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Прогнозирование сахарного диабета 1 типа в семьях больных (проспективное 16-летнее наблюдение). Акцент на будущее

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В течение 40 лет, с тех пор как была доказана аутоиммунная природа заболевания, продолжаются исследования по совершенствованию методов прогнозирования и разработки эффективных и безопасных методов профилактики сахарного диабета 1 типа (СД1).

Цель. *Проспективное наблюдение и прогнозирование развития СД1 в семьях больных.*

Материалы и методы. *В 224 конкордантных/дискордантных семьях больных СД1 у здоровых сибсов проведено исследование предрасполагающих и протективных гаплотипов (HLA-DRB1, DQ-генов) в комбинации с иммунологическими маркерами (ICA, IAA, GADA).*

Результаты. *При анализе распространенности HLA-генотипов у пациентов с СД1 выявлено, что гаплотипы высокого риска в составе генотипа DQ2 и/или DQ8 в сочетании с другими встречались в 78% случаев; из них генотипы DQ2/DQ8, DQ2/DQ2, DQ8/DQ8 – в 35%; DQ2/X* и DQ8/X* – в 43%; а генотип низкого риска X*/X* – в 22% случаях. При анализе встречаемости у больных СД1 HLA-генотипов отмечено, что генотипы высокого риска (DQ2/DQ8) достоверно чаще встречались у детей, заболевших до 5-летнего возраста, – 33%, по сравнению с детьми, у которых СД1 манифестировал старше 10 лет – 23% (p=0,05). За 16-летний период проспективного наблюдения ядерных семей манифестация заболевания произошла у 8,4% сибсов. При анализе частоты встречаемости аутоантител (а/т) получено, что а/т достоверно чаще определялись у заболевших до манифестации заболевания, чем у больных СД1 в начальном периоде и у здоровых сибсов (сибсы, не развившие заболевание в течение всего периода наблюдения): 90%, 48,6% и 31% соответственно.*

Заключение. *За 16-летний период проспективного наблюдения за ядерными семьями манифестация заболевания произошла у 8,4% родственников 1-й степени родства (брат/или сестра больного СД1), что превышает рассчитанный эмпирически 20 лет назад риск для сибсов – 6,4%. 90% заболевших сибсов являлись носителями гаплотипов высокого риска (DQ2 и/или DQ8) и иммунологических нарушений (персистенция положительных аутоантител), что подтверждает их высокую прогностическую значимость.*

Ключевые слова: *сахарный диабет 1 типа; генетическая предрасположенность; HLA-гаплотипы; генотипы; аутоантитела к β-клеткам*

The Prediction of Type 1 Diabetes in discordant and concordant families: 16 years of follow-up. Focus on the future

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For 40 years, research continues to improve the forecasting methods and the development of effective and safe methods of preventing type 1 diabetes mellitus (T1DM).

Aim. *Prediction of the early preclinical stage of T1DM.*

Materials and methods. *We studied the predisposing and protective haplotypes (HLA-DRB1, gene DQ) together with immunological markers (ICA, GADA, IAA) in 224 discordant/concordant families.*

Results. *At the Endocrinology Research Centre, population and family risks of the development of T1DM in Russia were calculated on the basis of population genetic approaches. The analysis of the prevalence of HLA genotypes among T1DM patients revealed that the high-risk haplotypes in the structure of genotype(s) DQ2 and/or DQ8 in combination with the others were 78%: of these genotypes DQ2/DQ8, DQ2/DQ2, and DQ8/DQ8 accounted for 35%; DQ2/X* and DQ8/X* accounted for 43%; and the low-risk genotype X*/X* accounted for 22%. The genotype X/X consisted of weaker predisposing haplotypes that were specific to the Russian population in combination with neutral haplotypes or those consisting of neutral haplotypes only. The analysis of patients with T1DM genotypes revealed that high-risk genotypes (DQ2/DQ8) were more common in ill children up to the age of 5 (33% of cases) than in T1DM children over 10 years (23%) (p=0.05). Conversely, the low-risk genotypes were significantly less likely to be found in children with*

manifestations of diabetes up to 5 years than in sick people over 10 years [5% and 13%, respectively (p <0.05)]. This is consistent with hereditary load of diabetes manifestations in young children and with the earlier data. The 16-year prospective surveillance showed that the manifestation of the disease occurred in 8.4% of siblings. The analysis of the frequency of autoantibodies revealed that autoantibodies were identified the most reliably prior to the manifestation of the disease compared with T1DM patients in the initial period and healthy siblings (eldest siblings without the disease during the whole monitoring period): 90%, 48.6% and 31%, respectively, p <0.05.

