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Review Article

Environmental factors in the etiology of type 1 diabetes, celiac disease, and narcolepsy

Lernmark Å. Environmental factors in the etiology of type 1 diabetes, celiac disease, and narcolepsy.
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The etiology of human leukocyte antigen (HLA)-associated organ-specific autoimmune diseases is incomplete. In type 1 diabetes and celiac disease, the strongest associations are with the HLA-DR3-DQ2 and DR4-DQ8 haplotypes, whereas the DQB1*06:02 allele has a strong negative association. In contrast, narcolepsy, especially as recently triggered by the Pandemrix® H1N1 vaccine (GlaxoKlineSmith (GSK), Brentford, Middlesex, UK), did not seem to develop without at least one copy of the latter allele. The overall hypothesis is that the role of these different HLA haplotypes, especially in Finland and Sweden, is related to the immune response to infectious agents that are common in these two populations. The high incidence of both type 1 diabetes and celiac disease in Scandinavia may be the result of the HLA-DR3-DQ2 and DR4-DQ8 haplotypes, and the DQB1*06:02 allele are common because they protected people from succumbing to common infections. The timing of dissecting the autoimmune response is critical to understand the possible role of environmental factors. First, an etiological trigger may be a common virus infecting beta cells or with antigens inducing beta-cell cross reactivity. Second, an autoimmune reaction may ensue, perhaps in response to beta-cell apoptosis or autophagy, resulting in autoantigen-specific T cells and autoantibodies. It is critical in at-risk children to dissect the immune response prior to the appearance of autoantibodies in order to identify cellular reactions in response to environmental factors that are able to induce an HLA-associated immune reaction.

Åke Lernmark

Department of Clinical Sciences, Lund University, Malmö, Sweden

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Corresponding author: Åke Lernmark,
Department of Clinical Sciences,
Lund University,
CRC 60:11,
Jan Waldenströms gata 35,
Skåne University Hospital SUS,
SE-205 02 Malmö,
Sweden.
Tel: (46) 40 39 19 01;
fax: (46) 40 39 11 22;
e-mail: Ake.lernmark@med.lu.se

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HLA-DR-DQ is strongly associated with type 1 diabetes, celiac disease, and narcolepsy as examples or organ-specific autoimmune diseases. The disease association with HLA-DR-DQ, known since the 1970 (1), has remained the strongest genetic factor for type 1 diabetes and celiac disease risk (2–4). Recent genome-wide association studies (GWAS) have discovered genetic factors or genes that would explain either type 1 diabetes (5, 6) or celiac disease (7, 8). Human leukocyte antigen (HLA) remains the greatest contributor to disease risk. Although the mechanisms by which HLA class II molecules are expressed and present antigen-peptides, the specific role of HLA-DR3-DQ2 and HLA-DR4-DQ8 and genetically linked molecules (9) in triggering an autoimmune response needs to be determined. Although 89% of type 1 diabetes patients have any of these two haplotypes (10, 11) or both, there has been a recent increase in patients with lower-risk HLA genotypes (12, 13). Also, the mechanisms by which HLA-DQA1*01:02-B1*06:02 was present in all patients developing narcolepsy after Pandemrix® vaccination is in conjunction with the recent 2009 H1N1 pandemic (14–16). In this brief review, on the occasion of the 20th anniversary of DIPP (Type 1 Diabetes Prediction and Prevention Project), the possible importance of environmental factors will be discussed along with the possible role of HLA-DR-DQ in population selection in response to common infectious agents, known antigens and induction of organ-specific autoimmunity, what we have learned from DIPP, what we have learned from The Environmental Determinants of Diabetes in the Young (TEDDY) and islet autoimmunity, and what we have learned from TEDDY celiac disease autoimmunity.

Did HLA-DR-DQ class II heterodimers contribute to population selection and risk for autoimmunity?

