The *PTPN22* 1858T Gene Variant in Type 1 Diabetes Is Associated With Reduced Residual β-Cell Function and Worse Metabolic Control

ANTONIO PETRONE, PHD[1]
MARIALUISA SPOLETINI, PHD[1]
SIMONA ZAMPETTI[1]
MARCO CAPIZZI, MD[1]
SARA ZAVARELLA, PHD[1]
JOHN OSBORN, PHD[2]
PAOLO POZZILLI, MD[4]
RAFFAELLA BUZZETTI, MD[1]
FOR THE IMMUNOTHERAPY DIABETES (IMDIAB) GROUP[3]

OBJECTIVE — Evidence has been reported for a new susceptible locus for type 1 diabetes, the protein tyrosine phosphatase nonreceptor type 2 (*PTPN22*), which encodes a lymphoid-specific phosphatase. The aim of the study was to evaluate the influence of the C1858T variant of the *PTPN22* gene on β-cell function as measured by C-peptide levels from time of disease diagnosis through 12 months follow-up in a prospective series of 120 consecutive type 1 diabetic subjects.

RESEARCH DESIGN AND METHODS — The C1858T polymorphism was genotyped using TaqMan. Fasting C-peptide, A1C, and insulin requirements were determined at diagnosis and every 3 months for 12 months; their change during follow-up was analyzed using the general linear model repeated-measures procedure.

RESULTS — Fasting C-peptide levels were significantly lower and A1C levels were significantly higher in subjects carrying the *PTPN22* 1858T variant than in subjects homozygous for C1858 from time of disease diagnosis through 12 months of intensive insulin therapy follow-up ($P = 0.008$ and $P = 0.01$, respectively). These findings were independent of age at onset, sex, and HLA risk groups. The trend in C-peptide and A1C levels in the 12-month period did not differ significantly between subjects with or without the 1858T variant. Insulin dose was similar in the 1858T carriers and noncarriers.

CONCLUSIONS — Type 1 diabetic subjects carrying the 1858T variant show significantly lower β-cell function and worse metabolic control at diagnosis and throughout the study period than subjects homozygous for C1858; these differences remain unchanged over the course of the first year after diagnosis.

Diabetes Care **31:1214–1218, 2008**

Type 1 diabetes is an immune-mediated disease leading to the destruction of insulin-producing β-cells (1,2). The degree of β-cell destruction and the consequent amount of residual β-cell function are heterogeneous and lead to variations in C-peptide secretion detectable at the time of disease diagnosis (3) and even after insulin administration (4). Although some type 1 diabetic subjects lose β-cell function completely soon after diagnosis, others retain partial function over a long period of time (3,4). Such findings suggest that in type 1 diabetes, the natural course of β-cell destruction may vary considerably, and it is possible that a different genetic background may influence the course of the disease. A new gene susceptible to type 1 diabetes, the protein tyrosine phosphatase nonreceptor type 2 (*PTPN22*) (5), which encodes a lymphoid-specific phosphatase known as lymphoid tyrosine phosphatase (LYP), a powerful inhibitor of T-cell activation (6), has recently been identified outside the HLA region. Several studies have shown that a missense single nucleotide polymorphism (SNP), C1858T, in the *PTPN22* gene is associated with type 1 diabetes (5) and other autoimmune diseases (7–9). So far, it is unclear how the 1858T allele can influence the activity of LYP. In a recent study, Vang et al. (10) demonstrated that the ^{620}Trp variant (which corresponds to the 1858T allele) is a gain-of-function form of the protein, but the mechanism by which the *PTPN22* ^{620}Trp variant exerts its disease-promoting effect has yet to be established. Altered LYP function in peripheral $CD4^+CD25^+$ T regulatory cells, making them less potent in suppressing immune responses against autoantigens, has recently been suggested (11). Finally, evidence has been provided regarding a permissive role played by the *PTPN22* 1858T variant on disease progression from pre–type 1 diabetes to overt disease (12). In view of these considerations, the present study was designed to investigate whether the missense SNP C1858T of the *PTPN22* gene may have an effect on β-cell function as measured by C-peptide levels in type 1 diabetic subjects at diagnosis and in the course of the 12 months after disease onset.

