Genotypes of Cytosolic Low–Molecular-Weight Protein-Tyrosine-Phosphatase Correlate With Age at Onset of Type 1 Diabetes in a Sex-Specific Manner

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We investigated the possible role of cytosolic low–molecular-weight protein-tyrosine-phosphatase (cLMWPTP or acid phosphatase locus 1 [ACP1]) in the mediation of age at onset of type 1 diabetes. ACP1 is an enzyme involved in signal transduction of T-cell receptors, insulin, and other growth factor receptors. We studied acid phosphatase polymorphism in 189 consecutive children with type 1 diabetes admitted to the Pediatric Clinic of Sassari University (Sardinia) and in 86 adolescent patients with recently diagnosed type 1 diabetes from continental Italy. In both populations, females with medium-high activity acid phosphatase genotypes had onset of disease significantly earlier than males. The data suggest that acid phosphatase genotype affects the age of onset and probably also the sex ratio in type 1 diabetes. Sex hormones might modulate the susceptibility to autoimmune diseases, including type 1 diabetes, through the influence of signal transduction pathways involved in immune functions. Elucidation of the molecular basis for gender differences in the course and severity of type 1 diabetes could have important implications for treatment as well, because there might be gender-specific effects in the response to immunotherapy.

TYPE 1 DIABETES IS AN autoimmune disease with a slight prevalence of males over females, whereas other autoimmune organ-specific diseases are characterized by a female preponderance.[1] It has been proposed that sex hormones could affect the immune system by influencing the signal transduction pathway involved in immune functions.[2] The clinical presentation and disease progression of many autoimmune disorders differ between males and females. Elucidation of the molecular basis for gender differences could have important implications for treatment, because there may be gender-specific effects also in the response to various forms of therapy.[2]

We investigated the possible effect of the genetic variability of cytosolic low–molecular-weight protein-tyrosine-phosphatase (cLMWPTP or acid phosphatase locus 1 [ACP1]), a polymorphic enzyme involved in various signal transduction pathways,[3] on the age of onset of type 1 diabetes.

SUBJECTS AND METHODS

The study was performed in 189 consecutive children with type 1 diabetes admitted to the Pediatric Clinic of Sassari University (Sardinia) and in 86 adolescent patients with recently diagnosed type 1 diabetes from continental Italy diagnosed by the Immunotherapy of Diabetes (IMDIAB) group in Rome. Clinical features of these patients are reported in Table 1. There are differences between the 2 samples. The sample from Sardinia was collected in a single pediatric hospital, was strictly consecutive and ethnically homogeneous, and the clinical parameters for all subjects refer to the moment of diagnosis. The sample from continental Italy was collected in various hospitals and was not strictly consecutive. Informed consent was obtained from patients or from their parents.

ACP1 is a high polymorphic enzyme controlled by an autosomal locus showing 3 codominant alleles named ACP1*A, *B, and *C.[4] The 3 ACP1 alleles show single base substitutions located at 3 specific sites: ACP1*A and *B alleles differ for 2 base substitutions, a silent C-T transition at codon 41 (exon 4), and an A-G transition at codon 105 (exon 6). The ACP1*C allele differs from the *A and *B alleles at codon 43 (exon 3).[5]

Total genomic DNA was extracted from frozen whole-blood samples collected in NA_2EDTA using the procedure described by Kunkel et al[6] with slight modifications. All polymerase chain reactions (PCRs) were set up in 30 μL, 0.2 μmol/L of both primers, 0.1 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 0.5 U of Taq polymerase (AmpliTaq, Applied Biosystem, Mannheim, Germany) 1× AmpliTaq buffer (PE), and 50 ng of DNA template. The amplification conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 45 seconds, 54°C for 45 seconds, 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. The annealing temperature, extension time, and primer concentration for the 2-kb amplification product were 57°C, 120 seconds, and 0.1 μmol/L, respectively.

Oligonucleotide primers used for PCR amplification are reported in Table 2.