It is well known that HLA class II heterodimers bind specific peptides in its pocket that are related to infectious agents. The Scandinavian Peninsula was repopulated more than 9500 years ago after the last in-land ice receded (Fig. 1). The survival of these early inhabitants would be dependent on how their HLA-DR-DQ haplotypes were able to present viral and bacterial peptides to mount an immune response for survival. Heritability of susceptibility to several infectious diseases is an important aspect of the functional importance of HLA class II molecules (17). Hence, a lower risk of thoracic tuberculosis was found in carriers of DR3-DQ2.5 and DR7-DQ2.1. Carriers of DR8-DQ4 were at higher risk for thoracic tuberculosis (18). Tuberculosis is but one example of a well-documented infection that plagued people. DR3 as well as DR7 individuals are poor responders to measles. During thousands of years in the absence of antibiotics and vaccination, people survived on their own ability to mount an effective immune response to common virus infections, particularly those affecting children. Therefore, intuitively, the better the HLA class II presentation of viral peptides to T Cell Receptor (TCR) and subsequent T cell and antibody reactions to measles, rubella, rubeola, mumps etc., the greater the chance for survival. In addition, mothers with high titer antibodies to virus infections were more likely to protect their offspring from deadly viral infections during the first year of life, something that may have changed after the second World War (19). It may be speculated that the high haplotype frequencies of DR3-DQ2, DR4-DQ8, and DQ6.2 in the Finnish and Swedish populations were selected during evolution because they represented survival to common infectious diseases that plagued the early inhabitants of Scandinavia. HLA-DR-DQ types with poor protection, i.e., responding with low antibody titers, would die out. The HLA-DR3-DQ2, DR4-DQ8 haplotypes common in the Finnish and Swedish may be because of selection by survival to particular infectious agents. This needs to be taken into account when analyzing the possible role of HLA-DR-DQ in immune responses to infectious agents that may induce type 1 diabetes, celiac disease, or narcolepsy.

The study of HLA in type 1 diabetes and celiac disease may in part be complicated by the fact that the majority of studies have only included subjects with the disease. However, recent investigations in the DIPP (20) and TEDDY (21) studies indicate that the association with disease may be secondary to the association with the autoantibodies that precede the clinical onset. Hence, DR3-DQ2 is associated with the risk for autoantibodies against GAD65 (GADA) and DR4-DQ8 with autoantibodies against insulin (IAA). Such associations between HLA and autoantibodies have previously also been observed at the time of clinical diagnosis (22, 23), but the increased risk of HLA-DR-DQ for islet autoantibodies as well as tissue transglutaminase autoantibodies (tTGA) (24) suggest that the importance of these class II molecules may rest with the ability to present autoantigen peptides to CD4+ T cells in order to induce an autoimmune response. The importance of the TEDDY-pilot studies DIPP (25), BABYDIAB (26), DAISY (27), and DiPiS (28) and the TEDDY study itself (29) underscore the importance to follow children at increased genetic risk from birth to disclose the time of seroconversion. As it is believed that autoantigen peptides are not presented spontaneously to induce autoimmunity, it is speculated that a trigger such as an environmental agent is needed. The etiological factor may be an environmental agent, but the contribution of other phenomena, such as beta-cell apoptosis or autophagy, cannot be excluded. So far, there is no evidence in type 1 diabetes that

Fig. 1. Migration into the Scandinavia Peninsula after the in-land receded some 9500 BC. Gatherers and hunters migrated into the peninsula, being selected for survival based on their HLA-DR-DQ in response to common infectious agents affecting humans including childhood infectious diseases. In both Finland and Sweden, the most common haplotypes are HLA DR3-DQ2, DR4-DQ8, and DR15-DQ6.2, which may be related to survival to environmental factors such as childhood virus diseases as well as tuberculosis.