RESEARCH DESIGN AND METHODS

— This study was performed in 120 consecutive subjects (64 men and 56 women) with type 1 diabetes diagnosed in the Lazio region of Central

From [1]Endocrinology, Department of Clinical Science, University "Sapienza," Polo Pontino, Rome, Italy; the [2]Department of Public Health Sciences, University "Sapienza," Rome, Italy; [3]Endocrinology, University "Campus Bio-Medico," Rome, Italy; and the [4]Centre of Diabetes and Metabolic Medicine, Barts and the London School of Medicine and Dentistry, Queen Mary, London, U.K.

Corresponding author: Professor Raffaella Buzzetti, Department of Clinical Science, "Sapienza" University of Rome, Viale del Policlinico 155, 00161 Rome, Italy. E-mail: raffaella.buzzetti@uniroma1.it.

Received for publication 19 June 2007 and accepted in revised form 26 January 2008.

Published ahead of print at http://care.diabetesjournals.org on 5 February 2008. DOI: 10.2337/dc07-1158.

A.P. and M.S. contributed equally to this work.

*A complete list of the members of the Immunotherapy Diabetes (IMDIAB) Group can be found in the APPENDIX.

Abbreviations: IMDIAB, Immunotherapy Diabetes; LYP, lymphoid tyrosine phosphatase; *PTPN22*, protein tyrosine phosphatase nonreceptor type 2; SNP, single nucleotide polymorphism.

Table 1—*Clinical characteristics in type 1 diabetic subjects according to* PTPN22 *C1858T genotypes*

	C1858T genotype	
	CC	CT+TT
Sex (male/female)	47/41	17/15
Age at diagnosis (years)	14.78 ± 7.7	15.48 ± 7.9
Ketonuria (%)	75	78
BMI (kg/m^2)	18.34 ± 3.52	17.84 ± 3.13

Data are means ± SEM unless indicated otherwise. CC vs. CT+TT: $P > 0.05$ for all comparisons made.

Italy within the framework of the Immunotherapy Diabetes (IMDIAB) Group (13). All subjects were Caucasians with parents of Italian origin. The age of patients ranged from 5 to 36 years (mean ± SEM age 14.9 ± 7.8 years). Type 1 diabetes was diagnosed according to the American Diabetes Association classification criteria. The study was approved by the ethical committees at Universities "Sapienza" and "Campus Bio-Medico." Written informed consent was obtained from all participating subjects.

In type 1 diabetic subjects intensive insulin therapy with three injections of regular insulin plus glargine insulin once a day was implemented from the time of diagnosis for optimization of metabolic control. Regular adjustments were made every 3 months, with frequent telephone consultations with the subjects (or parents if the subject was a child).

Fasting blood samples were collected in the morning within 1 week of disease diagnosis and with plasma glucose levels <180 mg/dl. Blood samples for genomic extraction were stored at −20°C before use. Genomic DNA was extracted using a QIAamp DNA Blood Kit (QIAGEN Genomics, Bothell, WA). The missense SNP C1858T was genotyped using the fluorogenic 5′ nuclease assay application of the ABI PRISM 7900HT Sequence Detection System (ABI, Foster City, CA). Genotyping was performed using the following primers: forward, 5′-CAACTGCTCCAAGGATA GATGATGA-3′; reverse, 5′-CCAGCTTCCTCCTCAACCAATAAATG-3′; and the TaqMan MGB probes Fam TCAGGTGTCCGTACAGG and Vic TCAGGTGTGTCCATACAGG. Of the 10 ng/μl of DNA, 4 μl were dispensed into 384-well PCR plates using a Biomek FX robot (Beckman Coulter, Fullerton, CA) to which 2 μl of a mix containing primers, MGB probes, and TaqMan Universal PCR Master Mix (ABI) was added in accordance with manufacturers' instructions. These were sealed with optical seals (ABI) and incubated at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min before analysis on an 7900HT plate reader (ABI). Fasting C-peptide levels, A1C (normal range 4–6%), and insulin requirements were evaluated at diagnosis and every 3 months for 12 months afterward. C-peptide was determined by a radioimmunoassay using a commercial kit (Bio-Rad, Milan, Italy) validated through the international C-peptide workshop in 150 control subjects (aged 5–40, median age 18 years: 0.35–1 nmol/l) with intra-assay and interassay coefficients of variation of 10 and 15%, respectively; A1C was measured by a column assay (Bio-Rad). Ketonuria was defined as presence of ketones at >20 mg/dl in the urine.