Conclusion. *The 16-year prospective surveillance in families with T1DM showed that the frequency of recurring diabetes cases was 8.4%, which exceeds the siblings' rate of risk of 6.4% that had been empirically calculated 20 years ago. This may be due to an increase in the incidence of T1D in the population and different methodological approaches (one-time screening versus long-term monitoring).*

Keywords: *type 1 diabetes; genetic susceptibility; HLA-haplotypes; genotypes; phenotypes; autoantibodies to beta-cells*

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The incidence of type 1 diabetes mellitus (T1DM) is increasing in many countries throughout the world. Over the past 40 years, the incidence has increased by 3.5% per year worldwide and by 2.8% per year in Russia. According to the State Registry of Patients with Diabetes Mellitus, as of 1 January 2013, there were 19,548 children and 9,942 adolescents diagnosed with T1DM in Russia. In 2013, the International Diabetes Federation (IDF) reported an incidence of 12.1 cases per 100,000 children [1, 2]. The highest increase in the incidence of T1DM has occurred in younger children. During 2001–2011, the incidence increased by 8.15% and 10.3% in children aged 0–4 years and 5–9 years, respectively, compared with 4.7% in adolescents (Shiryaeva T.Yu., 2013). Furthermore, the incidence of T1DM in young children in Europe is predicted to increase 2-fold by 2020 [3, 4]. Therefore, research into methods to predict and prevent T1DM is particularly important and relevant.

Over recent years, new research has resulted in changes to George Eisenbarth's widely accepted theory on the pathogenesis of T1DM (Fig. 1). Approximately 50–60% of T1DM cases are associated with polymorphisms of HLA class 2 genes and the other 40% are associated with polymorphisms of other genes, including insulin, CTLA4 and PTPN22, which perform different roles in the coordination of the immune response. Genetic factors are involved in the development of T1DM, not only during autoimmune induction but also throughout the progression of the disease. Disease development is influenced by both T1DM-predisposing genes and T1DM-protecting genes. In addition, the quantitative and qualitative impact of a number of environmental factors, present from birth to disease onset, is also important in the development and progression of T1DM. The destruction of β-cells varies over time and depends on the presence of insulitis and the ability of β-cells to regenerate [5].

The increasing incidence of T1DM observed in recent decades may be a result of new and/or increasingly potent negative environmental factors and a possible time-dependent change in the contribution of genetic factors to the development of T1DM.

Aim

The aim of this study was to conduct prospective surveillance of families of T1DM patients and to predict the development of T1DM in siblings.

