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HLA-DQ and risk gradient for celiac disease

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ABSTRACT

Celiac disease (CD) is a rare example of multifactorial disorder in which a genetic test is of great clinical relevance, as the disease rarely develops in the absence of specific HLA alleles. We typed *DR-DQ* genes in 437 Italian children with celiac disease, 834 first-degree relatives, and 551 controls. Of patients, 91% carried DQ2 and/or DQ8 heterodimers, 6% only had β2 chain, 2% was α5 positive, and four were DQ2/DQ8/β2/α5 negative. Only the presence of α5 resulted negatively associated to disease ($p = 2 \times 10^{-4}$), whereas we confirmed the effect of the β half of DQ2 dimer on CD predisposition ($p = 4 \times 10^{-12}$). Considering 1:100 disease prevalence, we obtained a risk gradient ranging from 1:7 for DQ2 and DQ8 individuals down to 1:2518 for subjects lacking all predisposing factors. The *DQB1*02* and *DQB1*0302* concurrence ($p = 9 \times 10^{-4}$), besides the *DQB1*02/*02* homozygosity, had an additional role in disease genetic determination. The CD prevalence rose to 17.6% in sisters, 10.8% in brothers, and 3.4% in parents. In the three groups, the subjects carrying high-risk HLA molecules were 57%, 71%, and 58%; among them, 29%, 15%, and 6% respectively had CD. Those siblings and parents with no susceptible factors were not affected. These findings indicate the impact of the HLA test for CD in clinical practice.

1. Introduction

Celiac disease (CD, MIM 212750) is a chronic intestinal inflammation resulting in villous atrophy and flattening of the mucosa. The disease occurs in genetically predisposed individuals in response to the dietary ingestion of wheat gluten and similar proteins in barley and rye. The treatment consists of lifelong gluten exclusion from the diet that leads to histologic and clinical remission and prevents the development of refractory CD and long-term complications such as malignancy, osteoporosis, infertility, and autoimmunity.

Originally considered a rare malabsorption syndrome in childhood, CD is now recognized as a common disorder that may arise at any age, with a growing proportion of new cases diagnosed in adults and in patients with extraintestinal manifestations. Recent accurate epidemiologic studies have revealed that CD affects approximately 1% of the general population, both in Europe and in North America [1]. The prevalence of the disease increases among patients with anemia or autoimmune diseases, with short stature, or with Down, Turner, or Williams syndrome [2]. Moreover, CD clusters in families with a prevalence among first-degree relatives ranging from 2.8% to 17.2% in different series [3].

Although CD is one of the most common lifelong diseases in western countries, most affected individuals remain undiagnosed [1]. This is apparently because many patients have atypical symptoms or none at all. The disease is characterized by the production of anti-tissue transglutaminase (anti-tTG) and anti-endomysial (EmA) antibodies. Serologic screening for the presence of these autoantibodies in individuals with characteristic symptoms of CD or with associated conditions is usually the initial step in detecting new cases. Although anti-tTG and EmA appear to be good markers of the active phase of the disease, the definitive diagnosis requires a small-bowel biopsy showing the typical histologic abnormalities (villous atrophy, crypt hyperplasia, and leukocyte infiltration).

CD mostly develops in HLA-DQ2 (*DQA1*05* and *DQB1*02*)–positive individuals, whereas most of the remaining cases are HLA-DQ8 (*DQA1*03* and *DQB1*0302*) positive. The close association can be explained by the fact that the DQ2 and DQ8 α/β heterodimers mediate the activation of gluten-reactive $CD4^+$ T cells in the gut. In particular, the disease-associated HLA-DQ molecules expressed on antigen-presenting cells specifically bind gluten-derived peptides, modified by the enzyme tTG, and present them to intestinal T cells. The resulting T response leads to the production of the disease-specific antibodies and to the secretion of pro-inflammatory cytokines with consequent mucosa atrophy and clinical manifestations.

