Abstract

Genetic susceptibility to T1D depends mainly on differences in HLA-risk. However, non HLA T1D susceptibility genes have been identified: CTLA4, PTPN22, INSVNTR, IL-6, and PPARG. The aim of the present study was to investigate the impact of the Pro12Ala of PPARG and the -174G/C of the IL-6 promoter on disease progression in 275 children and adolescents with new onset type 1 diabetes.

At 1, 6, and 12 months after diagnosis, residual beta-cell function was estimated from meal stimulated C-peptide (Boost test), and serum was analyzed for: pro-insulin, ICA, GADA, IA-2A, and IA as well as IL-6, RANTES/CCL-5, MIP1-beta/CCL-4, and MIF. At 1, 3, 6, 9, and 12 months after diagnosis the HbA_{1c} % and the daily insulin dose were recorded. Genotyping for the -174G/C and the Pro12Ala variants was carried out by PCR-RFLP.

IL-6 -174G/C. Multiple regression analysis adjusted for sex, age, HLA-risk, and HCO3 at disease onset, showed no significant association between the -174G/C polymorphism and HbA_{1c} %, blood glucose, C-peptide or pro-insulin during follow-up. No association of the -174G/C with serum levels of RANTES/CCL-5, MIF, MIP1-beta/CCL-4 and IL-6 levels was observed.

Pro12Ala. Multiple regression analysis showed that the stimulated C-peptide was significantly lower in Ala carriers at 1 month after diagnosis ($p = 0.02$). Regression analyses of repeated measurements showed that Ala carriers throughout the study period received a daily insulin dose that was 0.08 IU/kg lower than in Pro/Pro carriers ($p = 0.04$). This difference in insulin dose was not secondary to a better glycemic control, as the HbA_{1c} was similar in both groups. We found no association of the Pro12Ala with auto-antibodies or cyto-/chemokine levels at any time.

We conclude that the association of the Pro12Ala with insulin dose in new onset T1D is secondary to its effect on peripheral insulin sensitivity/resistance. Therefore, this variant may also add to the T1D susceptibility already conferred by the HLA risk genes. In the present study the IL-6 -174G/C variant did not associate with disease progression.

Association of the Pro12Ala of PPARgamma2 Gene with Residual Beta-cell Function and Insulin Requirements but not Auto-Immune Activity in Children and Adolescents with New Onset Type 1 Diabetes. Results from the Hvidoere Study Group

Sven Poerksen[1],✉, Lotte Nielsen[1], Philip Hougaard[2], Christian Pfleger[3], Nanette Schloot[3], Reinhard Holl[4], Peter Swift[5], Steen Gammeltoft[1], Henrik Mortensen[1], Lars Hansen[6] and the Hvidoere Study Group on Childhood Diabetes. [1]Glostrup University Hospital, Denmark; [2]University of Southern Denmark, Denmark; [3]German Diabetes Research Institute, Germany; [4]The University of Ulm, Germany; [5]Children's Hospital, Leicester Royal Infirmary, UK and [6]Novo Nordisk A/S, Denmark. ✉ svepor01@glostruphosp.kbhamt.dk

www.hvidoeregroup.org

Background

The Pro12Ala polymorphism of PPARgamma is a type 2 diabetes susceptibility gene, associated with insulin resistance, and is found to be associated with type 1 diabetes too. As in type 2 diabetes, the more common proline allele seems to confer an increased risk for the development of diabetes.

The genetic variant -174G/C in the promoter of the IL-6 gene has been associated with serum levels of IL-6 in systemic-onset juvenile chronic arthritis and with increased risk of developing autoimmune type 1 diabetes in young females.

Aims and Hypotheses

Traditional genetic studies of type 1 diabetes focus on disease susceptibility. We investigated how genetic variation might influence the progression of the disease. Therefore, the aim of this study was to evaluate the impact of the genetic variants Pro12Ala of PPARgamma and -174G/C of the IL-6 gene on:

- residual beta-cell function
- glycemic control
- autoantibody status
- inflammatory status
- the daily insulin dose

We hypothesized that

1. The protective Ala allele gives rise to a lower inflammatory state and therefore is associated with lower auto-antibody titres and improved beta-cell function
2. The protective Ala allele is associated with higher insulin sensitivity that may show as improved HbA_{1c} and reduced insulin dose
3. The IL-6-174G/C variant has influence on serum IL-6 levels, possibly in an age- and sex-dependent manner
4. The suspected functional IL-6 variant has impact on disease presentation at onset and during disease progression in patients with newly diagnosed type 1 diabetes

Methods

Multicenter longitudinal investigation with 18 participating paediatric centres from 15 countries in Europe and Japan. 275 children up to 16 years with newly diagnosed type 1 diabetes were followed for 12 months.