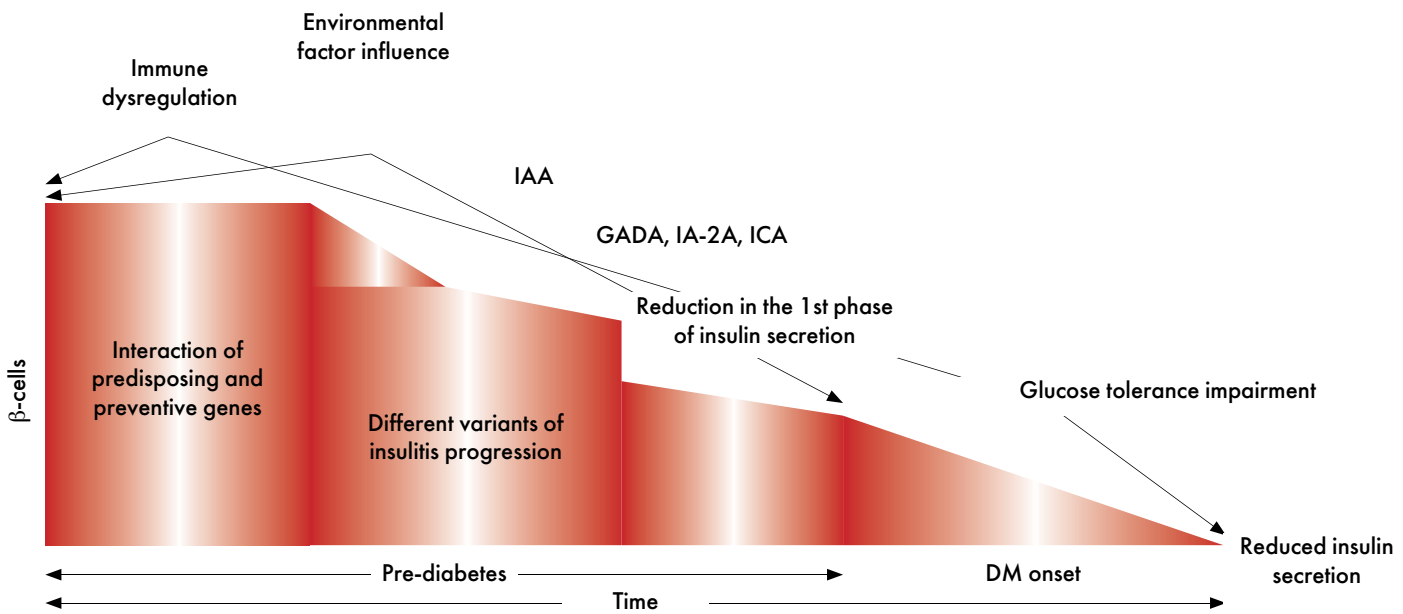

Fig. 1. A model of the T1DM pathogenesis.

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Materials and methods

Healthy siblings from 224 concordant/discordant families of T1DM patients (brother/sister of the T1DM patient; N = 223; mean age: 10.9 ± 5.9 years) were studied for predisposing and protective haplotypes (HLA-DRB1 and DQ genes) in combination with immunological markers [islet cell cytoplasmic autoantibodies (ICA), insulin autoantibodies (IAA) and glutamic acid decarboxylase autoantibodies (GADA)]. In addition, molecular genetic studies (study of the HLA-DRB1 and DQ genes) were performed in a random sample of 649 T1DM patients (mean age: 7.8 ± 6.2 years) living in the Russian Federation. The control group consisted of 300 healthy individuals living in Moscow or the Moscow region, with no past history of T1DM or other autoimmune diseases. All participants were ethnically homogeneous Russians (according to passport data) who were not relatives.

All participants provided informed consent prior to taking part in the research program.

HLA genotyping was performed in the Laboratory of Immunology and Genetics (headed by O.N. Ivanova, MD) using allele-specific polymerase chain reaction (DNA Technology LLC, Russia). Enzyme-linked immunoassay with Isletest-ICA, -GADA and -IAA kits (Biomerica, Germany) was used to investigate immunological markers.

Data processing and statistical analysis were performed using STATISTICA (version 6, StatSoft, www.statsoft.com) and Microsoft Office Excel 2003 software. Statistical analysis of the frequency and distribution of genotypes was performed using contingency tables and chi-square test (χ^2) with Yates' correction for continuity to assess the significance of differences (p) in the frequency distribution. Differences were considered significant at $p < 0.05$. The odds ratio (OR) was calculated using the method described by Bland (2000).

Results and discussion

Here we report the findings of a long-term (16-year) prospective surveillance study on the development of T1DM in concordant/discondarnt families with ≥1 T1DM patients, conducted at the Paediatric Endocrinology Department of the Endocrinology Research Centre in Moscow, Russia. This study used population genetic approaches to calculate population and family risks of developing T1DM in Russia [6].

