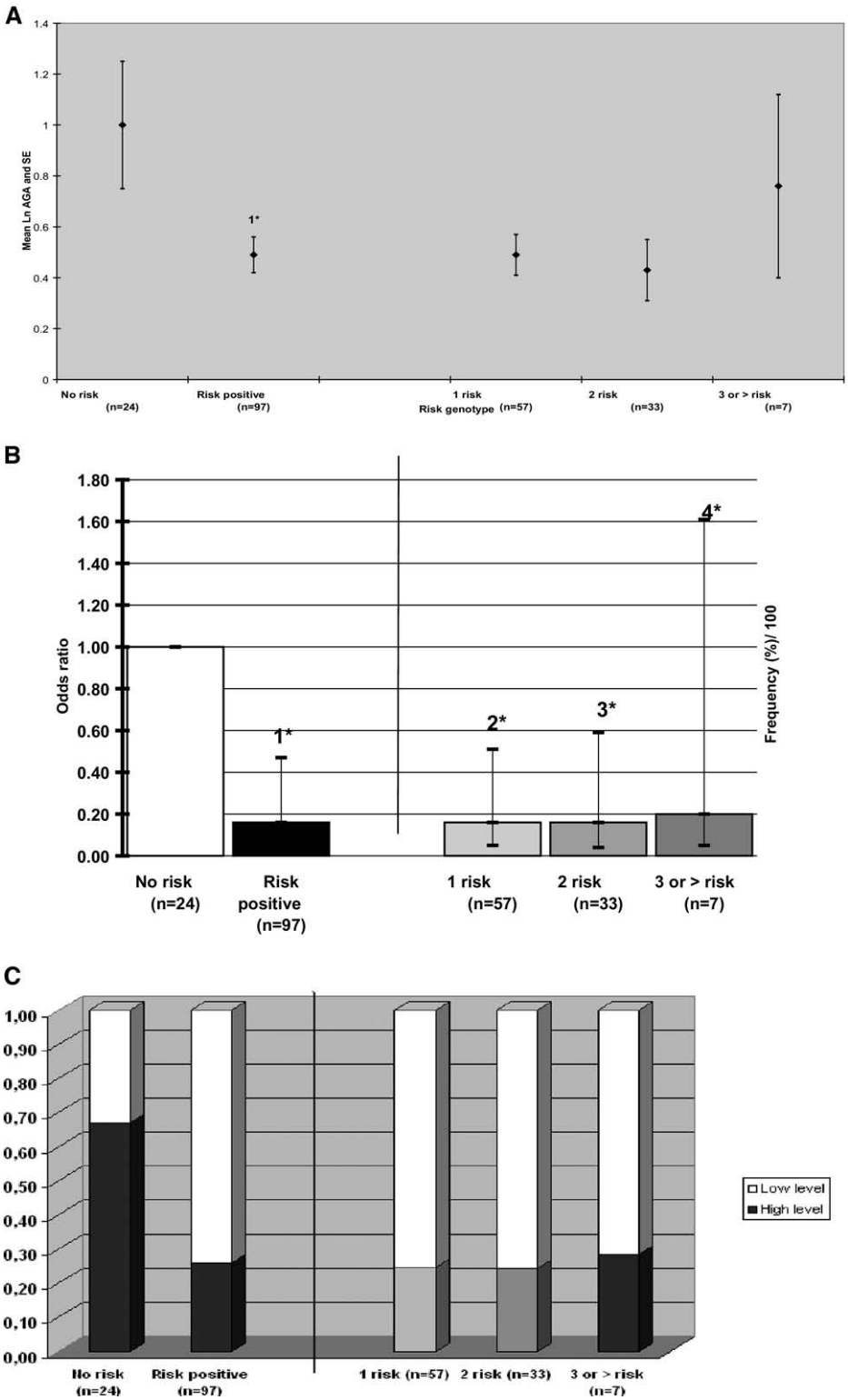

Fig. 2. Association of genotype risk dosage with permeability measured by AGA (A) Qualitative: mean IgA-AGA with standard error of mean (*1 significance: Trend test $p = 0.007$). (B) Quantitative: odds ratio with 95% confidence interval of IgA-AGA levels (elevated vs. normal AGA) (Significance: $^{1*}p = 9.3 \times 10^{-5}$; $^{2*}p = 0.003$; $^{3*}p = 0.001$; $^{4*}p = 0.07$; p overall 2, 3, 4 = 0.008). (C) Frequency of quantitative levels of IgA-AGA levels (elevated vs. normal AGA; high vs. low).

group of patients ($n = 97$) with one or more risk genotypes showed a lower mean ln IgA-AGA levels (0.49 vs. 1.01 U/ml, p trend = 0.007, Fig. 2A). When we correlated the genetic risk load to normal or elevated levels of IgA-AGA, we found that patients with normal levels of IgA-AGA were significantly more often a carrier of one or more genetic variants associated with permeability, yielding an odds ratio of 0.16 (95% CI, 0.06–0.47) (Fig. 2B, C, $p = 9.3 \times 10^{-5}$). Interestingly, all recessive genotypes or haplotypes, which have been associated with CD showed a lower level of IgA-AGA [3,4]. (See supplementary Tables 4 and 5.)