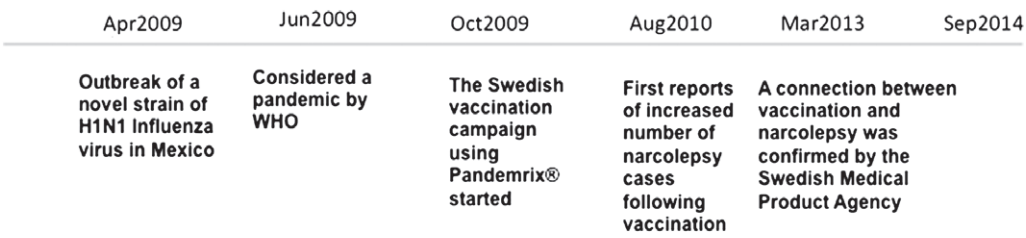

Fig. 2. Time line of the H1N1 pandemic in April 2009, the Swedish mass vaccination campaign (also taking place in Finland) with Pandemrix®, and the detection of patients with narcolepsy.

iatrogenic effects would explain the 2–5% increase in incidence rate that has been reported in Finland shortly after the second World War and in Sweden through registries established in the early 1980s (30, 31). A number of environmental exposures such as dietary factors, enteroviruses, and toxins as well as psychosocial factors have been considered throughout the years (32). In celiac disease, Sweden experienced a marked increased incidence rate in the 1980s after the recommendations on gluten intake were changed (33). The epidemic was thought in part to be because of a change among three factors within the area of infant feeding: amount of gluten given, age at introduction of gluten, and whether breastfeeding was ongoing or not when gluten was introduced. A more recent iatrogenic disease was the sudden increase in young children with narcolepsy following the vaccination in Finland and Sweden with Pandemrix, a H1N1 (swine flu) vaccine.

What did vaccine-induced narcolepsy tell us about organ-specific autoimmunity?

In October 2009–March 2010, a mass vaccination campaign with Pandemrix took place in Sweden (Fig. 2). The mass vaccination may mimic HLA-dependent association with an infectious disease. It is noted that it may not only be the H1N1 proteins in the vaccine but also the combination, for example, with the adjuvant shark liver oil (squalene) that was used. The outcome of the mass vaccination was a marked increase in young patients with narcolepsy both in Finland (14) and Sweden (15, 34). The two countries used the same vaccine, and all investigators found that the patients with Pandemrix-induced narcolepsy were positive for HLA-DQB1*06:02. Heterozygocity was sufficient (16). Hence, it is a rare occasion to study the induction of an autoimmune disease directed against the hypocretin neurons in the hypothalamus that was induced by a vaccine of known composition, inducing disease only in HLA DQB1*06:02 subjects. Although the autoantigen is yet to be identified, it was found that A/H1N1 antibody levels were higher among the <13 years old compared with patients who were older than 30 years (16). Being HLA-DQB1*06:02 positive was associated with higher A/H1N1 antibody levels in both patients and controls (16). Further studies are needed, particularly to clearly identify the autoantigen involved as well as which virus protein (hemagglutinin, nucleoprotein, or NS1) might have been the primary trigger and the possible importance of a necessary second hit such as the adjuvant to trigger the DQB1*06:02-dependent autoimmunity.

What have we learned from 20 years with DIPP?

The DIPP study initiated in 1994 used novel technologies for rapid and reliable HLA typing of cord blood samples (35, 36). As the frequency of newly diagnosed children was primarily in families without type 1 diabetes, the newborn screening was focused on all children born whether or not there was a family member with type 1 diabetes (25). The two-step screening approach enhanced the sensitivity for type 1 diabetes risk in Finnish newborns up to 85.4%. In the children of the general population, 24% were identified for prospective follow-up, and it was expected that 2.6% would be diagnosed with type 1 diabetes before the age of 15 (25). The proportion of specific high-risk DR3-DQ2/DR4-DQ8; DR4-DQ8/DR4-DQ8, DR3-DQ2/DR2-DQ2, and DR8-DQ4/DR4-DQ8 genotypes in Finland represents about 5.5% of all newborn, and these genotypes would be found in 45–50% of the children who are diagnosed with type 1 diabetes by 15 years of age (37). The specific aim in DIPP was to test whether intranasally administered insulin would reduce progression to clinical onset (38). However, the data showed that in children with HLA-conferred susceptibility to diabetes, administration of nasal insulin, started soon after detection of autoantibodies, could not prevent or delay type 1 diabetes (39). The DIPP study has, on the other hand, generated a wealth of information on the pathogenesis, i.e., the progression of disease to clinical diagnosis once one or several autoantibodies have formed. This data include studies on the loss of beta-cell function in islet autoantibody-positive infants (40, 41). More interestingly for the present discussion was the analysis of which autoantibody was first detected during follow-up from 3 months of age and onwards (20). Three