The population risk of developing T1DM was 0.2% (Fig. 2). At a family level, the more T1DM cases present in a family, the higher was the risk of other family members developing T1DM. In families affected by T1DM, on average, the risk of another family member developing T1DM was 5%. In families with 2 T1DM children, the risk of a sibling developing T1DM was 9.5%. If both parents had T1DM, the risk of a child developing T1DM was increased to 34%. The family risk of developing T1DM also depended on the age of disease onset in the proband (the affected individual in the family). The earlier a child developed T1DM, the higher was the risk for that child's siblings. For example, if disease onset in a proband occurred before 20 years of age, the risk of developing T1DM for a sibling was 6.4%. If the onset occurred after 20 years of age, the risk for a sibling was 1.2%. These results are consistent with the findings of similar studies in other countries [7].

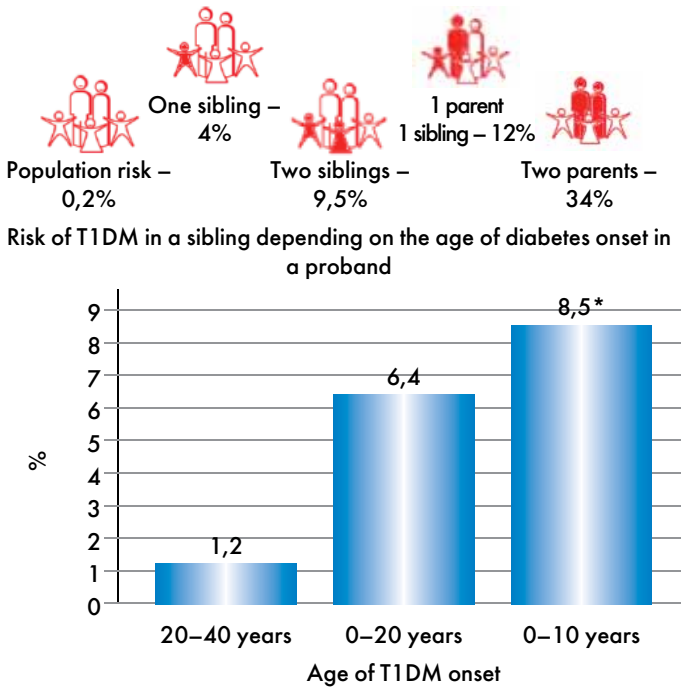

Fig. 2. Risk of T1DM in the Russian Federation.

The first step in predicting the development of T1DM is to determine the genetic risk for the proband. Up to 60% of the genetic predisposition to T1DM is determined by HLA class 2 genes [8, 9]. Therefore, it is important to identify HLA haplotypes and genotypes. In T1DM children and adolescents in the Russian population, 5 predisposing and 3 protective haplotypes were identified.

- According to the risk for T1DM, of the predisposing haplotypes, the DQ8 - DRB1*4-DQA1*301-DQB1*302 haplotype, typical of northern Europe, was placed first (OR = 4.7).
- The DRB1*4-DQA1*301-DQB1*304 haplotype, specific for the Russian population, was placed second (OR = 4).
- The DQ2 - DRB1*17-DQA1*501-DQB1*201 haplotype, typical of southern Europe, was placed third (OR = 2.7).
- The DRB1*16-DQA1*102-DQB1*502/4 haplotype, unique to the Russian population, was placed fourth (OR = 2.4).
- The DRB1*-DQA1*101-DQB1*501 haplotype, typical of the Russian population and Europe, was placed fifth (OR = 1.9).

Protective haplotypes in the Russian population were identical to those of European populations [10]:

- DRB1*15-DQA1*102-DQB1*602/8 (OR = 0.16);
- DRB1*11-DQA1*501-DQB1*301 (OR = 0.14);
- DRB1*13-DQA1*103-DQB1*602/8 (OR = 0.08).

