Role of the *ENPP1* K121Q Polymorphism in Glucose Homeostasis

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OBJECTIVE—To study the role of the *ENPP1* Q121 variant on glucose homeostasis in whites from Italy.

RESEARCH DESIGN AND METHODS—We conducted case-control studies in 764 adults (from two independent samples of 289 nonobese and 485 obese individuals) and 240 overweight/obese children undergoing oral glucose tolerance testing (OGTT). Early-phase insulin secretion and insulin sensitivity (the insulinogenic index and the insulin sensitivity index) and their interplay (the disposition index) were calculated.

RESULTS—In adult subjects, glucose profiles during OGTT were significantly ($P = 2 \times 10^{-2}$) different across K121Q genotype groups and higher in QQ than KK individuals ($P = 5 \times 10^{-2}$). The insulinogenic index was significantly reduced in QQ (18.5 ± 3.4) compared with both KK (31.6 ± 1.0; $P = 2.2 \times 10^{-7}$) and KQ (30.5 ± 1.5; $P = 3.2 \times 10^{-6}$) individuals. KQ individuals also showed a reduced insulin sensitivity index compared with KK subjects ($P = 3.6 \times 10^{-2}$). The disposition index was lower in QQ carriers than in KQ and KK individuals ($P = 8 \times 10^{-3}$ and 4×10^{-4}, respectively) and lower in KQ than in KK individuals ($P = 3 \times 10^{-2}$). Data obtained in overweight/obese children were very similar to those observed in adults, with QQ individuals showing (compared with KQ and KK subjects) a reduced insulinogenic index ($P = 7 \times 10^{-3}$ and 2×10^{-2}, respectively) and disposition index ($P = 2 \times 10^{-2}$ and 7×10^{-3}, respectively).

CONCLUSIONS—Homozygous carriers of the *ENPP1* Q121 variant are characterized by an altered glucose homeostasis. Reduced early-phase insulin secretion and inefficient interplay between insulin secretion and sensitivity, which occur at early ages, are major determinants of this defect. ***Diabetes* 57: 3360–3364, 2008**

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Insulin resistance and inadequate insulin secretion are pathogenic for type 2 diabetes (1). Insulin-resistant subjects who eventually develop type 2 diabetes maintain normal or near-normal glycemia for many years because of compensatory hyperinsulinemia (2). Type 2 diabetes eventually ensues only when β-cells fail to secrete sufficient insulin to adequately counteract insulin resistance (3). Thus, an inefficient interplay between insulin secretion and sensitivity is the key pathogenic factor for developing type 2 diabetes. In agreement with this tenet, the disposition index (the product of insulin secretion × insulin sensitivity) is the best predictor of type 2 diabetes (4).

Ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) (5) downregulates insulin signaling by direct inhibition of insulin receptor signaling (6–8). We previously described an *ENPP1* missense polymorphism (K121Q, rs1044498) (9). Compared with the more common K121, the Q121 variant is a stronger inhibitor of insulin receptor signaling in cultured cells (7,9) and has been associated in most, although not all, studies with insulin resistance–related abnormalities (5,6). Several groups have investigated whether the Q121 variant is also associated with type 2 diabetes (5,6). Although results have not been homogeneous across studies, a recent and comprehensive meta-analysis has concluded that Europeans who are homozygous for the Q121 allele (i.e., carrying the QQ genotype) have a 38% increased risk of type 2 diabetes (10). More recently, the Q121 allele has been reported to be associated with hyperglycemia and insulin resistance in 2,511 Framingham Heart Study participants (11). To gain insight about the mechanisms underlying these associations, we studied insulin secretion, insulin sensitivity, and their interplay in whites from Italy across *ENPP1* K121Q genotype groups.

RESEARCH DESIGN AND METHODS

A total of 764 adult (age 18–70 years) and 240 child (age 8–17 years) unrelated subjects from Eastern Sicily, with fasting plasma glucose <7.0 mmol/l and not taking medications known to interfere with glucose and lipid metabolism, were recruited at the Endocrine Unit of Garibaldi Hospital (Catania, Italy) (see Methods in the online appendix [available at http://dx.doi.org/10.2337/db07-1830] for details of their clinical characteristics, the study design, and oral glucose tolerance testing [OGTT]-derived measurements). People from Eastern Sicily have been reported to be genetically homogeneous (12), which is a feature that minimizes the risk of population stratification.