The residual beta-cell function (stimulated C-peptide) was measured in response to a liquid meal (BOOST test) 1, 6, and 12 months after diagnosis. Figure 1 shows the study flow chart and investigated parameters at different time points. Cyto- and chemokine-analyses were performed by double-sandwich ELISA, HbA_{1c} by HPLC.

C-peptide and HbA_{1c} were analysed by multiple regression, adjusted for sex and age. The insulin dose was analysed by a compound symmetric repeated measurements model adjusted for sex and age. Non-parametric Wilcoxon two-sample test was used to evaluate differences in autoantibody titres, IL-6, MIP1-beta/CCL-4, RANTES/CCL-5 and MIF.

Basic information on genotype distribution is given in Table 1.

Figure 1. Flow chart for the remission phase study

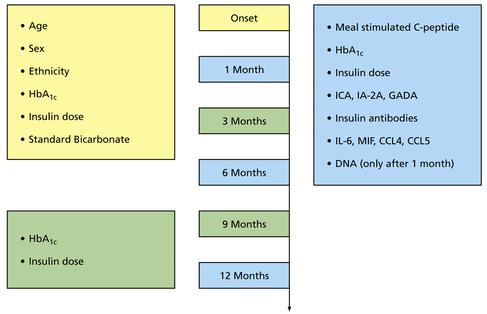


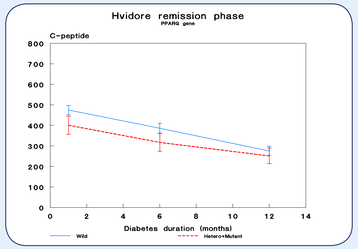
Table 1. Basic information on genotype distribution in PPARG and IL-6

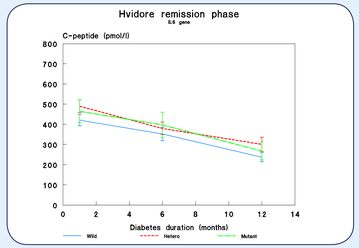
	PPARgamma	
	Pro12Pro	Ala carriers
Number	205	52
Age (yrs)	9.2	8.7
Sex (M/F)	104 / 101	27 / 25
Height (cm)	136	132
Weight (kg)	32	30

	IL-6-174		
	GG	GC	CC
Number	98	116	43
Age (yrs)	9.0	9.5	8.4
Sex (M/F)	49 / 49	60 / 56	17 / 26
Height (cm)	134	138	130
Weight (kg)	31	33	30

Results

Pro12Ala of PPARgamma | **IL-6-174G/C**

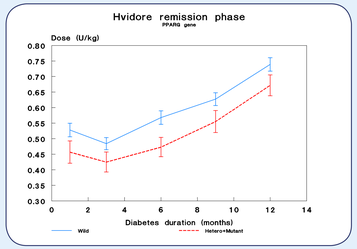


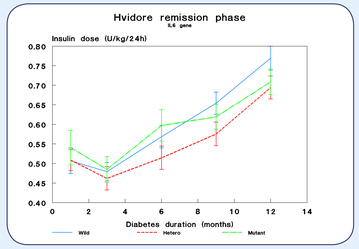


Beta-cell function

PPARgamma 12Ala carriers had significantly ($p = 0.02$) lower stimulated C-peptide values than Pro12Pro carriers one month after diagnosis, but not after 6 and 12 months.

There was no association of the *IL-6* variant with residual beta-cell function.

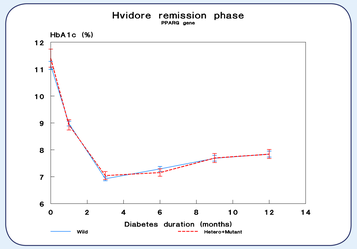


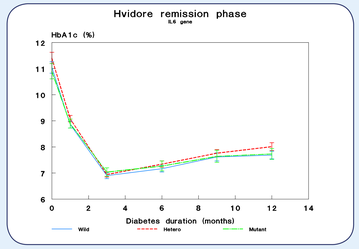


Insulin dose

PPARgamma 12Ala carriers received 0.08 units/kg/day less insulin than Pro12Pro carriers throughout the 12-month period ($p = 0.04$).

There was no association of the *IL-6* variant with the insulin dose.





HbA_{1c}

Throughout the 12-month period there were no differences in glycemic control between PPARgamma 12Ala or *IL-6* -174G/C and wild type carriers.

Conclusions

- The Ala allele of PPARgamma is associated with reduced insulin requirements in children and adolescents during the first year after onset of type 1 diabetes. As glycemic control was similar in both groups and the Ala carriers had even lower C-peptide levels we conclude that the association is secondary to a higher peripheral insulin sensitivity/resistance. Therefore, this variant may also add to the T1D susceptibility already conferred by the HLA risk genes.
- In the present study the IL-6 -174G/C variant did not associate with disease progression. Possibly because of a limited sample size.
- Neither the Pro12Ala of PPARgamma, nor the -174G/C of IL-6 was associated with cyto-/chemokine activity or autoantibody status.