Analysis of the prevalence of HLA genotypes revealed that 78% of T1DM patients had high-risk haplotypes in the DQ2 and/or DQ8 genotypes in combination with other genotypes (DQ2/DQ8, DQ2/DQ2 and DQ8/DQ8: 35%; DQ2/X* and DQ8/X*: 43%). The low-risk genotype X*/X* was present in 22% patients. The X/X genotype was associated with weaker predisposing haplotypes specific for the Russian population in combination with neutral haplotypes or with only neutral haplotypes.

Data on the frequency of predisposing and protective HLA haplotypes/genotypes in T1DM patients should be taken into account when assessing risk in their healthy siblings. The highest genetic risk of developing T1DM is associated with the heterozygous DQA1*0501-DQB1*0201\DQA1*0301-DQB1*0302 - DQ2/DQ8 genotype. Medium or moderate risk is determined by a combination of the high-risk haplotype with other haplotypes (DQ2; DQ8). Low genetic risk (X/X) is determined by the absence of high-risk haplotypes and the presence of protective and neutral haplotypes or low-risk haplotypes [6, 10].

Based on an individual assessment of the genetic risk in the families with T1DM children, the following disease-risk categories were determined:

- High-risk group: 15% (N = 33, 11.5 ± 6.0 years)
- Medium-risk group: 50% (N = 114, 11.6 ± 5.6 years)
- Low-risk group: 35% (N = 76, 12.5 ± 5.9 years)

During the 16-year prospective surveillance of concordant/discondarnt families, the onset of T1DM occurred in 8.4% of first-degree relatives (brother or sister) of the T1DM patient, which is higher than the risk for siblings (6.4%) calculated empirically 20 years ago. As shown in Fig. 3, over the years of the study, the number of affected individuals in the high-risk group remained high and increased (13%, 19% and 21%). However, the numbers of affected individuals in the medium- and low-risk groups remained virtually unchanged (7%, 9% and 9% and 3.2%, 2.6% and 2.6%, respectively). Analysis of siblings who developed T1DM revealed that 90% were carriers of high-risk haplotypes. The remaining 10% of the affected siblings had weakly predisposing or neutral haplotypes.

Analysis of cases of T1DM developing in the siblings of T1DM patients revealed a positive correlation between the age of T1DM onset in a sibling and in a proband ($R = 0.33$, $p < 0.05$) and a negative correlation between the age of T1DM onset in a sibling and the genetic risk (high, medium or low; $R = -0.34$, $p < 0.05$).

A previous study found that in young children, the presence of multiple autoantibodies in combination with highly predisposing HLA haplotypes and genotypes was associated with a very high risk of developing T1DM, reaching 90% during a 10-year observation period [5]. In the present study, autoantibodies were significantly more likely to be detected in affected siblings before the onset of disease compared with T1DM patients in the initial period and healthy siblings who remained disease-free throughout the observation period (90%, 48.6% and 31%, respectively; $p < 0.05$). A similar pattern was observed for several autoantibodies in affected siblings before disease onset and healthy siblings compared with patients in the initial period of T1DM in whom insulitis had settled over time (the mean disease duration at the time of blood sampling for antibodies was 3 months); autoantibodies were detected in 74%, 14.6% and 8.1%, respectively ($p < 0.05$). These findings identified an autoimmune process that is most active in affected siblings during the preclinical stage before the onset of T1DM and confirmed the importance of immunological markers in predicting T1DM (Fig. 4).

In light of the increasing onset of T1DM in younger children and the fact that the genotype of 10% of the affected siblings lacks highly predisposing HLA haplotypes, the frequency of markers of different genetic risk categories was analysed by patient age and year of diagnosis. As shown in Fig. 5, the high-risk genotypes (DQ2/DQ8) were significantly more prevalent in children who developed T1DM before the age of 5 years (33%) compared with children who developed T1DM after the age of 10 years (23%; $p = 0.05$). Conversely, the low-risk genotypes were significantly less prevalent in children who developed T1DM before the age of 5 years compared with those who were affected after the age of 10 years (5% and 13%, respectively; $p < 0.05$). These findings were consistent with a large hereditary load of T1DM in young children and were consistent with previous data.

