Role of the ENPP1 K121Q Polymorphism on Glucose Homeostasis

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ABSTRACT

Objective: To study the role of the ENPP1 Q121 variant on glucose homeostasis in Whites from Italy.

Research Design and Methods: Case-control studies in 764 adults (i.e. from two independent samples of 289 non-obese and 485 obese individuals) and 240 overweight/obese children undergoing OGTT. Early-phase insulin secretion and insulin sensitivity (i.e. the insulinogenic index, IGI and the insulin sensitivity index, ISI) and their interplay (i.e. the disposition index, DI) were calculated.

Results: Adult subjects. Glucose profiles during OGTT were significantly ($p=2 \cdot 10^{-2}$) different across K121Q genotype groups, being higher in QQ vs. KK individuals ($p=5 \cdot 10^{-2}$). The IGI, was significantly reduced in QQ (18.5±3.4) as compared to both KK (31.6±1.0; $p=2.2 \cdot 10^{-7}$) and KQ (30.5±1.5; $p=3.2 \cdot 10^{-6}$) individuals. KQ individuals also showed a reduced ISI as compared to KK subjects ($p=3.6 \cdot 10^{-2}$). The DI was lower in QQ carriers vs. KQ and KK individuals ($p=8 \cdot 10^{-3}$ and $4 \cdot 10^{-4}$, respectively) and in KQ vs. KK individuals ($p=3 \cdot 10^{-2}$).

Overweight/obese children. Data obtained in this sample were very similar to those observed in adults with QQ individuals showing, as compared to KQ and KK subjects, reduced IGI ($p=7 \cdot 10^{-3}$ and $2 \cdot 10^{-2}$, respectively) and DI ($p=2 \cdot 10^{-2}$ and $7 \cdot 10^{-3}$, 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.
Insulin resistance and inadequate insulin secretion are pathogenic for type 2 diabetes (T2D) (1). Insulin resistant subjects who eventually develop T2D, maintain normal or near-normal glycemia for many years because of compensatory hyper-insulinemia (2). T2D eventually ensues only when beta-cell fails to secrete sufficient insulin to adequately counteract a insulin resistance (3). Thus, an inefficient interplay between insulin secretion and sensitivity is the key pathogenic factor for developing T2D. In agreement with this tenet, the disposition index (DI; i.e. the product of insulin secretion x insulin sensitivity), is the best predictor of T2D (4).

The ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) (5) down-regulates insulin signalling by direct inhibition of the insulin receptor (IR) signalling (6-8). We previously described an ENPP1 missense polymorphism (K121Q, rs1044498) (9). As compared to the more common K121, the Q121 variant is a stronger inhibitor of IR signalling 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 T2D (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 T2D (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 get insights 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**

**Study subjects.** A total of 764 adult (age: 18-70 years) and 240 children (age: 8-17 years) unrelated subjects from Eastern Sicily, with fasting plasma glucose <7.0 mmol/l, 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 online-appendix for details of their clinical characteristics, study design and OGTT-derived measurements). People from Eastern Sicily have been reported to be genetically homogeneous (12), a feature that minimizes the risk of population stratification.

The study protocol was approved by the institutional review board and performed according to the Helsinki Declaration. A 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 RFLP (9), with a failure rate <1%. Genotyping quality was checked by directly sequencing 10% of randomly selected samples. The agreement rate of re-sequenced samples was >99%. The proportion of the K121Q genotypes was in Hardy-Weinberg equilibrium (HWE) in both samples studied.

**Statistical analysis.** Values are provided as mean±SEM. All the analyses are adjusted for age, gender 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-patients 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 gender. Chi-square was used to test for association between categorical variables and genotypes and to test the HWE as well. Not normally distributed variables were log-transformed for analyses. A p value <0.05 was considered as significant. All analyses were performed using SPSS Version 15.0 (Chicago, IL, USA) and SAS Release 9.1 (SAS Institute, Cary, NC).

**RESULTS**

**ADULT SUBJECTS.**
**Baseline characteristics.** Clinical baseline characteristics of non-obese and obese subject pooled together and stratified according to the ENPP1 K121Q genotype are shown in table 1 and indicate that the Q121 variant was associated with fasting glucose, being higher in QQ individuals. Data are also given separately for non-obese and obese individuals (table 1, online-appendix).

**Glucose and insulin profiles during OGTT.** In both non-obese and obese individuals, plasma glucose levels tended to be different across genotype groups, with QQ carriers showing the worst profile (figure 1A and B, respectively; online-appendix), which reached statistical significance in the obese group (age, gender, BMI adjusted overall \(p\)-value=4.8·10^{-2}). Since 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}, figure 1A). At post-hoc analysis, glucose profile was higher in QQ vs. KK carriers (adjusted \(p=5·10^{-2}\)). Although no significant difference was observed in insulin profiles across genotype groups, a clear genotype-time interaction was observed (adjusted \(p=2·6·10^{-3}\), figure 1B). As a matter of fact, plasma insulin levels at 30 min were lower in QQ vs. KQ (adjusted \(p=2·10^{-4}\)) and KK (adjusted \(p=4·10^{-4}\)) carriers.