Statistical analysis was performed using SPSS (version 12; SPSS, Chicago, IL). $P < 0.05$ was considered to be statistically significant. Data are expressed as means ± SEM.

The variances of fasting C-peptide, A1C, and insulin requirements during follow-up were analyzed using the general linear model repeated-measures procedure. This provided the analysis of groups of related variables representing different measurements of the same attribute (within-subjects factor) (14). The model included between-subjects factors that divided the population into groups according to their genotype and time of follow-up by genotype interaction, using age, sex, and HLA risk groups as covariates. HLA genotypes were introduced as a dichotomous variable in the analysis as follows: high and moderate risk = 1 and low risk = 0 (high-risk genotype: DRB1*03-DQB1*0201/DRB1*04-DQB1*0302; moderate-risk genotypes: DRB1*04-DQB1*0302/DRB1*04-DQB1*0302, DRB1*03-DQB1*0201/DRB1*03-DQB1*0201, DRB1*04-DQB1*0302/X, and DRB1*03/X [X different from DRB1*03, DRB1*04-DQB1*0302, or DQB1*0602/03]; and low-risk genotypes: all other genotypes). C-peptide and A1C were $\log_{10}$ transformed to normalize their distributions. On the basis of a previous study (15) in which C-peptide levels were twice as high among class III/III genotype as in class I/I and in class I/III of the *IDDM2* gene, the present study should be able to detect a doubling in C-peptide levels in subjects homozygous for the C1858 variant compared with subjects carrying the 1858T variant of the *PTPN22* with a power of 99% and significance level of 5%, using our sample size of 32 CT+TT patients and 88 CC patients.

RESULTS — The clinical characteristics of subjects with type 1 diabetes grouped according to their *PTPN22* C1858T genotype are reported in Table 1.

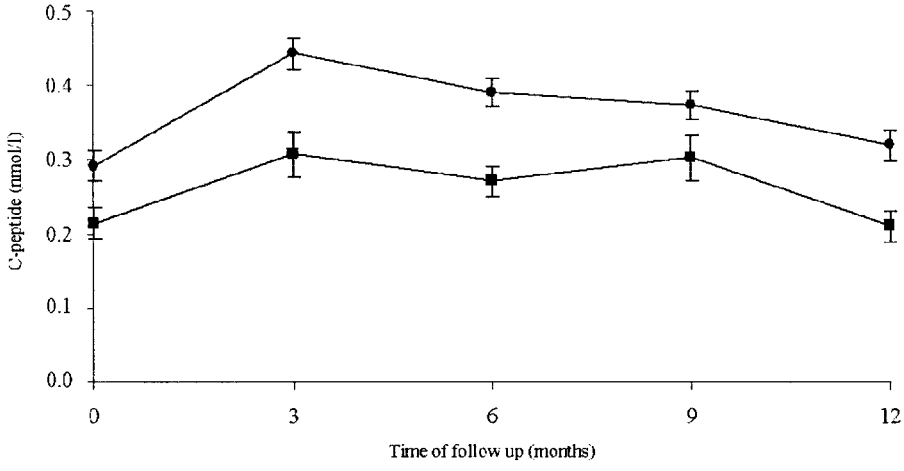

Figure 1—*Residual β-cell function as measured by C-peptide (nanomoles per liter) in 88 type 1 diabetic subjects with the CC (●) genotype and 32 type 1 diabetic subjects with the CT+TT (■) genotype. CC vs. CT+TT:* P = *0.008.*