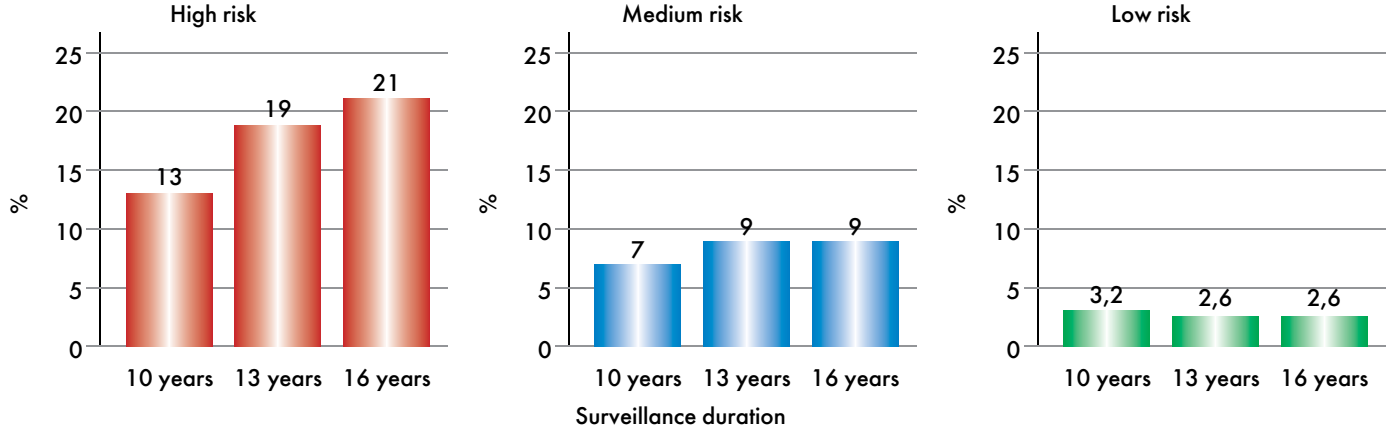

Fig. 3. Dynamics of the DM frequency in siblings from different risk groups depending on the surveillance duration (T1DM onset in siblings for the 16-year period is 8.4%).

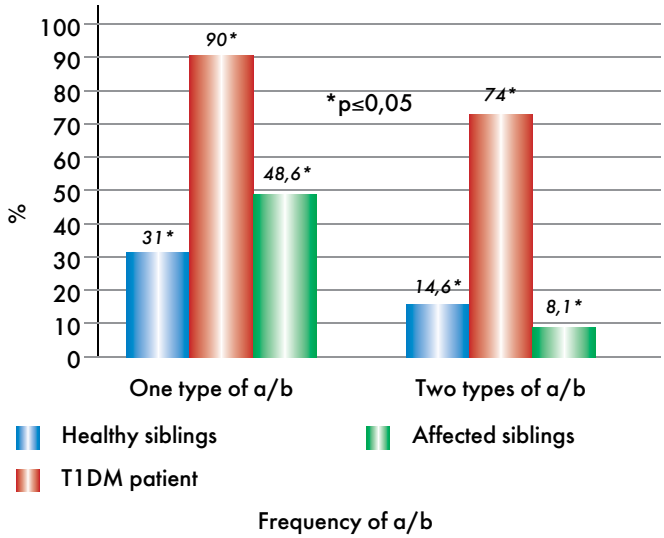

Fig. 4. Autoimmunity in affected siblings compared to healthy siblings.