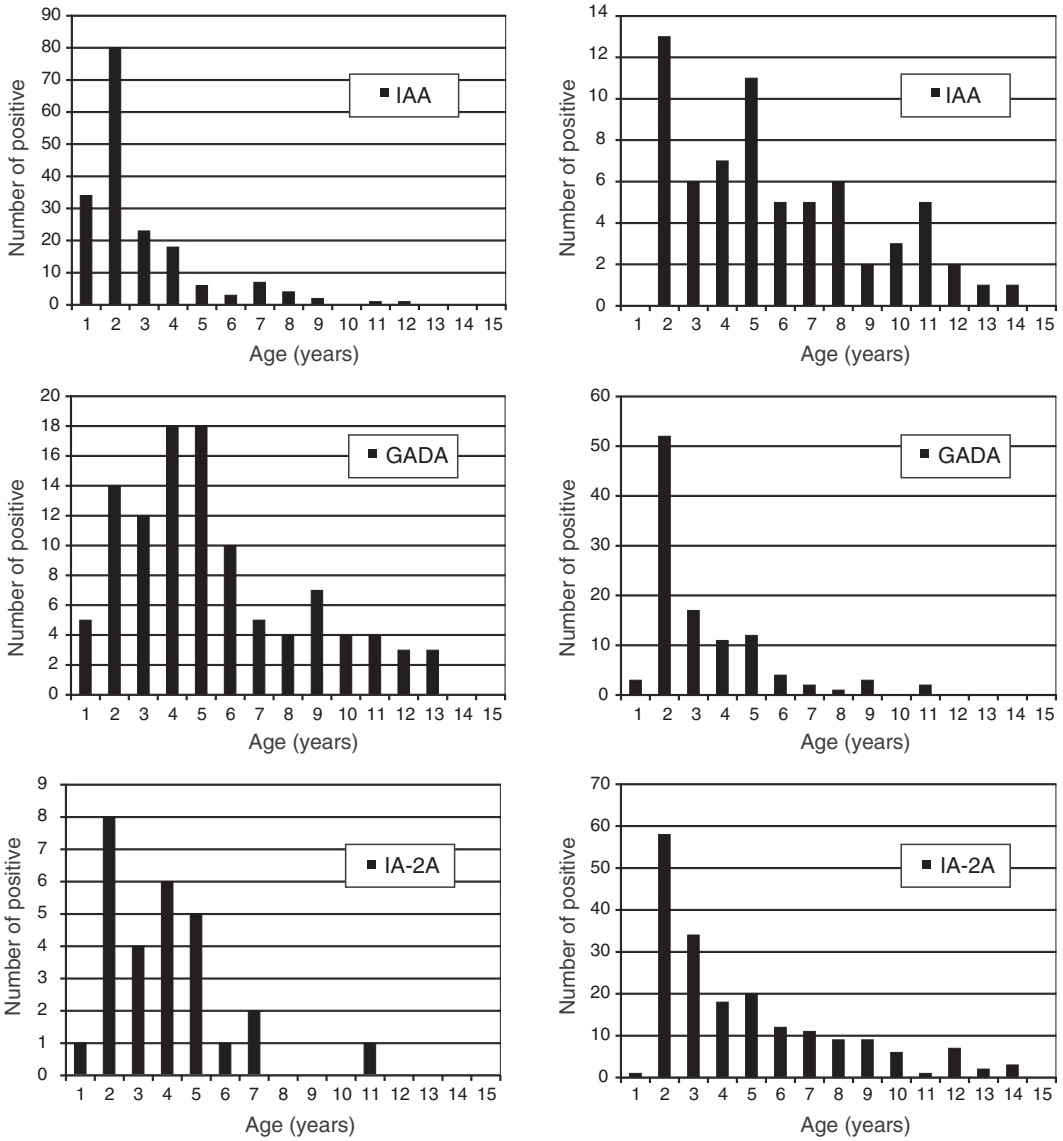

Fig. 3. Appearance of the first and second islet autoantibody in human leukocyte antigen (HLA)-high-risk children followed quarterly from 3 months of age. Note that the *y*-axis of the different panels is widely different. The patterns indicate that insulin antibodies (IAA) is the first autoantibody at young age, that GADA is appearing later, and that IA-2 (IA-2A) is less often seen as the first islet autoantibody. GAD65 autoantibodies (GADA) and IA-2A are commonly seen as the second autoantibody. Reproduced with permission from ref. (20).

patterns emerged (Fig. 3). The first was the early appearance of insulin antibodies (IAA) during the first years of life. The second was that GADA came later as the fist islet autoantibody. The third was that IA-2 (IA-2A) was less often the first islet autoantibody. These data clearly indicate that there may be two different triggers of islet autoimmunity. One pattern was the autoimmune response to insulin resulting in IAA during the first years of life. The second was GADA appearing later in life but with a tendency to sustain. These data suggest that much research on type 1 diabetes has followed the street light effect (42). Research has been looking were the light is, i.e., at the time of diagnosis, and thereby missing (i) the etiology prior to seroconversion and (ii) the pathogenesis once islet autoimmunity has been established through the first persistent autoantibody, to be followed by a second, third, and fourth autoantibody heralding the clinical onset of disease (42).

What have we learned from TEDDY so far?