The study protocol was approved by the institutional review board and performed according to the dictates of the Helsinki Declaration. Written informed consent was obtained from each adult participant and from a parent of each child.

Genotyping. DNA was extracted from whole blood by standard methods. Genotyping was performed by restriction fragment–length polymorphism (9), with a failure rate <1%. Genotyping quality was checked by directly sequencing 10% of randomly selected samples. The agreement rate of resequenced

TABLE 1
Subjects clinical features and fasting biochemical parameters according to the *ENPP1* K121Q polymorphism

	Adult subjects (n = 764)			Children (n = 240)		
	KK	KQ	QQ	KK	KQ	QQ
n (%)	528 (69)	215 (28)	21 (3)	166 (69)	64 (27)	10 (4)
Male/female	186/342	73/142	6/15	67/99	22/42	2/8
Age (years)	37.1 ± 0.53	37.1 ± 0.84	34.5 ± 2.25	12.9 ± 0.22	13.0 ± 0.31	13.4 ± 0.65
BMI (kg/m^2)	35.5 ± 0.46	36.6 ± 0.77	34.4 ± 2.24	—	—	—
BMI z score (kg/m^2)	—	—	—	2.23 ± 0.03	2.36 ± 0.12	2.30 ± 0.10
Plasma glucose (mmol/l)	5.17 ± 0.03	5.25 ± 0.05	5.47 ± 0.19*	4.97 ± 0.10	4.92 ± 0.10	4.68 ± 0.16
IRI (pmol/l)	85.5 ± 2.32	86.9 ± 3.87	71.9 ± 8.88	124.6 ± 4.70	144.4 ± 8.54†	144.7 ± 23.0
HDL cholesterol (mmol/l)	1.18 ± 0.02	1.17 ± 0.02	1.14 ± 0.07	1.05 ± 0.02	1.02 ± 0.03	0.97 ± 0.12
Triglycerides (mmol/l)	1.29 ± 0.03	1.32 ± 0.05	1.12 ± 0.14	1.02 ± 0.04	0.95 ± 0.06	0.79 ± 0.08

Data are means ± SEM unless otherwise indicated. Significances are given after adjusting for age, sex, and BMI or BMI z score. *$P = 1.6 \times 10^{-2}$ vs. KK adults and †$P = 1.5 \times 10^{-2}$ vs. KK children and adolescents. IRI, immunoreactive insulin.

samples was >99%. The proportion of the K121Q genotypes was in Hardy-Weinberg equilibrium in both samples studied.

Statistical analysis. Data are provided as means ± SEM. All the analyses are adjusted for age, sex, and BMI. Continuous variables were compared between groups using one-way or repeated-measurements ANCOVA. In particular, repeated-measurements ANCOVA models to assess differences over time were carried out via hierarchical linear models, and within-patient correlation was accounted for with an unstructured correlation type matrix (13). Simple and multiple regression models were used to test for correlations between continuous variables. The interaction between genotype and BMI in modulating OGTT-derived indexes was tested by the general linear model analysis after adjusting for age and sex. χ^2 was used to test for association between categorical variables and genotypes and to test the Hardy-Weinberg equilibrium as well. Variables not normally distributed were log transformed for analyses. A P value <0.05 was considered significant. All analyses were performed using SPSS (version 15.0; SPSS, Chicago, IL) and SAS (release 9.1; SAS Institute, Cary, NC).

RESULTS

Adult subjects

Baseline characteristics. Clinical baseline characteristics of nonobese and obese subjects pooled together and stratified according to the *ENPP1* K121Q genotype are shown in Table 1. Fasting plasma glucose was associated with the Q121 variant and was higher in QQ individuals. Data are also given separately for nonobese and obese individuals (Table 1 of the online appendix).