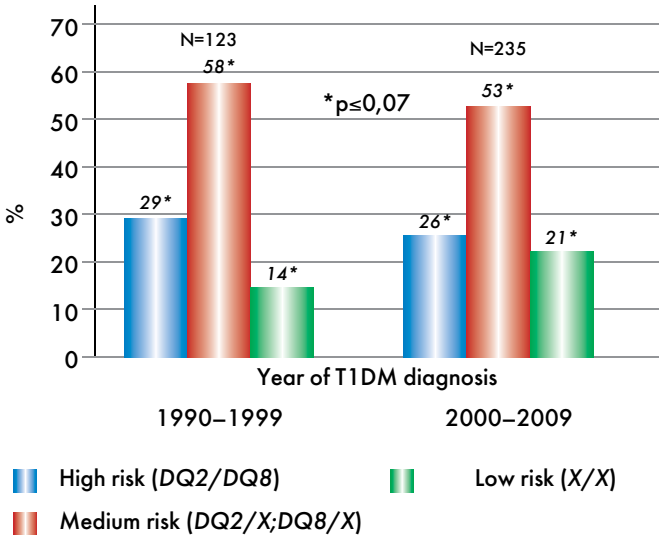

Fig. 6. Frequency of HLA genotypes in T1DM patients depending on the year of the diagnosis.

Results of the analysis of the frequency of HLA genotypes in T1DM patients for the past 20 years, depending on the year of diagnosis, are shown in Fig. 6. In patients who developed T1DM during 2000–2009, there was a trend towards a lower prevalence of high-risk (DQ2/DQ8) and medium-risk (DQ2/X and DQ8/X) genotypes compared with patients who developed T1DM during 1990–1999 (high risk: 28.5% and 26%, respectively; medium risk: 57.7% and 53%, respectively; p >0.05).

Analysis of the low-risk X/X genotypes revealed a clear trend towards a higher prevalence of low-risk genotypes in patients diagnosed with T1DM in 2000–2009 compared with patients diagnosed in 1990–1999 (21.5% and 13.8%, respectively; p = 0.07).Similar studies have been conducted in Finland and Australia, using a larger sample of patients (Fig. 7). In patients who were diagnosed in 2000, the prevalence of high- and medium-risk genotypes

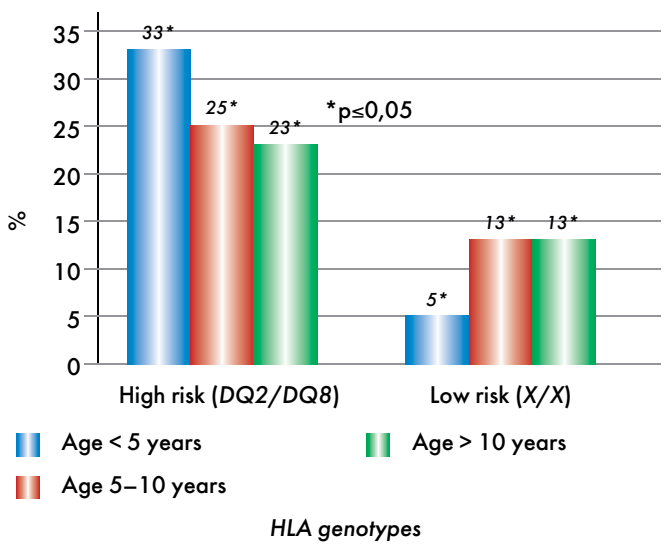

Fig. 5. Frequency of HLA genotypes in T1DM patients depending on the age of disease onset in a T1DM patient.

was lower than that in patients who were diagnosed before 1970 (18% and 25%, respectively; p = 0.07). Conversely, the low-risk genotypes, particularly the protective genotypes, were more prevalent in T1DM patients diagnosed in 2000 than in patients diagnosed before 1970 (13% and 6%, respectively; p = 0.0004) [11, 12].

The increase in the number of individuals with low-risk genotypes developing T1DM over the last 20 years may be indirect evidence of an increasing role of the environment in the pathogenesis of T1DM. Another possible explanation for the development of T1DM in the 22% of patients without high-risk haplotypes is the involvement of other genetic systems currently being investigated. Insulin resistance in the increasing number of obese/overweight children may be a trigger for the development of islet autoimmunity under the influence of environmental factors in T1DM patients with low genetic risk. The stability of the high-risk genotype (DQ2/DQ8) over many years suggests that it is resistant to environmental influences and is central to the autoimmune response in the development of T1DM.

Therefore, the development of T1DM can be represented as shown in Fig. 8. The younger the patient, the greater is the role of predisposing genotypes and multiple autoantibodies, which determine a more aggressive progression of the disease and a lower level of insulin secretion at onset [13].