While the DIPP study was launched in 1994, the TEDDY study began screening nearly 440 000 newborns in 2004 (37, 43). The newborn screening was successful, including more than 8600 children 3–4.5 months at their first visit. The strength of the study is that the inclusion criteria only encompassed four genotypes (additional genotypes were included in case the child had a first degree relative with the

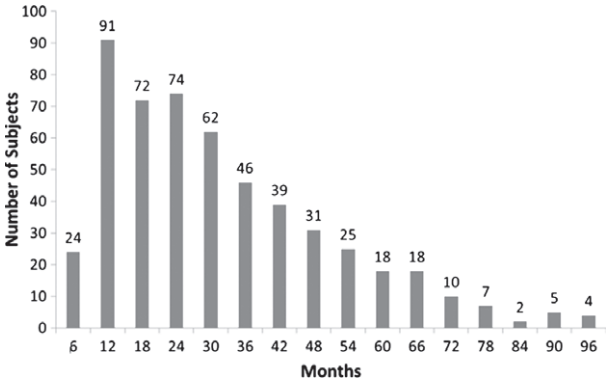

Fig. 4. Time of seroconversion in the TEDDY study. Number of subjects is shown in relation to the age of the child in months. Data are obtained from ref. (21).

disease, only 11% in TEDDY): DR3-DQ2/DR4-DQ8; DR4-DQ8/DR4-DQ8, DR3-DQ2/DR2-DQ2, and DR8-DQ4/DR4-DQ8. At the time of the DIPP's 20th anniversary, the TEDDY children were 4–10 years of age, and major observations have already been made (Figs. 4 and 5). Autoantibodies against insulin (IAA), GADA and IA-2A are measured in two different reference laboratories, and seroconversion is defined by persistence at two different visits and a positive score in both laboratories (44). The data in Fig 4 illustrate that the time of seroconversion in this group of children from the USA, Sweden, Finland, and Germany already occurred to the greatest extent at 1–3 years of age (21). However, a major finding was that the incidence rate of the first islet autoantibody differed by age and HLA genotype (21). Hence, the trigger for IAA occurred early in life and primarily in children positive for DR4-DQ8. It is noted that the incidence rate for IAA tapers off with increasing age as if children acquire protection against a hypothetical trigger with increasing age. GADA as the first islet autoantibody follows a different pattern. This islet autoantibody appeared later. There is no decline in GADA incidence rate; rather, it seems to remain stable through the years of follow-up. GADA as the first islet autoantibody seems to primarily affect children with the DR3-DQ2 haplotype.