The European Genetic Cluster on Celiac Disease has demonstrated that practically all CD patients carry HLA-DQ2 and/or HLA-DQ8 molecules or one chain of the DQ2 heterodimer, coded by *DQA1*05* (α5 chain) or *DQB1*02* (β2 chain) alleles, and that CD occurs only exceptionally in the absence of at-risk DQ factors [4]. Moreover, a gene dosage effect for the *DQB1*02* allele has been

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described in several studies [5,6]. Given the strong association, the HLA typing is routinely used as a genetic test for CD; the presence of susceptible DQ variants does not predict certain developments of the disease but strongly modifies the risk, whereas their absence makes CD very unlikely with a negative predictive value close to 100% [7]. We have recently reported evidence of gender differences in this association, with a different negative predictive value for the HLA test in female and male subjects, indicating the need to consider the gender in the disease-risk calculation [8].

Despite the fact that the diagnostic significance of the HLA test for CD is not absolutely certain, it is generally considered that it may be of help in the definition of uncertain cases. The analysis is also recommended in at-risk groups, such as first-degree relatives of patients, to decide the follow-up [2]. Indeed, the HLA genes are lifelong stable markers and their typing may discern subjects genetically susceptible or nonsusceptible to the disease long before the possible appearance of clinical or serologic signs.

The HLA-DQ association with the risk of CD has been extensively discussed, but knowledge of the practical usage of the HLA typing as a genetic test for the disease remains limited and the clinical implications of the results are still not clearly defined. We present here our experience with the HLA testing in a cohort of Italian pediatric celiac patients and first-degree relatives collected over the last 20 years, with the aim to contribute to the development of clinical practice guidelines for the use of the HLA typing as a predictive test for CD.

2. Subjects and methods

2.1. Patients and relatives

All subjects ($N = 1271$) except two siblings were described in a previous report (8). Briefly, they included 145 CD patients and 292 nuclear families (292 index cases, 34 affected and 216 unaffected siblings, and 20 affected and 564 unaffected parents).

The 437 index cases had a median age of 5 years 8 months at sample collection. Age in siblings ranged from 1 to 20 years with a median of 10 years. No differences were observed between affected and unaffected cohorts.

All relatives were tested for anti-tTG and EmA antibodies, and selected individuals underwent a small-intestinal biopsy, as previously described [9].

Informed consent was obtained from each participant.

2.2. Controls

The control sample ($N = 551$) included 292 healthy Italian individuals and 259 affected family based controls, as previously reported [8].

2.3. HLA typing

All individuals were typed for *DRB1*, *DQA1,* and *DQB1* genes by sequence-specific primer–polymerase chain reaction (SSP-PCR) using commercial kits (Dynal Biotech, Bromborough, UK).

2.4. Nomenclature

The term DQ2 belongs to the serologic HLA nomenclature, and it specifies an epitope on the β2 chain. However with time, in CD, the term has usually referred to a particular α5β2 DQ2 dimer encoded by *DQA1*05* and *DQB1*02* alleles. Therefore, we only use DQ2 to indicate subjects carrying both the alleles, whereas individuals *DQA1*05* negative/*DQB1*02* positive, in which the β2 chain forms dimers with a different α chain, are simply named β2. A single or double dose of *DQB1*02* is indicated as *B1*02/X* or *B1*02/*02,* respectively. The phenotype coded by *DQA1*05* allele in absence of *DQB1*02* is designated as α5.

To specify a particular haplotype, we adopted a code in which the first two digits represent *DRB1*, the third *DQA1,* and the fourth *DQB1* alleles. The fifth digit, when present, denotes the *DQB1*03* variants. For example the code 0352 stands for *DRB1*03-DQA1*05-DQB1*02*; 0432 stands for *DRB1*04-DQA1*03-DQB1*02*; 04332 stands for *DRB1*04-DQA1*03-DQB1*0302*; and 11531 stands for *DRB1*11-DQA1*05-DQB1*0301*.

2.5. Statistical analysis

Statistical significance was calculated by Fisher's exact test using 2×2 contingency tables. Values of $p < 0.05$ were considered significant.

Disease risks are expressed as 1:N, where N is the number of individuals among which one patient is present. Considering a disease prevalence of 1:100 in the general population, for each HLA-DQ category, N is calculated as a percentage of controls with that particular HLA-DQ status multiplied by 100 and divided by percentage of patients with the same DQ typing.

The risks in female and male subjects were calculated using the DQ frequencies previously reported in the two genders and considering the 1.8:1 female to male ratio [8].