Glucose and insulin profiles during OGTT. In both nonobese and obese individuals, plasma glucose levels tended to be different across genotype groups, with QQ carriers showing the worst profile (Fig. 1*A* and *B*, respectively in the online appendix), which reached statistical significance in the obese group (age-, sex-, and BMI-adjusted overall P value 4.8×10^{-2}). Because glucose profiles across genotypes were similar in the two groups, data were pooled and analyzed together. Plasma glucose levels during OGTT were different across genotypes (adjusted overall P value 2×10^{-2}) (Fig. 1*A*). At post hoc analysis, the glucose profile was higher in QQ than in KK carriers (adjusted $P = 5 \times 10^{-2}$). Although no significant difference was observed in insulin profiles across genotype groups, a clear genotype-time interaction was observed (adjusted $P = 2.6 \times 10^{-3}$) (Fig. 1*B*). As a matter of fact, plasma insulin levels at 30 min were lower in QQ than in KQ (adjusted $P = 2 \times 10^{-4}$) and KK (adjusted $P = 4 \times 10^{-5}$) carriers.

Insulin secretion and sensitivity. First-phase insulin secretion, as indicated by the insulinogenic index, was different across genotypes (adjusted overall P value 1.2×10^{-6}) (Fig. 1*C*). At post hoc analysis, the insulinogenic index was reduced in QQ (18.5 ± 3.4) compared with KK (31.6 ± 1.0; adjusted $P = 2.2 \times 10^{-7}$) and KQ (30.5 ± 1.5; adjusted $P = 3.2 \times 10^{-6}$) individuals. The insulin sensitivity index was different across genotypes (adjusted overall P value 5×10^{-3}). At post hoc analysis, insulin sensitivity index was reduced in KQ (4.3 ± 0.2; adjusted $P = 3.6 \times 10^{-2}$) but not QQ individuals (5.1 ± 1.1) compared with that of KK (4.9 ± 0.2) subjects (Fig. 1*D*). The disposition index was different across genotypes (adjusted overall P value 7.9×10^{-5}). At post hoc analysis, the disposition index was lower in KQ (142.9 ± 13.4) and QQ (97.2 ± 28.7) compared with that of KK (166.6 ± 9.8) individuals (adjusted $P = 3 \times 10^{-2}$ and 4×10^{-4}, respectively) and lower in QQ than in KQ individuals (adjusted $P = 8 \times 10^{-3}$) (Fig. 1*E*).

Gene-BMI interaction. In the whole sample, insulin sensitivity index was inversely related to BMI (β value for log-transformed BMI −8.40; $P < 1 \times 10^{-10}$). The linear slope was steeper in QQ (β = −11.68) than in KK (β = −8.95) or KQ (β = −6.86) individuals, indicating a gene-BMI interaction (age- and sex-adjusted $P = 5.6 \times 10^{-3}$) in modulating insulin sensitivity (online appendix Fig. 2). In contrast, no gene-BMI interaction was observed in modulating either insulinogenic index (adjusted $P = 0.72$) or disposition index (adjusted $P = 0.50$).

Overweight/obese children. In subjects carrying the KQ genotype, baseline fasting plasma insulin was higher (adjusted $P = 1.5 \times 10^{-2}$) than in KK individuals (Table 1). Although not reaching a nominal statistical significance with the present sample size ($P = 0.21$), plasma glucose levels during OGTT tended to be different across K121Q genotype groups, with QQ individuals showing the worst profile (Fig. 2*A*). Plasma insulin levels during OGTT were different across genotypes (adjusted overall P value 3.5×10^{-3}) and higher, at post hoc analysis, in KQ than in KK subjects (adjusted $P = 2.4 \times 10^{-3}$) (Fig. 2*B*). Insulin secretion and sensitivity data obtained in this sample were very similar to those observed in adults, with QQ individuals showing, compared with KQ and KK subjects, a reduced insulinogenic index ($P = 7 \times 10^{-3}$ and 2×10^{-2}, respectively) (Fig. 2*C*) and disposition index ($P = 2 \times 10^{-2}$ and 7×10^{-3}, respectively) (Fig. 2*E*).