The C-T transition at codon 43 and the A-G transition at codon 105 (indicated respectively here as C and A in Fig 1) generate, respectively, a Cfo I and a Taq I restriction site that, together, were used for PCR-based genotyping (modified from Lazaruk).[5] A 341-bp segment spanning the entire exons 3 and 4 was amplified using primers #263 to #264 (Table 2). A 299-bp segment including exon 6 was amplified using primers #267 and #268. Ten microliters of the 341-bp exon 3 amplicon was fully cleaved by Cfo I at 37°C for 1 hour according to the manufacturer's instructions and then electrophoresed on 1.8% agarose gels. The digestion created 2 fragments of 255 and 86 bp for the ACP1*A and ACP1*B alleles, while the ACP1*C allele was not cut. Similarly, the 299-bp PCR product was digested by Taq I at 65°C for 1 hour according to the manufacturer's instructions, generating 2 frag-

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Table 1. Clinical Features of Patients From Sardinia and Continental Italy

	Sardinia	Rome
Males		
%	60.2	62.8
SE	3.5	5.2
Age at onset of diabetes (yr)		
Mean	7.4	11.4
SE	0.29	0.71
HbA_{1c} (%)		
Mean	8.21	11.32
SE	0.49	0.24
Blood glucose (mg/dL)		
Mean	409.4	381.2
SE	12.2	13.2

NOTE. HbA_{1c} and blood glucose are values at time of diagnosis.

ments of 100 and 199 bp for the ACP1*A allele, but not for the *B and the *C alleles (modified from Lazaruk).[5]

Three-way contingency table analysis was performed according to Sokal and Rohlf.[7] Other statistical analyses were perfromed using the SPSS package (SPSS, Inc, Chicago, IL).

RESULTS

The clinical parameters studied in our populations are reported in Table 1. Differences between the 2 populations are probably due to differences in the sampling procedure. In Sardinia, the age at onset ranges from 1 to 21 years, while in Rome it ranges from 3 to 32 years. It is likely that, being collected in a pediatric hospital, the Sardinian sample includes mainly subjects with early onset of diabetes.

Following indications from previous studies[8] that showed differences between low activity genotypes (*A/*A and *A/*B) and medium-high activity genotypes (*B/*B,*A/*C,*B/*C, and *C/*C) in diabetic patients, we grouped ACP1 genotypes of Sardinian patients in 2 categories for low and medium-high activity. Table 3 shows the association between the age at onset of type 1 diabetes and gender in relation to the ACP1 genotype. There is a significant 3-way interaction. Among genotypes with medium-high activity, the proportion of females is much higher in children with age at onset ≤6 years than in those with age at onset greater than 6 years ($P < .001$).

Table 4 shows the mean age of onset according to gender and category of ACP1 enzymatic activity for patients from Sardinia. The effect of gender on age at onset is dependent on the ACP1 genotype. Thus, while among ACP1 genotypes with low activity there is no difference between the sexes, among those with medium-high activity the mean age at onset is lower in females than in males (5.8 *v* 8.6 years; $P < .0001$) .

Table 2. Primers Used for ACP1 SNPs Analysis

Primer	Target Amplification	Nucleotide Sequence 5′-3′
#263	Exon 3	AGGCCAACCTGAACTCCTCT
#264	Exon 3	CCTGTCTTGCTTTATGGGCT
#267	Exon 6	TTCAGAAGACCCTAGCAGATG
#268	Exon 6	TGGCAAAACCTGCATAACAA

Abbreviation: SNPs, single-nucleotide polymorphisms.

We also performed a correlation analysis between ACP1 activity (considering each genotype separately) and age at onset in patients from Sardinia. A positive correlation between age at onset and ACP1 activity is observed in males, whereas the correlation is negative in females (Table 5).