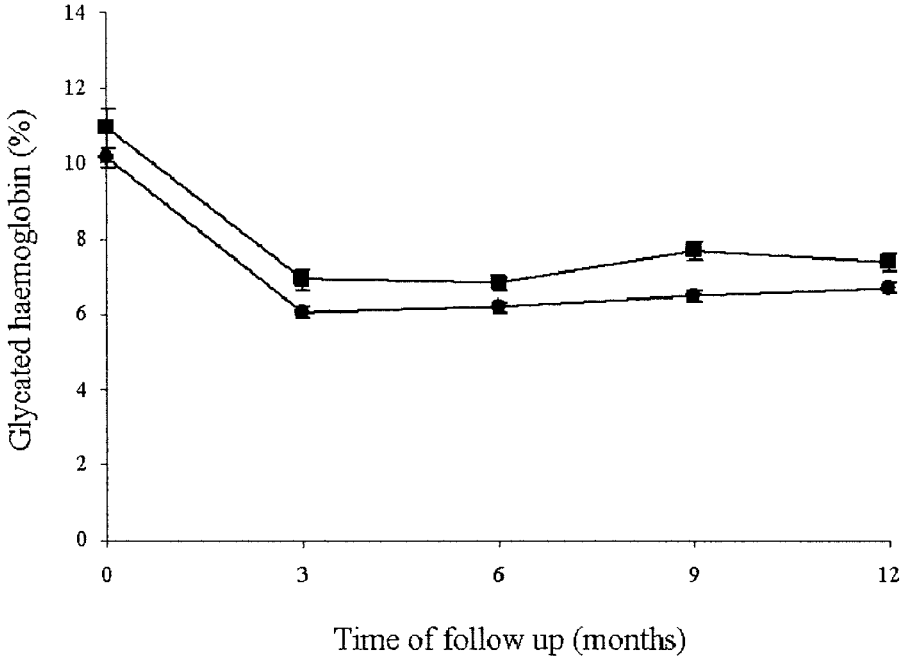

Figure 2—*A1C values (percent) in 88 type 1 diabetic subjects with the CC (●) genotype and 32 type 1 diabetic subjects with the CT+TT (■) genotype. CC vs. CT+TT:* P = *0.01.*

Fasting C-peptide levels in type 1 diabetic subjects carrying the 1858T variant of the *PTPN22* gene were significantly lower than those in subjects homozygous for the C1858 allele from time of disease diagnosis through the 12-month follow-up ($P = 0.008$; means ± SEM for C-peptide at diagnosis and after 12 months were 0.29 ± 0.02 and 0.31 ± 0.02, respectively, in CC subjects and 0.21 ± 0.02 and 0.21 ± 0.02, respectively, in CT+TT subjects) (Fig. 1). Moreover, A1C levels in subjects carrying the 1858T allele variant were significantly higher than those in subjects homozygous for the C1858 allele during the 12-month period ($P = 0.01$; means ± SEM for A1C at diagnosis and after 12 months were 10.14 ± 0.24 and 6.72 ± 0.14, respectively, in CC subjects and 10.94 ± 0.52 and 6.86 ± 0.23, respectively, in CT+TT subjects) (Fig. 2). These findings were independent of age at onset, sex, and HLA risk groups. Nonetheless, the changes in C-peptide and A1C levels over the observational period did not differ significantly between 1858T carriers and noncarriers. The insulin dose at diagnosis and after 3, 6, 9, and 12 months did not differ between subjects with or without the 1858T variant of the *PTPN22* gene (means ± SEM for insulin requirement at diagnosis and after 12 months were 0.66 ± 0.06 and 0.45 ± 0.04, respectively, in CC subjects and 0.72 ± 0.03 and 0.51 ± 0.03, respectively, in CT+TT subjects) (Fig. 3).

CONCLUSIONS — This study shows that in type 1 diabetic subjects the 1858T variant of the *PTPN22* gene is significantly associated with lower β-cell function as measured by C-peptide levels and higher A1C levels independent of age at diagnosis, sex, and HLA risk groups from disease diagnosis through the 12-month follow-up compared with C1858 homozygous subjects. These differences remain unchanged in the course of the first year after diagnosis.

We can speculate that, after diagnosis, other factors could interfere with the progression of the disease. Intensive insulin therapy implemented at diagnosis and during follow-up in all patients is able to alter the natural course of β-cell destruction (16), and, perhaps, by means of such treatment, a different trend in C-peptide levels between subjects carrying the two genetic variants cannot be described.

It can be argued that the *PTPN22* 1858T individuals had experienced more destructive β-cell damage before the clinical onset of diabetes and then maintained significant lower levels of C-peptide compared with C1858 homozygotes during the first year after diagnosis. However, because we have no information on the status of β-cell function before the onset of diabetes in the two groups, it remains to be established why the 1858T gene variant determines a more aggressive autoimmune process.

We have previously demonstrated that C-peptide levels are significantly higher in patients with low HLA DRB1-DQB1 risk genotypes compared with those with high and moderate risk at disease diagnosis (17), underlining the importance of the genetic component in the preservation of β-cell function.