3. Results

The case-control study of the 437 celiac children and 551 controls was previously described [8]. Briefly, 91.1% patients and 29.0% controls carried DQ2 and/or DQ8 heterodimers. Among the DQ2/DQ8-negative individuals, the frequencies of cases carrying *DQB1*02* (β2), *DQA1*05* (α5), or neither of the two alleles were 66.7%, 23.1%, and 10.2%, respectively versus 14.1%, 53.4%, and 32.5% of the controls, showing a positive association with CD of the β2 phenotype ($p = 4.3 \times 10^{-12}$) but a negative association of the α5 phenotype ($p = 2.2 \times 10^{-4}$).

Two alleles coding for at-risk DQ beta chains (**02/*02*, **02/*0302,* or **0302/*0302*) were found in 30.4% cases and 3.8% controls, leading to a very high p value ($p = 5.5 \times 10^{-32}$). The *DQB1*02/*02* combination was found in 107 of 437 patients and 15 of 551 controls (1.8×10^{-26}), and *DQB1*02/*0302* was observed in 24 of 437 and 5 of 551 (1.8×10^{-5}). Two case patients and one control were *DQB1*0302* homozygous. Of the total 56 DQ8 cases, only one lacked the *DQA1*03* allele carrying the *DRB1*03-DQA1*05-DQB1*02* haplotype and an unusual *DRB1*11-DQA1*05-DQB1*0302* combination.

Based on the HLA-DQ typing, we obtained a gradient of disease risk ranging between 1:7 and 1:2518. Figure 1 reports the values estimated from the whole sample and in the two genders separately. Looking at the total data set, the highest risk value was for DQ2- and DQ8-positive subjects (1:7), followed by the other three categories coding for two β susceptibility chains: DQ2, *B1*02/*02* (1:10), DQ8, *B1*02* positive (1:24), and β2, *B1*02/*02* (1:26). DQ2 subjects carrying a second *DQB1* allele different from **02 or *0302* (*B1*02/X*) resulted in a risk of 1:35. In this category, cis/trans arrangements were considered together because of the lack of statistical significance though the trend was similar to that described in other studies [10,11]. The presence of DQ8, when *DQB1*02* negative, led to a disease likelihood of 1:89; individuals *DQB1*0302* homozygous were considered together with the heterozygous ones because of their small number (two patients and one control). In the remaining three categories, the disease probability was lower than in the general population (1:100), resulting in 1:210 for β2 (*B1*02/X)* subjects, 1:1842 for the α5 ones, and 1:2518 in the absence of all the susceptible HLA-DQ factors. In this gradient, a single or double dose of the *DQA1*05* allele was not specified because a significant different contribution to CD predisposition was not revealed. The F:M proportion in the whole cohort of patients was 1.8; however, when the patients were stratified by HLA-DQ typing, the risks in the presence of β2 phenotype (*B1*02/*02* or *B1*02/X*) gave very similar values in the two genders, and the α5 status, even if rarely found in patients, was mostly present in male subjects [8].

Disease risk

	Patients%	Controls%	Risk	F	M
DQ2 and DQ8	2.5	0.2	1:7	1:7	1:8
DQ2, *B1*02/*02*	23.1	2.4	1:10	1:8	1:13
DQ8, *B1*02* pos.	3.0	0.7	1:24	1:16	1:52
β2, *B1*02/*02*	1.4	0.4	1:26	1:27	1:26
DQ2, *B1*02/X*	55.1	19.2	1:35	1:26	1:54
DQ8, *B1*02* neg.	7.3	6.5	1:89	1:62	1:157
β2, *B1*02/X*	4.6	9.7	1:210	1:211	1:208
α5	2.1	37.9	1:1842	1:8327	1:1027
Other	0.9	23.0	1:2518	1:2530	1:2497

Fig. 1. Risk gradient. Risks were evaluated from unrounded case-control DQ frequencies, considering a disease prevalence of 1:100 (arrow) and a female:male gender ratio of 1.8. DQ2 = *DQA1*05* and *DQB1*02*; DQ8 = *DQA1*03* and *DQB1*0302*; β2 = *DQB1*02* in the absence of *DQA1*05*; α5 = *DQA1*05* in the absence of *DQB1*02*. X, different from *DQB1*02* or *DQB1*0302*.