Table 6 shows the association between the age at onset of type 1 diabetes and gender in patients from continental Italy. The analysis is similar to that reported in Table 3 for the Sardinian sample. Because the range of age at onset in the continental sample is greater than in the Sardinian sample, we grouped the subjects into 3 categories and tested for linear association. Among children with medium-high activity of ACP1 there is a negative linear association between the proportion of females and age at onset. As in the Sardinian sample (Table 3), in the sample from continental Italy among genotypes with medium-high activity the proportion of females is much higher in children with age at onset ≤6 years than in those with age at onset greater than 6 years.

DISCUSSION

This is the first study to demonstrate that the ACP1 genotype affects the age of onset of type 1 diabetes. The pattern of association is similar in 2 series of patients sampled with different criteria from 2 Italian populations, reducing the likelihood that these findings are mere sampling artifacts.

Indeed, the fact that among children aged 6 years or less, ie, within the range of age in which the Sardinian sampling was certainly strictly consecutive, there is a significant association between gender and ACP1 genotype and that a similar pattern is present also in the continental sample represents a strong argument against a possible bias. This is because the Sardinian sample probably does not include subjects older than 14 years at the onset of disease.

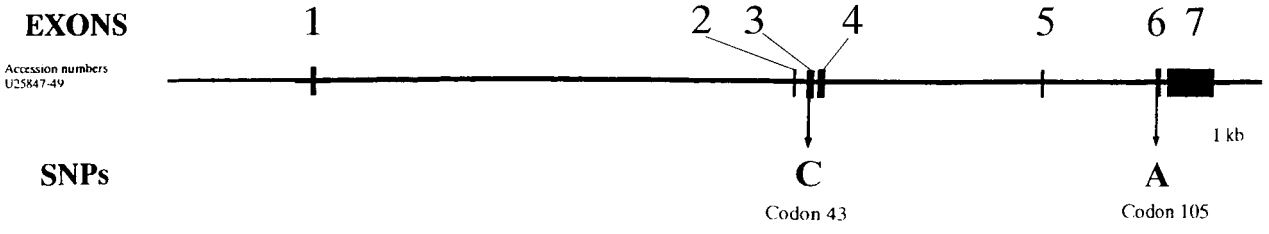

Fig 1. ACP1 genomic organization and SNPs.

Table 3. Age at Onset and Gender in Type 1 Diabetes Patients From Sardinia: Interaction With ACP1 Genotype

	ACP1			
	Low Activity (*A/*A and *A/*B)		Medium-High Activity (*B/*B, *A/*C, *B/*C, *C/*C)	
	Age at Onset			
	≤6 yr	>6 yr	≤6 yr	>6 yr
Females	35%	39%	59%	26%
Total no.	31	33	59	66
Three-way contingency table analysis by a log-linear model				
Interaction between ACP1, age at onset, and sex	$P < .02$			
Chi-square test				
Association between age at onset and sex				
*A/*A and *A/*B genotypes	NS			
Other ACP1 genotypes	$P < .001$			

Abbreviation: NS, not significant.

Although the biochemical functions of ACP1 point to a causal involvement of the enzyme in the pattern of observed association, at present the effect of a gene strictly linked to ACP1 and in linkage disequilibrium with it cannot be excluded.

ACP1 is located on chromosome 2p25. On the same chromosome loci involved in type 1 diabetes have been found in 2q33 and in 2q12-21,[9,10] and loci involved in glucose intolerance have been found in 2q37.3 and in 2p13.[11,12] All of these loci are located very far from the ACP1 locus.