Conclusion

- A 16-year prospective surveillance study of concordant/discondarnt families of T1DM patients found that the risk of siblings developing T1DM was 8.4%, which was higher than the risk empirically calculated 20 years ago (6.4%). This discrepancy may be the result of an increase in the incidence of T1DM in the population, although it may also reflect different methodological approaches (one-stage screening vs. prospective surveillance).

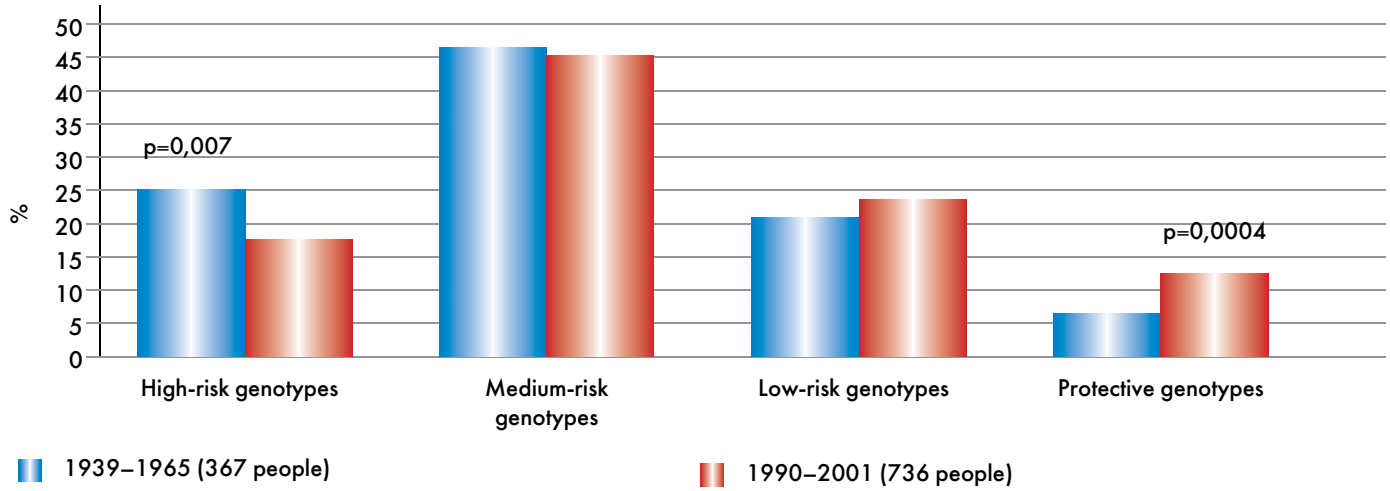

Fig. 7. Distribution of HLA DR-DQ genotypes in T1DM patients.

- Among the siblings who developed T1DM, 90% were carriers of the high-risk haplotypes DQ2 and/or DQ8 and were positive for autoantibodies, confirming the high predictive value of these markers.

Information on funding and conflicts of interest

The authors declare no apparent and potential conflicts of interest in connection with the publication of this article.

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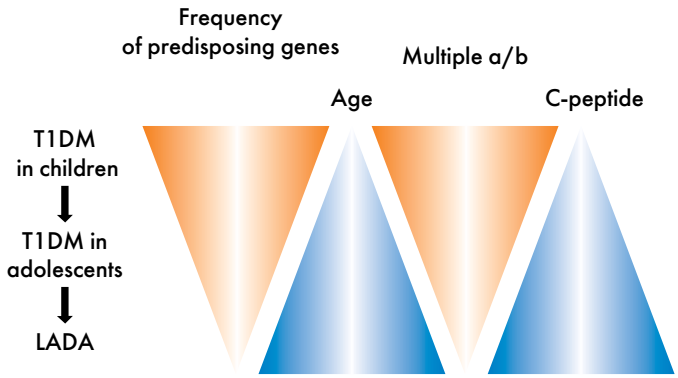

Рис. 8. Autoimmune diabetes.

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