Host Genetic Resistance to Symptomatic Norovirus (GGII.4) Infections in Denmark

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A total of 61 individuals involved in five norovirus outbreaks in Denmark were genotyped at nucleotides 428 and 571 of the FUT2 gene, determining secretor status, i.e., the presence of ABH antigens in secretions and on mucosa. A strong correlation (P = 0.003) was found between the secretor phenotype and symptomatic disease, extending previous knowledge and confirming that nonsense mutations in the FUT2 gene provide protection against symptomatic norovirus (GGII.4) infections.

Volunteer studies have shown that a subset of individuals are not affected by vomiting and diarrhea after challenges with noroviruses (1, 16, 19), and this information, together with the fact that attack rates seldom exceed 70% (4), suggests that inherited factors act to prevent certain individuals from symptomatic norovirus disease. Indeed, experimental and volunteer studies have indicated a correlation between secretor status and susceptibility to Norwalk virus (genogroup I norovirus) infections (3, 12, 14). These observations were recently confirmed and extended to also include authentic outbreaks by genogroup II viruses (17), which is the clinically most common genogroup of norovirus (9). Current knowledge thus suggests that secretor status determined by polymorphisms in the FUT2 gene is strongly associated with resistance to norovirus infections (3, 11, 12, 17). Further support for this hypothesis is also the fact that nonsecretor and Lewis a-positive individuals have significantly lower antibody prevalence and titer to norovirus GGII than secretor and Lewis b-positive individuals (10). The association between secretor status and norovirus susceptibility is not fully understood, but transfection of the FUT2 gene to nonpermissive cells has been shown to increase cell susceptibility to norovirus infection (14), suggesting that the H antigen or a related structure acts as a receptor for the virus.

FUT2 encodes an α(1,2)fucosyltransferase, which adds a fucose molecule to the H-type 1 precursor, giving the H-type 1 antigen. H-type 1 can, in contrast to its precursor, work as a precursor for the A and B blood group antigens. FUT2 determines the secretor status, which is the presence or absence of blood group ABH antigens on mucosal surfaces and in secretions such as saliva (5). Individuals carrying the secretor genotypes (SeSe or Sese) secrete the H antigen with or without A and/or B antigens, while individuals of the nonsecretor genotype (sese) do not. The polymorphisms found in the FUT2 gene show high ethnic specificity (8, 13), with a nonsense mutation at nucleotide 428 associated with the Caucasian populations (5, 8) and the nonsense mutation se571 allele mainly found in Pacific Islanders (2).

High number of norovirus outbreaks in Denmark 2004 to 2005. The winter season of 2004 to 2005 in Denmark was characterized by a high number of norovirus gastroenteritis outbreaks. From September 2004 to the end of May 2005, 499 of 1,309 patient samples (38%) were positive for norovirus by PCR. The majority of the positive samples (90%) were submitted from hospitals, representing at least 30 different hospitals and situated in all parts of Denmark. For detection of norovirus in stool samples, purified viral RNA was reverse transcribed and PCR amplified (RT-PCR kit; QIAGEN, Hilden, Germany) by using the primers JV12 and JV13 essentially as described earlier (17, 18). The PCR products (285 bp after trimming of the primer sequences) were sequenced to determine the genotype. Briefly, sequencing was performed by using an ABI Prism BigDye Terminators v 3.0 cycle sequencing kit and an automatic ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA). The genotypes were established by comparison to sequences in GenBank. The dominating genotype was GGII.4, which was detected in 95 of 98 (97%) randomly selected positive samples.

Norovirus outbreak descriptions. The aim of the present study was to investigate why a significant fraction of individuals involved in several norovirus outbreaks in Denmark remained uninfected. To determine whether host genetics and, more specifically, secretor status affected susceptibility, five different outbreaks (1 to 5) occurring between November 2004 and April 2005 were investigated in greater detail. Norovirus was detected in ill (vomiting and diarrhea) patients associated with all five outbreaks. Eleven viral isolates representing the five outbreaks were also genotyped, and all viruses were found to

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belong to genotype II.4, thus leading to the conclusion that the five outbreaks were associated with GGI.4 norovirus.

Four of the five outbreaks occurred in separate wards in three different hospitals (outbreak 1 and outbreaks 3 to 5) and affected both patients and staff. The affected wards were two internal medicine wards, one geriatric ward, and one ward for orthopedic surgery (Table 1). The last outbreak occurred within a family (Table 1, outbreak 2), where all individuals were affected both patients and staff. The affected wards were two of which were studied in detail. These departments had each 21 beds in 9 to 10 rooms. The outbreaks lasted for 14 days, and a maximum of 13 ill patients and 7 staff was recorded 3 days after onset. Outbreak 1 occurred in two closely related wards with 22 and 20 beds and lasted approximately for 1 week, affecting at least eight patients and three staff. The last hospital outbreak (outbreak 5) occurred during a 2-week period in a ward with 20 beds with a total of 12 patients and 9 staff being ill (Table 1). Fourteen stool samples were collected from symptomatic individuals, and all were norovirus positive. Norovirus-positive samples were detected in each outbreak, but samples were not collected from all of the individuals involved in the outbreaks, and no samples from unaffected individuals were tested. Individuals were considered as “affected” when having symptoms of acute gastroenteritis, i.e., diarrhea and/or vomiting during an outbreak period at the specific setting.