ACP1 or cLMWPTP is an enzyme involved in T-cell receptor signal transduction.[13] It has also been shown to modulate insulin, as well as platelet-derived growth factor (PDGF) receptor signal transduction.[14] This enzyme is composed by 2 isoforms, F and S, which have different molecular and catalytic properties.[3,15] ACP1 is a polymorphic enzyme showing strong quantitative variations of total enzymatic activity and of F/S ratio among genotypes. In Caucasian populations, 6 genotypes are present: *A/*A, *A/*B, *B/*B, *A/*C, *B/*C, and *C/*C,[4] attributed to the presence of 3 codominant alleles—ACP1*A, ACP1*B, and ACP1*C—at the autosomal locus. Spencer et al[16] found the following activities (μmol *p*-nitrophenol produced in 30 minutes per gram of hemoglobin at 37°C): ACP1*A/*A, 122.4; ACP1*A/*B, 153.9; ACP1*B/*B, 188.3; ACP1*A/*C, 183.8; and ACP1*B/*C, 212.3. Intracellular signal transduction is mediated by balance between kinases and phosphatases and both have a crucial role in immunological and metabolic responses, and in cellular proliferation and growth.[17,18] Genetic variability of kinases and phosphatases involved in these signaling pathways could influence lymphocyte activation threshold, as well as cellular responses to cytokines and growth factors.

Table 4. Effect of ACP1 Genotype on the Relationship Between Gender and Age at Onset in Sardinian Population

	Age at Onset	
	Males	Females
*A/*A and *A/*B	7.1 (0.7)*	7.3 (0.9)
Other ACP1 genotypes	8.6 (0.5)	5.8 (0.4)
ANOVA		
Main effect	$P = .006$	
Interaction ACP1-gender	$P = .014$	
One-way		
Tukey test, males/other ACP1 genotypes *v* females/other ACP1 genotypes	$P < .0001$	

*SE is in parentheses.
Abbreviation: ANOVA, analysis of variance.

Table 5. Correlation Analysis Between ACP1 Activity and Age at Onset in the Sardinian Sample

Males	$r = .19$
Females	$r = -.14$
Difference between correlations in males and females	$P < .05$

Recently, great attention has been paid to gender differences in autoimmunity.[2] Experimental studies indicate that viruses that elicit Th1 or Th2 rensponses may have different clinical expression in males and in females.[19-21] In humans, different degrees of immune response have been observed between the sexes. Clinical disease expression including age at onset, progression, and response to treatment may also show gender differences.[2] Sex hormones could have an important role through modulation of immune response. Estrogens seem to enhance at low level but inhibit at higher level immune reactivity.[22-25] Testosterone exerts immunosuppressive effects in autoimmunity.[26,27] Pituitary hormones may also have importance: prolactine and growth hormone may enhance autoimmunity.[2]

Genetic factors too have a relevant role in autoimmunity. It has been suggested the besides major histocompatability complex (MHC)-linked candidate genes there are also non–MHC-linked candidate genes involved in autoimmunity.[28,29] Moreover, there could be unique genes for a given disease determining susceptibility of a target organ to an autoimmune attack.[2] There is a general consensus on the importance of elucidation of molecular mechanisms and in particular of the signal transduction pathways involved in the gender differences, age at onset, and the course and severity of autoimmune diseases.[2]

Following the suggestion of the Task Force on Gender, Multiple Sclerosis and Autoimmunity,[2] stratification on the basis of sex has revealed an important effect of the genetic variability in a system involved in T-lymphocyte activation and in signal transduction pathways from insulin and other growth factor receptors.[3] This suggests an interaction of ACP1 with some sex-specific factor in the pathogenesis of type 1 diabetes. However, further studies of the polymorphic systems controlling signal transduction in autoimmune disorders stratified by gender are warranted.

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Table 6. Age at Onset and Gender in Type 1 Diabetes Patients From Continental Italy: Interaction With ACP1 Genotype

	ACP1					
	Low Activity *A/*A and *A/*B			Medium-High Activity *B/*B; *A/*C; *B/*C; *C/*C		
	Age at Onset					
	≤6 yr	>6 ≤ 15 yr	>15 yr	≤6 yr	>6 ≤ 15 yr	>15 yr
Females	33%	45%	12%	57%	44%	0.0%
Total no.	9	20	8	7	34	8
Mantel-Haenszel test for linear association		NS			$P = .02$	

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