DISCUSSION

Here, we report that adult whites carrying the *ENPP1* Q121 variant show altered glucose homeostasis during OGTT due to an inefficient interplay between insulin secretion and insulin sensitivity (i.e., a reduced disposition index). In hyperglycemic individuals, disposition index

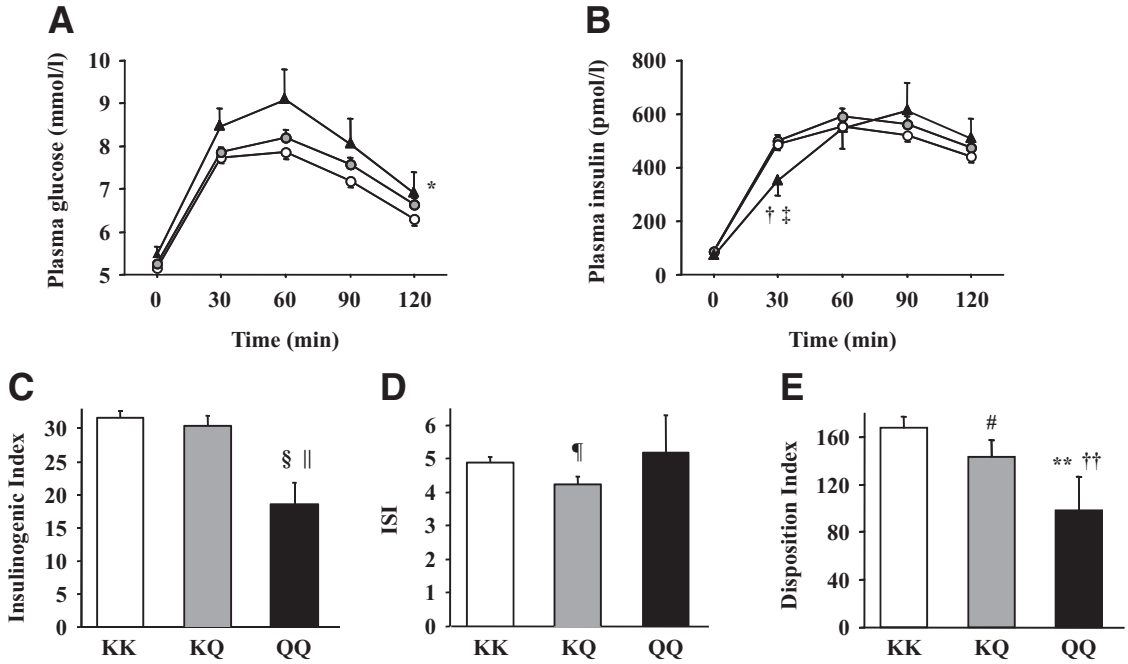

FIG. 1. Glucose, insulin, and derived indexes at OGTT in adult subjects. Glucose (*A*) and insulin (*B*) levels during OGTT in QQ (black triangles), KQ (gray circles), and KK (white circles) adult individuals. Insulinogenic index (*C*), insulin sensitivity index (ISI) (*D*), and disposition index (*E*) values in QQ (black bar), KQ (gray bar), and KK (white bar) adult individuals. All data were adjusted for sex, age, and BMI. *A*: Plasma glucose profiles in QQ vs. KK carriers, *$P = 5 \times 10^{-2}$. *B*: Plasma insulin levels at 30 min in QQ vs. KQ (†$P = 2 \times 10^{-4}$) and vs. KK carriers (‡$P = 4 \times 10^{-5}$). *C*: QQ vs. KK carriers, §$P = 3.2 \times 10^{-6}$; QQ vs. KK carriers, ‖$P = 2.2 \times 10^{-7}$. *D*: KQ vs. KK carriers, ¶$P = 3.6 \times 10^{-2}$. *E*: KQ vs. KK carriers, #$P = 3 \times 10^{-2}$; QQ vs. KK carriers, **$P = 4 \times 10^{-4}$; QQ vs. KQ carriers, ††$P = 8 \times 10^{-3}$.

may be reduced because of reduced insulin secretion, insulin resistance, or both. Our data show that QQ carriers have impaired first-phase insulin secretion, which entirely account for their reduced disposition index. Conversely, the reduction of disposition index in KQ subjects, who have a perfectly normal first-phase insulin secretion, is entirely due to insulin resistance. The insulin sensitivity index was not reduced in QQ individuals as a whole. It should be considered that insulin resistance inferred from increased endogenous insulin levels (as done in our case) may be underestimated in individuals with reduced insulin secretion such as QQ carriers seem to be. We observed a