Altered LYP function, codified by the 1858T variant, in $CD4^+CD25^+$ T regulatory cells, making them less potent in suppressing immune response, could explain more aggressive β-cell destruction and consequently a major loss in β-cell function in type 1 diabetic subjects carrying this genetic variant (11). The PTPN22 1858T variant has been found to be strongly associated with progression to β-cell–specific autoimmunity and clinical disease (12). This progression was demonstrated by a fourfold higher risk of developing an additional autoantibody carried by islet cell antibody–positive children possessing the *PTPN22* TT genotype compared with children with the CC

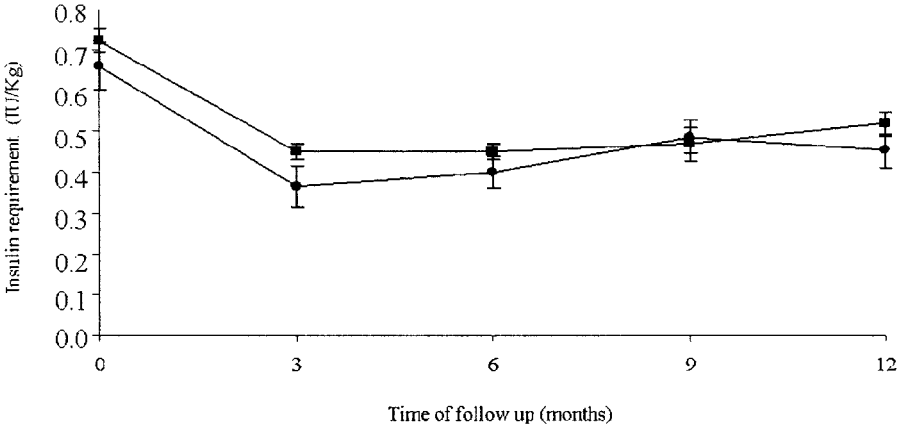

Figure 3—*Insulin requirement values (international units per kilogram) in 88 type 1 diabetic subjects with the CC (●) genotype and 32 type 1 diabetic subjects with the CT+TT (■) genotype.*

genotype. These findings could explain why, in our subjects carrying the *PTPN22*-susceptible variant, lower levels of C-peptide are detectable at diagnosis of type 1 diabetes.

Considering the clinical relevance of the present findings, Palmer et al. (18) reported that a difference of 0.1 nmol/l in C-peptide levels (as in the present study) would probably be considered meaningful in a clinical trial testing the efficacy of preserving β-cell function, although it would yield a small difference in A1C in intensively treated patients. Nonetheless, the Diabetes Control and Complications Trial (16) demonstrated that even relatively modest treatment effects on C-peptide will result in clinically meaningful benefits. In fact, even modest retention of β-cell function in individuals with type 1 diabetes is recognized to result not only in better metabolic control but also in reduced end-organ complications (especially retinopathy) and is associated with a significantly reduced risk of serious hypoglycemia.

The insulin dose required by type 1 diabetic subjects carrying different genotypes was not statistically significant between the groups from disease onset through follow-up. However, A1C levels were significantly higher in type 1 diabetic subjects carrying the *PTPN22* 1858T variant. This finding suggests that, because of the lower β-cell function, overall metabolic control is impaired in subjects carrying the 1858T variant compared with that in CC homozygotes, despite the fact that all subjects were treated with intensive insulin therapy, and every effort was made to keep glucose control within as normal ranges as possible.

In summary, the results from this study indicate that extension of β-cell destruction in type 1 diabetes could be controlled in part by the *PTPN22* gene. Future approaches aimed to prevent the progression of β-cell loss using C-peptide as the primary outcome should take into account the genetic background of subjects, with particular reference to the *PTPN22* gene variant; further studies are needed to replicate and extend these findings.

Acknowledgments— This work was supported in part by Grants FIRB2001 (to R.B.) and RBNE01C5S2_004 from the Italian "Ministero dell'Università e Ricerca Scientifica e Tecnologica" and in part by grants from "Fondazione Diabete, Endocrinologia and Metabolismo" and "Centro Internazionale Studi Diabete," Rome, Italy.

We thank subjects with type 1 diabetes and their families for participation in the study and the physicians of the IMDIAB Group for sample collection. We also thank Tracie Dornbusch for English editing and revision.