The major lessons to be learned from TEDDY are analyses of samples and questionnaire information obtained prior to seroconversion. The observation that IAA only and GADA only may be related to two different HLA risk haplotypes will make it necessary to reconsider the original hypothesis of one environmental trigger resulting in islet autoimmunity. Instead, two different etiological phenomena will have to be entertained, one for HLA DR3-DQ8 and another for HLA-DR4-DQ8. Interestingly enough, when parents were asked if they had given their child probiotics, it turned out that probiotic supplementation at the age of 0–27 days was associated with a decreased risk of islet autoimmunity when compared with probiotic supplementation after 27 days or no probiotic supplementation (45). Children with the DR3/4-DQ2/8 genotype had the strongest response to early probiotics (45). These data support the notion that a number of environmental determinants may affect the risk for a first islet autoantibody.

What have we learned from studying celiac disease in TEDDY?

Using the HLA DR3-DQ2/DR3-DQ2 and the DR3-DQ2/DR4-DQ8 genotypes as inclusion criteria in TEDDY in children from the general population opened the study for the two highest genetic risk groups of celiac disease. In TEDDY, autoantibodies to tTGA were used to screen all TEDDY children at 4 years of age. The fact that more TEDDY children had tTGA than islet autoantibodies and more children in fact were diagnosed with celiac disease allowed the investigators to analyze tTGA in all samples obtained prior to the fourth year sample in order to identify the visit where seroconversion was found to have taken place (46). The data demonstrated that TEDDY children with the HLA DR3-DQ2, especially homozygotes, were found to be at high risk for tTGA and celiac disease. There were 2% in the DQ4/8 genotype, 9% in DQ8/8, and 11% in DQ2/8 but 26% in the DQ2/2 children. The higher risk in Sweden than in other countries emphasizes the importance of identifying environmental factors associated with celiac disease (46). Such environmental factors may include, but are not limited to, gluten and may include virus infections as well. For example, the amount of gluten consumed until 2 years of age increased the risk of celiac disease at least twofold in TEDDY DR3-DQ2 children (47). The timing of the first introduction of gluten was not a risk factor for tTGA (48). The TEDDY data (46) suggest that a total of 300 TEDDY children are on a gluten-free diet. This fact will allow the TEDDY investigators to ask if the risk for a first islet autoantibody is affected. There are about 20 children with islet autoantibodies also on a gluten-free diet. What will be their risk for type 1 diabetes? Hence, studying tTGA and celiac disease in TEDDY is a unique opportunity to disclose which environmental factors are involved in triggering celiac disease autoimmunity. It will also be possible to determine the natural history of progression from tTGA induction until the diagnosis of celiac disease.

Conclusion

The appearance of islet autoantibodies as biomarkers of type 1 diabetes and tTGA of celiac disease has made it possible to restructure the research design of the disease etiology and pathogenesis. The DIPP and the TEDDY studies have identified a large number of