As expected on the basis of the known linkage disequilibria, very strong DR-DQ associations were found. Of the DQ2 cases, roughly 2/3 resulted *DRB1*03* positive and most of the remainder was *DRB1*07/*11* heterozygous; the remaining 22 children (6.2%) presented unusual DR-DQ combinations: 07233/1252 ($n = 1$); 1152/11531 ($n = 2$); 1315/1352 ($n = 1$); 1316/1352 ($n = 1$); 0432/11531 ($n = 2$); 11531/1322 ($n = 1$); 0722/12531 ($n = 3$); 0722/13531 ($n = 10$); 0722/14531 ($n = 1$) (Subjects and methodssee for codes). All DQ8 cases except two (0352/11532; 08332/1316) were *DRB1*04* and all the β2 patients typed as *DRB1*07*. The four patients classified as "other" were 0844/1516, 1316/1415, 1415/1415, and 1415/1516. The complete list of α, β combinations with the associated DR genotypes in each HLA-DQ group is added as supplementary material (Supplementary Table 1). In every class, the risks were found to be homogeneous without significant differences even between DR3/3 and DR3/7 or between DR3/X and DR7/11, except for the DQ8, *B1*02*–negative category, in which the DR4/x and DR4/11 genotypes gave disease risk values of 1:204 and 1:56 respectively ($p = 0.02$). No significant differences in the DR-DQ association were observed between cases and controls.

A total of 250 siblings and 584 parents were evaluated by clinical and serologic analyses and typed for HLA class II alleles; biopsies were performed in selected cases. In all, 18 of 102 sisters (17.6%) and 16 of 148 brothers (10.8%) were observed to be affected, with an overall prevalence of CD in siblings of 13.6%, significantly higher than the 3.4% found in parents ($p = 2.1 \times 10^{-7}$). Figure 2A shows the proportions of siblings and parents with HLA at high risk for CD (DQ2, DQ8, or β2 with a double dose of *DQB1*02*), with moderate disease risk (β2, *B1*02/X*), or negative for all susceptibility factors (HLA negative). The 65% of siblings and 58% of parents had high-risk HLA molecules; among them, 20% siblings and 6% parents had CD ($p = 2.7 \times 10^{-6}$). Of the 105 β2 (*B1*02/X*) relatives, only one sibling was affected, and none of the HLA-negative ones had CD. Sisters were less numerous than brothers ($p < 0.05$) and less often had high-risk HLA alleles ($p = 0.02$); however, when positive, twice the number of sisters had CD ($p = 0.03$) (Fig. 2B).

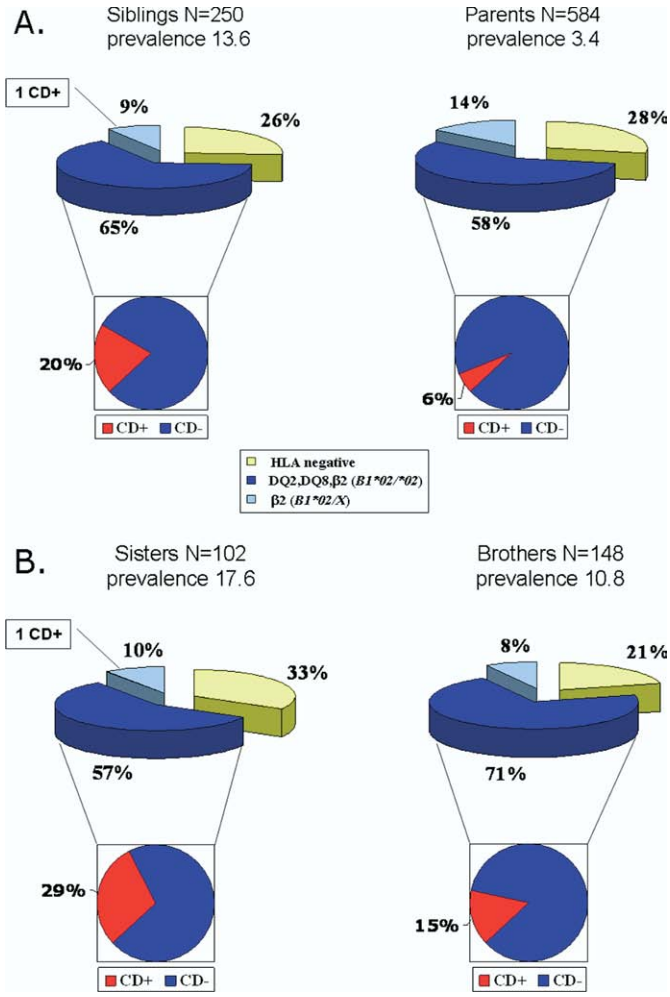

Fig. 2. At-risk DQ factors and celiac disease (CD) in relatives. (A) Siblings and parents. (B) Sisters and brothers. CD+, celiac; CD−, not celiac.