APPENDIX

Investigators of the IMDIAB group include Paolo Pozzilli, Natalia Visalli, Silvia Manfrini, Elvira Fioriti, Luciana Valente, Chiara Guglielmi, Giuseppina Beretta Anguissola, Flavia Costanza, Laura Cipolloni, Angelo Lauria Pantano, Antonio Picardi, Manon Khazrai Merola, M. Gisella Cavallo, Maria C. Matteoli, I. Patrizia Patera, Marco Cappa, Antonio Crinò, Stefania Corbi, Sabrina Spera, Riccardo Schiaffini, Concetta Suraci, Rita Cassone, Maria L. Manca Bitti, Carla Bizzarri, Dario Pitocco, and Giovanni Ghirlanda.

References

1. Notkins AL, Lernmark A: Autoimmune type 1 diabetes: resolved and unresolved issues. *J Clin Invest* 108:1247–1252, 2001
2. Atkinson MA, Eisenbarth GS: Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 358:221–229, 2001
3. The DCCT Research Group: Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). *J Clin Endcrinol Metab* 65:30–36, 1987
4. Scholin A, Bjorklund L, Borg H, Arnqvist H, Bjork E, Blohme G, Bolinder J, Eriksson JW, Gudbjornsdottir S, Nystrom L, Ostman J, Karlsson AF, Sundkvist G: Diabetes Incidence Study in Sweden: islet antibodies and remaining β-cell function 8 years after diagnosis of diabetes in young adults: a prospective follow-up of the nationwide Diabetes Incidence Study in Sweden. *J Intern Med* 255:384–391, 2004
5. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 36:337–338, 2004
6. Cloutier JF, Veillette A: Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *J Exp Med* 189:111–121, 1999
7. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang SY, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ, Gregersen PK: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75:330–337, 2004
8. Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VE, Chang M, Ramos P, Baechler EC, Batliwalla FM, Novitzke J, Williams AH, Gillett C, Rodine P, Graham RR, Ardlie KG, Gaffney PM, Moser KL, Petri M, Begovich AB, Gregersen PK, Behrens TW: Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *J Hum Genet* 75:504–507, 2004
9. Velaga MR, Wilson V, Jennings CE, Owen CJ, Herington S, Donaldson PT, Ball SG, James RA, Quinton R, Perros P, Pearce SH: The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J Clin Endocrinol Metab* 89:5862–5865, 2004
10. Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P, Nika K, Tautz L, Tasken K, Cucca F, Mustelin T, Bottini N: Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 37:1317–1319, 2005
11. Bottini N, Vang T, Cucca F, Mustelin T: Role of PTPN22 in type 1 diabetes and other autoimmune diseases. *Semin Immunol* 18:207–213, 2006
12. Hermann R, Lipponen K, Kiviniemi M, Kakko T, Veijola R, Simell O, Knip M, Ilonen J: Lymphoid tyrosine phosphatase (LYP/PTPN22) Arg620Trp variant regulates insulin autoimmunity and progression to type 1 diabetes. *Diabetologia* 49:1198–1208, 2006
13. Visalli N, Sebastiani L, Adorisio E, Conte A, De Cicco AL, D'Elia R, Manfrini S, Pozzilli P, IMDIAB Group: Environmental risk factors for type 1 diabetes in Rome and province. *Arch Dis Child* 88:695–698, 2003
14. GLM repeated measures. In *SPSS Advanced Models 12.0*. Chicago, SPSS. Vol. 2, 2003, p. 17–34
15. Nielsen LB, Mortensen HB, Chiarelli F, Holl R, Swift P, de Beaufort C, Pociot F, Hougaard P, Gammeltoft S, Knip M, Hansen L, Hvidore Study Group: Impact of IDDM2 on disease pathogenesis and progression in children with newly diagnosed type 1 diabetes: reduced insulin antibody titres and preserved β cell function. *Diabetologia* 49:71–74, 2006
16. Steffes MW, Sibley S, Jackson M, Thomas W: β-Cell function and the development of diabetes-related complications in the

Diabetes Control and Complications Trial. *Diabetes Care* 26:832–836, 2003
17. Petrone A, Galgani A, Spoletini M, Alemanno I, Di Cola S, Bassotti G, Picardi A, Manfrini S, Osborn J, Pozzilli P, Buzzetti R: Residual insulin secretion at diagnosis of type 1 diabetes is independently associated with both, age of onset and HLA genotype. *Diabete Metab Res Rev* 21:271–275, 2005
18. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW: C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β-cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes* 53:250–264, 2004