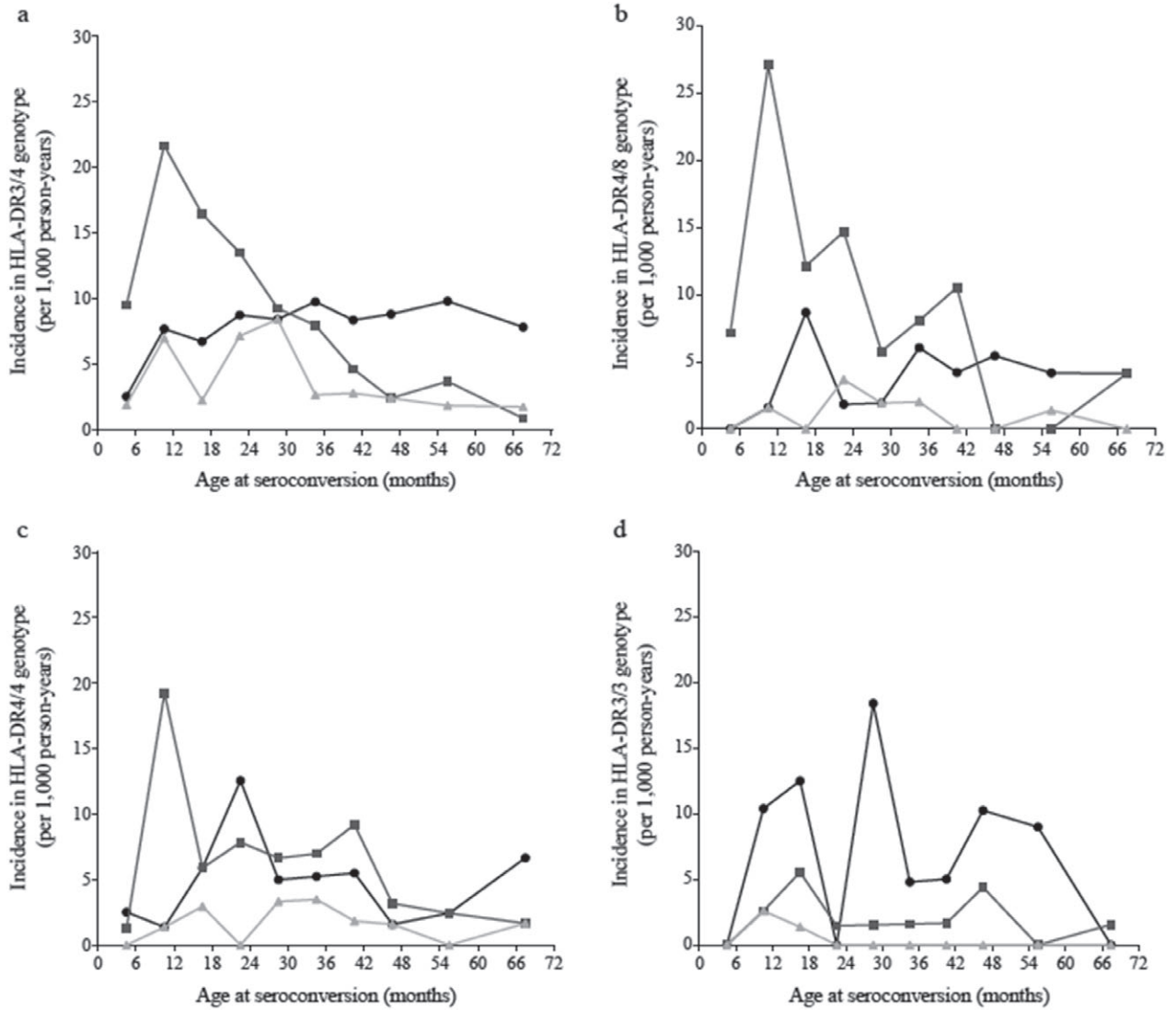

Fig. 5. Incidence of insulin autoantibodies (IAA) (filled squares) and GAD65 autoantibodies (GADA) (filled circles) in TEDDY children of four different genotypes as indicated. Note that children with DR4-DQ8 are predominantly IAA and children with DR3-DQ2 predominantly GADA positive. IAA appeared early and GADA later. Children with both IAA and GADA as the first autoantibody are also shown (filled triangles). Reproduced with permission from ref. (21).

children with the DR4-DQ8 haplotypes who tended to develop IAA during the first 2–3 years of life. In children with the DR3-DQ2 haplotype, GADA was the first autoantibody, but they tended to appear later and showed indications of a stable incidence rate while that of IAA decreased with increasing age. tTGA autoantibodies also developed earlier, and the overlap between the markers and their associated diseases was limited. Both DIPP and TEDDY have large sample repositories, and it should prove useful to analyze samples prior to seroconversion to detect environmental factors that are able to trigger either islet or celiac disease autoimmunity. It is a testable hypothesis that a trigger may not be alone. A combination of two triggers, such as a large amount of gluten in conjunction with a virus infection, may be the trigger of an immune response to tTGA.

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