**Insulin secretion and sensitivity.** First-phase insulin secretion, as indicated by the insulinogenic index (IGI), was different across genotypes (adjusted overall \(p\)-value=1.2·10^{-6}, figure 1C). At post-hoc analysis, the IGI was reduced in QQ (18.5±3.4) vs. KK (31.6±1.0; adjusted \(p=2.2·10^{-7}\)) and KQ (30.5±1.5; adjusted \(p=3.2·10^{-6}\)) individuals. The insulin sensitivity index (ISI) was different across genotypes (adjusted overall \(p\)-value=5·10^{-5}). At post-hoc analysis, ISI was reduced in KQ (4.3±0.0, adjusted \(p=3.6·10^{-2}\)), but not in QQ individuals (5.1±1.1), as compared to KK (4.9±0.2) subjects (figure 1D). The disposition index (DI) was different across genotypes (adjusted overall \(p\)-value=7.9·10^{-7}). At post-hoc analysis, the DI was lower in KQ (142.9±13.4) and QQ (97.2±28.7) vs. KK (166.6±9.8) individuals (adjusted \(p=3·10^{-2}\) and 4·10^{-4}, respectively) and in QQ vs. KQ individuals (adjusted \(p=8·10^{-3}\)) (figure 1E).

**Gene-BMI interaction.** In the whole sample, ISI was inversely related to BMI (beta for log-transformed BMI=−8.40, \(p<1·10^{-10}\)). The linear slope was steeper in QQ (beta=−11.68) than in KK (beta=−8.95) or KQ (beta=−6.86) individuals, indicating a gene-BMI interaction (age, gender adjusted \(p=5.6·10^{-3}\) in modulating insulin sensitivity (figure 2; online-appendix). In contrast, no gene-BMI interaction was observed in modulating either IGI (adjusted \(p=0.72\)) or DI (adjusted \(p=0.50\)).

**OVERWEIGHT/OBESE CHILDREN.**

**Baseline characteristics.** In subjects carrying the KQ genotype, fasting plasma insulin was higher (adjusted \(p=1.5·10^{-5}\)) than in KK individuals (table 1).

**Glucose and insulin profiles during OGTT.** Although not reaching a nominal statistical significance with the present sample size (\(p=0.21\)), plasma glucose levels tended to be different across K121Q genotype groups, with QQ individuals showing the worst profile (figure 2A). Plasma insulin levels were different across genotypes (adjusted overall \(p\)-value=3.5·10^{-3}), being higher, at post-hoc analysis, in KQ than KK subjects (adjusted \(p=2.4·10^{-3}\)) (figure 2B).

**Insulin secretion and sensitivity.** Data obtained in this sample were very similar to those observed in adults with QQ individuals showing, as compared to KQ and KK subjects, reduced IGI (\(p=7·10^{-3}\) and 2·10^{-2}, respectively, figure 2C) and DI (\(p=2·10^{-2}\) and 7·10^{-3}, respectively, figure 2E).

**DISCUSSION**

We here 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 DI). In hyperglycemic individuals, DI 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 DI. Conversely, the reduction of DI in KQ subjects, who have a perfectly normal first-phase insulin secretion, is entirely due to insulin resistance.

ENPP1 Q121 variant and diabetes-related traits
ISI 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 significant genotype-BMI interaction in determining insulin sensitivity which is in line with other evidence showing such interaction in modulating the risk of hyper-insulinemia (14), atherosclerosis (15) and T2D (16,17). By looking at the linear slopes indicating the relationships between ISI and BMI across genotype groups, it appears that QQ individuals tend to have a reduced ISI 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 like to 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 T2D. In contrast, in the presence of an ‘obesogenic’ background the deleterious effect of Q121 variant on insulin secretion is added on the top of that exerted on insulin sensitivity thus, eventually, increasing the risk of T2D.

Overall, the novel findings of our study are that a) the \textit{ENPP1} Q121 variant affects insulin secretion; b) for this effect to be evident, the homozygous state is necessary; c) the homozygous state is necessary also for a strong deleterious effect on glucose homeostasis and on DI which, in fact, are significantly worse in QQ than in KQ subjects. These data perfectly fit with, and possibly explain the, increased risk of T2D of QQ (but not KQ) individuals described by a large meta-analysis comprising all studies so far published in Whites (10).