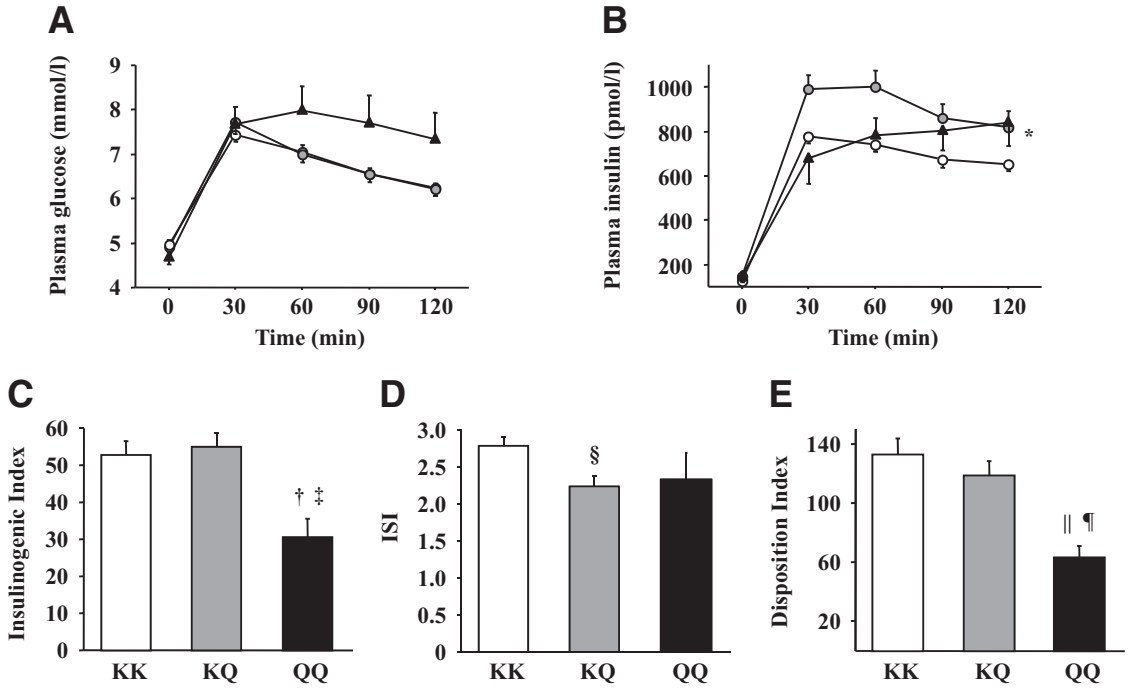

FIG. 2. Glucose, insulin, and derived indexes at OGTT in children. Glucose (*A*) and insulin (*B*) levels during OGTT in QQ (black triangles), KQ (gray circles), and KK (white circles) individuals. Insulinogenic index (*C*), insulin sensitivity index (ISI) (*D*), and disposition index (*E*) in KK (white bar), KQ (gray bar), and QQ (black bar) individuals. All data were adjusted for sex, age, and BMI *z* score. *B*: Plasma insulin levels in KQ vs. KK carriers, *$P = 2.4 \times 10^{-3}$. *C*: QQ vs. KQ carriers, †$P = 7 \times 10^{\times 3}$; QQ vs. KK carriers, ‡$P = 2 \times 10^{-2}$. *D*: KQ vs. KK carriers, §$P = 6 \times 10^{-3}$. *E*: QQ vs. KQ carriers, ‖$P = 2 \times 10^{-2}$; QQ vs. KK carriers, ¶$P = 7 \times 10^{-3}$.

significant genotype-BMI interaction in determining insulin sensitivity, which is in line with other evidence showing such interaction in modulating the risk of hyperinsulinemia (14), atherosclerosis (15), and type 2 diabetes (16,17). Based on the linear slopes indicating the relationships between insulin sensitivity index and BMI across genotype groups, it appears that QQ individuals tend to have a reduced insulin sensitivity index among frankly obese, but not among lean, subjects. This may be due to the protective role of the QQ genotype on BMI (18), which, when successfully operating (as it is in lean individuals), is likely to preserve insulin sensitivity. We hypothesize that among lean individuals, in the absence of a coexistent reduction of insulin sensitivity, the defective first-phase insulin secretion observed in QQ individuals is not sufficient by itself to increase the risk of type 2 diabetes. In contrast, in the presence of an obesogenic background, the deleterious effect of the Q121 variant on insulin secretion is added on top of that exerted on insulin sensitivity, thus eventually increasing the risk of type 2 diabetes.