4. Discussion

Celiac disease is a rare example of multifactorial disorder in which a genetic test is of great importance in clinical practice. From this point of view, the peculiarity of CD is due to several issues: the gluten ingestion is known to be the environmental triggering factor, and a gluten-free diet represents a valid therapy that leads to complete remission of the clinical signs; the disease is largely underdiagnosed, because most patients have silent or atypical forms; untreated CD significantly increases risk of developing long-term complications such as lymphomas or autoimmune disorders; thus an early diagnosis is important so as to start a therapeutic diet as soon as possible and to avoid severe consequences. The primary genetic susceptibility component has been well defined, and the disease rarely develops in the absence of specific HLA class II alleles.

We present here the results of a large, single-center HLA study carried out in a group of Italian children with CD and their relatives. The case-control data confirm the known strong association of the DQ2 molecules with CD; however the proportion of DQ2-positive patients observed in this Italian cohort is clearly lower than the 90–95% reported in a Northern European population (4). Indeed, we found that 80.8% of individuals with CD carried the $\alpha5\beta2$ DQ2 heterodimer, whereas 10.3% were DQ2 negative/DQ8 positive and 5.9% only had the $\beta2$ chain. Of the 3% DQ2, DQ8 and $\beta2$ negative, 2.1% were $\alpha5$ positive, and 0.9% lacked all HLA susceptibility factors. However, the only presence of the $\alpha5$ chain was observed in 37.9% of DQ2-, DQ8-, and $\beta2$-negative controls; thus this half of the DQ2 dimer did not prove to be a predisposing factor for the disease. The diagnosis of the four HLA-negative cases was accurately checked: these patients presented with typical symptoms, with mucosal atrophy and positive serology. Clinical re-evaluation, by gluten challenge, was performed in two patients: one individual has not relapsed so far, and the second experienced vomiting and diarrhea on gluten challenge (three attempts).

Other investigators have reported that CD rarely occurs in individuals carrying HLA-DQ alleles, which are unusual for the disease (4). A multicenter study should be carried out to finally establish whether gluten intolerance may really develop in the absence of the known DQ susceptibility markers and to define the molecular basis of this genetically distinct disease.

Comparison of DQ frequencies between this case cohort and controls and the respective odds ratios were previously described [8]. In the present work, we provide a detailed estimation of the CD risks related to each particular HLA-DQ status, considering a disease prevalence of 1:100. As expected, the DQ2 and DQ8 dimers in any case led to increased odds of developing the disease. However, different values were found for subjects carrying one or two copies of *DQB1*-predisposing alleles. The two more frequent classes of patients were represented by DQ2 subjects, carrying one (55%) or two (23%) copies of the *DQB1*02* allele; the homozygous effect was proved by the threefold CD risk value in the presence of a second *DQB1*02* allele (1:10 vs. 1:35). Although rarely present, the *DQB1*02/*02* condition in absence of the *DQA1*05* allele also gave a high likelihood of developing gluten intolerance (1:26). Ascertaining the *DQB1*02* homozygous status appears to be necessary also because the presence of a double dose of this allele was associated with the disease earlier onset, as well as increased anti-tTG levels, severity, and complications [5,12,13]. Moreover, we showed that the concurrence of *DQB1*02* and *DQB1*0302* alleles also had an additional role in disease genetic determination. In fact, the presence of both DQ2 and DQ8 molecules resulted in the highest genetic predisposition (1:7), and a *DQB1*02/*0302* effect was also evident in the absence of *DQA1*05*, as the occurrence of *DQB1*02* allele in DQ8 subjects raised the risk from 1:89 to 1:24. A synergic effect between DQ2 and DQ8 molecules was first reported in type 1 diabetes (T1D) and ascribed to trans-encoded dimers (*DQA1*05/DQB1*0302* and *DQA1*03/DQB1*02*) able to present diabetogenic peptides to T cells [14,15]. We suggest here that trans-encoded DQ heterodimers may also bind gluten-specific peptides and trigger a selective immune response in celiac patients. Another hypothesis is that the increased risk of CD development in the presence of both DQ2 and DQ8 molecules may be caused by an enlargement of the peptide repertory, as the two dimers are known to use different criteria for the selection of deamidated gluten epitopes [16,17].