Our findings are consistent with a role of altered insulin signalling in increasing the risk of T2D also through a direct negative effect on beta-cell function (19). As a matter of fact, cultured beta-cells and animals with deficient IR (20) or IR-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 vesicles trafficking (22) and release of intracellular calcium stores. (21). Insulin-stimulation of IR (7,9), IRS-1 (7) and PI3-K (7) activation is clearly reduced in non pancreatic cells carrying the Q121 variant. Thus, although entirely speculative, altered beta-cell function in Q121 carriers might be secondary to defective activation of the IR/IRS-1/PI3-K signalling 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 SNP, which is in perfect LD with K121Q ($r^2=1$), was not associated with neither IGI nor insulin resistance HOMA-IR index ($p=0.47$ and 0.34, respectively) among Scandinavians in the Diabetes Genetics Initiative (DGI; http://www.broad.mit.edu/diabetes) study (24). Several reasons 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 been reported also for the \textit{PPAR} $\Delta_2 P12A$ polymorphism, a well established genetic marker of T2D (25). Thus a population specific effect cannot be \textit{a priori} excluded and should be formally tested in further multi-centre studies. Unfortunately, neither ISI nor DI values are available in the DGI data-base.

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 T2D reported in QQ individuals (10) render unlikely a false positive result, our present findings have to be formally replicated in independents cohort before they can be considered as definitive.

In conclusion, carriers of the ENPP1 Q121 variant, especially in the homozygous state, show altered glucose homeostasis. A 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 T2D displayed by these individuals (10).

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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         |
| **Number (%)**      |            |            |            |            |            |            |
| KK                  | 528 (69%)  | 215 (28%)  | 21 (3%)    | 166 (69%)  | 64 (27%)   | 10 (4%)    |
| KQ                  | 215 (28%)  | 73 (29%)   | 6 (3%)     | 64 (27%)   | 22 (9%)    | 2 (1%)     |
| QQ                  | 21 (3%)    | 6 (3%)     | 10 (4%)    | 10 (4%)    | 2 (1%)     | 2 (1%)     |
| **Males/Females**   | 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/m²)**     | 35.5±0.46  | 36.6±0.77  | 34.4±2.24  | -          | -          | -          |
| **BMI z-score (kg/m²)** | -         | -          | -          | 2.23±0.03  | 2.36±0.12  | 2.30±0.10  |
| **PG (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 (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  |
| **TG (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 mean±SEM. Significances are given after adjusting for age, gender and BMI or BMI z-score. PG: plasma glucose; IRI: immunoreactive insulin; HDL: high-density lipoprotein cholesterol; TG: triglycerides.

*p=1.6·10⁻² vs. KK adults and †p=1.5·10⁻² vs. KK children and adolescents.
**Figure 1. Glucose, insulin and derived indexes at OGTT in adult subjects**

Glucose (panel A) and insulin (panel B) levels during OGTT in QQ (black triangles), KQ (grey circles) and KK (white circles) adult individuals. Insulinogenic index (IGI, panel C), insulin sensitivity index (ISI, panel D) and disposition index (DI, panel E) values in QQ (black bar), KQ (grey bar) and KK (white bar) adult individuals. All data were adjusted for gender, age and BMI.

Panel A. Plasma glucose profiles in QQ vs. KK carriers: \( *p=5\times10^{-2} \).

Panel B. Plasma insulin levels at 30 min in QQ vs. KQ (\( \uparrow p=2\times10^{-4} \)) and vs. KK carriers (\( \uparrow \uparrow p=4\times10^{-5} \)).

Panel C. QQ vs. KQ carriers: \( \uparrow \uparrow p=3.2\times10^{-6} \); QQ vs. KK carriers: \( \parallel p=2.2\times10^{-7} \).

Panel D. KQ vs. KK carriers: \( \uparrow \uparrow p=3.6\times10^{-2} \).

Panel E. KQ vs. KK carriers: \( p=3\times10^{-2} \); QQ vs. KK carriers: \( ** p=4\times10^{-4} \); QQ vs. KQ carriers: \( \uparrow \uparrow p=8\times10^{-3} \).
Figure 2. Glucose, insulin and derived indexes at OGTT in children
Glucose (panel A) and insulin (panel B) levels during OGTT in QQ (black triangles), KQ (grey circles) and KK (white circles) individuals. Insulinogenic index (IGI, panel C), insulin sensitivity index (ISI, panel D) and disposition index (DI, panel E) in KK (white bar), KQ (grey bar) and QQ (black bar) individuals. All data were adjusted for gender, age and BMI z-score.
Panel B. Plasma insulin levels in KQ vs. KK carriers: *$p=2.4\cdot10^{-3}$.
Panel C. QQ vs. KQ carriers: † $p=7\cdot10^{-3}$; QQ vs. KK carriers: ‡ $p=2\cdot10^{-2}$.
Panel D. KQ vs. KK carriers: § $p=6\cdot10^{-3}$.
Panel E. QQ vs. KQ carriers: || $p=2\cdot10^{-2}$; QQ vs. KK carriers: ¶ $p=7\cdot10^{-3}$.