Overall, the novel findings of our study are that *1*) the *ENPP1* Q121 variant affects insulin secretion; *2*) for this effect to be evident, the homozygous state is necessary; and *3*) the homozygous state is also necessary for a strong deleterious effect on glucose homeostasis and disposition index, which, in fact, are significantly worse in QQ than in KQ subjects. These data perfectly fit with, and possibly explain, the increased risk of type 2 diabetes of QQ (but not KQ) individuals described by a large meta-analysis comprising all studies thus far published in whites (10).

Our findings suggest a role of altered insulin signaling in increasing the risk of type 2 diabetes as well as having a direct negative effect on β-cell function (19). As a matter of fact, cultured β-cells and animals with deficient insulin receptors (20) or insulin receptor substrate-1 (IRS-1) (21) have reduced insulin secretion. This defect is mainly mediated by the impaired insulin stimulation of the downstream phosphatidylinositol 3-kinase (PI3-K) activity, which alters vesicle trafficking (22) and the release of intracellular calcium stores (21). Insulin stimulation of insulin receptors (7,9), IRS-1 (7), and PI3-K (7) activation is clearly reduced in nonpancreatic cells carrying the Q121 variant. Thus, although the suggestion is entirely speculative, altered β-cell function in Q121 carriers might be secondary to defective activation of the insulin receptor/IRS-1/PI3-K signaling pathway.

A second important finding of our study is that reduced first-phase insulin secretion and the inefficient interplay between insulin secretion and sensitivity occur at early ages in obese QQ individuals. Although children of normal weight would have been an interesting group to examine in order to investigate the role of the Q121 variant on glucose homeostasis, our present finding is, nonetheless, of clinical importance given the recent emergence of hyperglycemia in the early stage of life (23).

The rs7767502 single nucleotide polymorphism, which is in perfect linkage disequilibrium with K121Q ($r^2 = 1$), was not associated with either the insulinogenic index or the homeostasis model assessment of insulin resistance index ($P = 0.47$ and 0.34, respectively) among Scandinavians in the Diabetes Genetics Initiative (DGI) (http://www.broad.mit.edu/diabetes) (24). Several factors may underlie this apparent discrepancy. First, the DGI did not evaluate the recessive genetic model, which is clearly accounting for defective insulin secretion in our population. In addition, some heterogeneity in the diabetogenic effect of the Q121 variant has been reported among whites (10). A similar heterogeneity among Europeans has also been reported for the peroxisome proliferator–activated receptor γ (*PPARγ*) P12A polymorphism, a well-established genetic marker of type 2 diabetes (25). Thus, a population specific effect cannot be excluded a priori and should be formally tested in further multicenter studies. Unfortunately, neither insulin sensitivity index nor disposition index values are available in the DGI database.

The risk of false positive results can affect the genetic investigation of complex traits. Thus, despite the strong *P* values observed (i.e., in the range of the genome-wide significance for glucose profiles and early insulin secretion), the very similar results obtained in a cohort of overweight/obese children, the genetic homogeneity of the samples studied (12), and the clear consistency with the risk of type 2 diabetes reported in QQ individuals (10) render unlikely a false positive result. Our present findings have to be formally replicated in independent cohorts before they can be considered definitive.

In conclusion, carriers of the *ENPP1* Q121 variant, especially in the homozygous state, show altered glucose homeostasis. Reduced first-phase insulin secretion and an inefficient interplay between insulin secretion and sensitivity, which occur at early ages, are major determinants of this defect and are likely to play a role also in the increased risk of type 2 diabetes displayed by these individuals (10).

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