A nonsense mutation at nucleotide 428 provides protection against symptomatic GGI.4 norovirus infection. To investigate any association between susceptibility to norovirus disease and polymorphism in FUT2, DNA from saliva (n = 61) was purified, PCR amplified, and subjected to pyrosequencing at nucleotide 428 as described previously (6). For nucleotide 571, the PCR forward primer 5′-BIOTIN-GCACCTTTGTAAGGTCCA-3′ was used together with the reverse primer 5′-CTTCCACACCTTGGCATGAC-3′ (CyberGene AB, Huddinge, Sweden). For sequencing of nucleotide 571 the primer 5′-TGGACATAGTCTCCCTC-3′ was used.

Genotyping revealed that none of the 61 individuals examined carried the mutation at position 571, while 15% (9 of 61) were homozygous nonsecretors carrying the allele mutated at nucleotide 428 (se428se428), 44% (27 of 61) were homozygous secretors (SeSe), and 41% (25 of 61) were heterozygous secretors (Sese428). The allelic distribution among symptomatic and asymptomatic individuals is illustrated in Table 1 and shows that homozygous and heterozygous carriers of the secretor wild-type allele were both found to be susceptible to symptomatic infections. In the family outbreak (Table 1, outbreak 2), all four family members presented with vomiting and diarrhea that lasted for a few days, and FUT2 genotyping revealed that all of the family members were carriers of the secretor phenotype. The father was homozygous for the wild-type allele, while the mother was heterozygous. Both children inherited two wild-type alleles from their parents and thus became homozygous secretors.

We found a strong association (P = 0.003 [Fisher exact test, two-tailed, 95% confidence interval]) between nonsecretors and resistance to symptomatic norovirus GGI.4 infections. Indeed, we could not find any nonsecretor with symptomatic norovirus GGI.4 infection. This observation is similar to a previous study from Sweden (17). Our results thus extend previous reports showing that nonsecretors appear resistant against experimental and authentic infections with genogroup I and II viruses (3, 12, 17).

The fact that 20% of Swedes (6, 17) and 15% of the Danes in the present study were nonsecretors shows that a significant part of the population in Scandinavia carry this null mutation at position 428 in the FUT2 gene. A prerequisite to detect an association with a disease is, however, that the particular mutation must be reasonable common and, second, the disease to be studied should be common and preferably occur in all age groups. Although the 428G→A screening gives a very precise information about nonsecretor phenotype among Caucasians, it should be noted that in other populations other inactivating mutations in the FUT2 gene give rise to the nonsecretor phenotype, such as the 571C→T nonsense mutation (7, 15) investigated in the present study.

Genotyping allows, in contrast to phenotyping, the possibility of determining the role of homozygosis and heterozygosis. The advantage of genotype characterization is exemplified with the family outbreak (Table 1), illustrating that susceptibility to disease is fulfilled in the presence of only one functional allele. Furthermore, genotyping allows allele frequencies to be determined and compared. In the present study the mutated 428G→A allele frequency among the ill individuals was 0.26 compared to 0.44 among unaffected subjects. This should be compared to 0.26 for symptomatic and 0.48 for unaffected Swedes (17). Twenty-three secretor-positive individuals remained unaffected in the present study. This could have been due to protective immunity or that they remained unexposed in spite of the close settings where these outbreaks occurred.

In conclusion, this is the second report observing a strong correlation (P = 0.003) between a nonsense mutation at nu-

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**Table 1.** Nonsecretors (se428se428) have inherited resistance to symptomatic norovirus (GGI.4) infections in Denmark*

<table>
<thead>
<tr>
<th>Outbreak no. (location or circumstance)</th>
<th>No. of saliva samples investigated</th>
<th>No. of symptomatic patients</th>
<th>No. of asymptomatic and/or unexposed patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>SeSe</td>
<td>Sese428</td>
</tr>
<tr>
<td>1 (geriatric ward)</td>
<td>20</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>2 (within family)</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3 (orthopedic surgery)</td>
<td>13</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>4 (ward of internal medicine)</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5 (ward of internal medicine)</td>
<td>13</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total (%)</td>
<td>61</td>
<td>29</td>
<td>48</td>
</tr>
</tbody>
</table>

* a, Number of samples; SeSe, homozygous wild type; Sese428, heterozygous for the mutated FUT2 allele 428G > A; se428se428, homozygous for the mutated FUT2 allele.

b P = 0.003 (Fisher exact test).
cleotide 428 in the FUT2 gene and resistance to symptomatic norovirus GGII.4 infections, which is the clinically most common genotype.

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