4. Discussion

This is the first study reporting a correlation between genetic variants in genes that might be involved in intestinal permeability and food antigen antibodies. In patients with Down syndrome, we found that the levels of AGA and ASCA are strongly correlated with each other and that AGA levels were significantly decreased in carriers of one or more variants of CD-associated risk genotypes [3,4].

We hypothesized that suspected CD-associated permeability genes are associated with an increased intestinal permeability that would be marked by elevated AGA. Hence, genetic variants in these genes might play a role in intestinal permeability and precede the development of CD. These results suggest the presence of alternative mechanisms for the elevated levels of AGA in patients with Down syndrome. In other words, our findings suggest that the levels of AGA may not be solely explained by the presences of *MYO9B*, *MAGI2*, and *PARD3* genes.

Rather, it suggests that there are other factors that influence the penetrance of these genetic risk variants in permeability. Our findings raise the question of what other phenomena might play a role in the increased levels of AGA in patients with Down syndrome. This is not yet clear, though there might be several explanations for our findings. First, in our study we excluded all patients who had CD (as measured by EMA and tTGA). Therefore, the genetic structure underlying the increased permeability in CD may have been excluded from this analysis. This is in sharp difference with those studies that have reported the association between tight junction genes and CD [3,4], where CD patients were compared with controls. CD might overrule permeability function whenever it develops. Second, one may speculate that the elevation of AGA may be mainly determined by an immunologic disturbance rather than by solely alterations in the genetics of tight junctions, possibly at the level of tolerance induction and/or maintenance, because of the chromosomal aberration causing Down syndrome. The increased prevalence of autoimmune disease and susceptibility to infections in Down syndrome supports this hypothesis. Derangements of immunoglobulin levels, phytohemagglutinins responsiveness and T- and B-cell markers are also reported in patients with Down syndrome. Both thymus-dependent and thymus-independent functions are impaired in Down syndrome patients with a characteristic age distribution. IgA and IgG immunoglobulin levels in Down syndrome patients older than 6 years are definitely increased [22]. This elevation is particularly found for food antigen antibodies. This immunologic disorder might also explain why patients with Down syndrome have an increased prevalence of elevated AGA.

There are some limitations to our study. First, although it was an effort to collect 126 patients with Down syndrome, the interpretation of our data was difficult because of the relatively small number of patients with elevated AGA. Second, we did not perform sugar absorption tests with lactulose-mannitol, the other marker that, besides AGA, can be used to study intestinal permeability. However, there is evidence that both tests are correlated and that mild intestinal permeability alteration as represented by an increased lactulose/mannitol ratio also causes elevated AGA levels [10–12]. Furthermore, a celiac study showed that the lactulose/mannitol ratio was significantly related to IgA-AGA suggesting an increased intestinal permeability in AGA-positive patients [12]. Another study showed a positive trend between IgG-ASCA and intestinal permeability in (tau = 0.16, $p = 0.06$) in Crohn's disease [23]. The lactulose-mannitol test in our cohort was not feasible because of most patients being inaccessible and high costs. Additionally, in literature there is controversial on the value of this test as a reliable marker for intestinal permeability. The test seems to be too sensitive and frequently influenced by environmental factors [24–27]. Furthermore, ideally we need a test that represents tight junction function and the lactulose-mannitol test does only partially. Only very recently claudin 3 in urine was discovered and might be used as a reliable marker of tight junction distortion [28]. However, further confirming studies are needed. Third, we may not have investigated all the relevant genetic variations, as many more genes might be involved in intestinal permeability.

We found that patients with Down syndrome had elevated levels of AGA, which is in agreement with earlier reports [15]. However, these publications showed that the hypergammaglobulinemia of IgA and IgG type was observed in Down syndrome patients older than 6 years and that serum immunoglobulin levels were normal in patients aged less than 5 years [22,29]. Our study could not support this finding; most of our patients with elevated immunoglobulins were younger than 6 years, except for IgG-ASCA (all four patients with elevated IgG-ASCA were older than 7 years). Furthermore, we found no elevation in the levels of ASCA, which might be explained by the fact that this is a very large food antigen and it might be too large to penetrate the intestinal barrier. This is supported by earlier studies showing that even in young CD patients, with a disturbed gut barrier, ASCA could only be detected in 18% of patients [30].

5. Conclusion

There is a strong correlation between the levels of AGA and ASCA in Down syndrome. We conclude that the levels of AGA are increased in patients with Down syndrome but this cannot be explained by the presence of tight junction (genetic) risk variants. In contrast, patients who carry at least one or more risk genotypes even had lower AGA levels. Thus, other immunologic phenomena play a role in the increased prevalence of elevated AGA in patients with Down syndrome, possibly involving altered induction and/or maintenance of tolerance. Future studies on more (Down) patients and with more sensitive markers for intestinal permeability are needed to unravel the pathogenesis of CD and other autoimmune diseases in this patient group.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.humimm.2010.01.016.

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