Overall, a risk of CD higher than 1:100 was found for all individuals DQ2 and/or DQ8 positive or $\beta2$ with two *DQB1*02* alleles, whereas values lower than disease prevalence in the general population were ascertained for the remaining three categories: $\beta2$ *(B1*02/X)*, $\alpha5$, and "other" (*i.e.,* different from DQ2, DQ8, $\beta2$, and $\alpha5$). Nevertheless, $\beta2$ subjects, *DQB1* heterozygous, were observed to be at higher risk (1:210) than the other two categories based on the observation that *DQB1*02*, but not *DQA1*05,* was significantly associated with CD in DQ2/DQ8-negative individuals. Indeed, subjects with $\alpha5$ phenotype resulted as having extremely low odds of developing gluten intolerance (1:1842), and those of the "other" category, comprising the four patients who were DQ2, DQ8, $\beta2$, and $\alpha5$ negative, showed a risk of 1:2518. We also calculated the CD risks in the two genders separately considering the assessed female:male proportion of 1.8:1. The risk gradient did not turn out to be completely homogenous, according to the strongest DQ2/DQ8 association in female subjects and the male predominance in DQ2/DQ8-negative cases [8]. Indeed, the presence of DQ2 and/or DQ8 dimers led to higher F than M risks with a mean ratio of 2.0, whereas the gender ratio resulted 1.0 in $\beta2$ (*B1*02/*02* or *B1*02/X*) and 0.1 in $\alpha5$-positive subjects. Therefore we believe gender to be a variable that must be taken into consideration in disease risk evaluation.

The CD risk gradient here reported was calculated in the general population; however, the HLA-DQ typing is not typically used as a screening strategy, because DQ2/DQ8 is present in approximately 30% of healthy individuals. Nevertheless, the gradient could be applicable to high-risk groups, only correcting the values by *a priori* disease risk. In these cohorts, HLA typing has a practical impact in selecting individuals who must undergo subsequent tests, and the risk gradient could be of help in programming the intervals of the clinical and serologic follow-up. In particular, subjects with $\alpha5$ or "other" phenotypes should be reassured that they are unlikely to develop gluten intolerance, whereas the presence of DQ2 and/or DQ8 (especially in female subjects) or $\beta2$, *B1*02/*02* implies the need to periodically repeat blood and clinical tests. The borderline risk value of the $\beta2$ status would suggest that less frequent checks are necessary.

As recommended by the guidelines of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition [2], HLA testing is particularly useful in first-degree relatives because of the high disease prevalence in this group. Our family-based study shows that CD develops almost exclusively in the presence of DQ2, DQ8 dimers or in *DQB1* homozygous $\beta2$ subjects. Indeed, 1:5 siblings carrying HLA high-risk molecules had CD, with twice the number of sisters affected compared with brothers, and CD was present in roughly 1:20 parents positive for the HLA markers. No affected relatives without DQ2, DQ8 or *DQB1*02/*02* $\beta2$ status were observed except one sister with the *DQB1* heterozygous $\beta2$ condition. These data confirm the low genetic predisposition conferred by the presence of one copy of the *DQB1*02* allele in the absence of *DQA1*05* and the nonsusceptible effect of the $\alpha5$ status.

In conclusion, our findings provide additional evidence that DQ polymorphism significantly modulates the likelihood that CD will develop, highlighting the impact of HLA typing in detecting individuals potentially at risk. The test appears especially useful for discriminating siblings who could be reassured about the unlikelihood of developing the disease from those who must be monitored for development of CD. Although we do not have offspring data because of the pediatric index cases, it seems reasonable to assume that this cohort also must be carefully monitored.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.humimm.2008.10.018.

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