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Original article

Functional glucokinase regulator gene variants have inverse effects on triglyceride and glucose levels, and decrease the risk of obesity in children

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Abstract

Objective. – Recently, the association of the natural variants rs1260326 and rs780094 of the glucokinase regulatory protein (*GCKR*) gene with increased fasting triglycerides and decreased fasting plasma glucose in diabetic adults was reported; the minor alleles were also found to reduce the risk of type 2 diabetes. The present study examined the possible associations of these variants with triglycerides and glucose levels, their allele distribution and their possible effects on childhood obesity.

Methods and results. – A total of 221 obese children and 115 healthy normal-weight children as controls were genotyped using PCR–RFLP methods. Both functional *GCKR* variants were found in association with elevated serum triglycerides and lower fasting plasma glucose levels. Results of logistic regression revealed that, despite higher triglyceride levels, the carriers of the *GCKR* variants were more protected against the development of obesity; the adjusted models confirmed the lower risk of obesity for both variants (rs1260326: OR, 0.46; 95% CI, 0.25–0.83; rs780094: OR, 0.41; 95% CI, 0.23–0.74).

Conclusion. – Our findings confirm the inverse modulating effect of functional *GCKR* variants on triglycerides and glucose levels in obese paediatric patients and healthy normal-weight controls. The results of our study strongly suggest that the minor alleles confer protection against the development of obesity in children. The findings also suggest that the minor alleles of functional *GCKR* may protect against diabetes and the metabolic syndrome in adults.

Keywords: *GCKR*; Glucokinase Regulatory Protein; Children; Obesity; Serum triglyceride; Fasting plasma glucose

Résumé

Les variants du gène régulateur de la protéine glucokinase *GCKR* ont des effets opposés sur les triglycérides et la glycémie et diminuent le risque de l'obésité chez l'enfant.

Objectif. – Il a été rapporté récemment une association entre les variants naturels rs1260326 et rs780094 du gène régulateur de la protéine glucokinase (*GCKR*, *glucokinase regulatory protein*) et une augmentation des triglycérides et une diminution de la glycémie à jeun chez des diabétiques adultes. Il a également été montré que ces allèles mineurs réduisaient le risque de diabète de type 2. Dans cette étude, nous avons examiné chez l'enfant l'association possible de ces variants avec les triglycérides et la glycémie, leur distribution allélique et étudié leur effet éventuel sur l'obésité.

Méthodes et résultats. – Le génotypage par PCR-RFLP de 221 enfants obèses et 115 enfants témoins de poids normal a été réalisé. Les deux variants fonctionnels de *GCKR* étaient associés à des triglycérides plus élevés et une glycémie plus faible. L'analyse par régression logistique a montré qu'en dépit de l'augmentation des triglycérides, les porteurs des variants de *GCKR* étaient protégées vis-à-vis du développement de l'obésité. Après ajustement, la diminution du risque d'obésité était confirmée pour les deux variants (rs1260326 : OR 0,46, IC à 95 % 0,25–0,83 ; rs780094 : OR 0,41, IC à 95 % 0,23–0,74).

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Conclusions. – Ces résultats indiquent que les variants fonctionnels de *GCKR* exercent un effet inverse sur les triglycérides et la glycémie chez l'enfant obèse et chez l'enfant de poids normal, et qu'ils confèrent une protection vis à vis du développement de l'obésité. Ils suggèrent que les allèles fonctionnels mineurs de *GCKR* pourraient avoir un effet protecteur chez l'adulte vis-à-vis du développement du diabète et du syndrome métabolique.

Mots clés : *GCKR* ; Glucokinase Regulatory Protein ; variants fonctionnels ; Enfant ; Obésité ; Triglycérides ; Glycémie

1. Introduction

The presence of obesity in children is a critical risk factor for the early development of atherosclerosis and the metabolic syndrome, both of which significantly contribute to the early onset of type 2 diabetes (T2D) and cardiovascular disease [1–3]. T2D is a classic example of a complex disease, as both environmental and genetic factors, as well as interactions among these factors, all contribute to disease development [4–7].

The glycolytic enzyme glucokinase plays a central role in maintaining blood glucose homeostasis [8,9]. The activity of this key enzyme is allosterically controlled in hepatocytes and pancreatic cells by glucokinase regulatory protein at the cellular level (*GCKR*) [9–14]. This regulator inhibits the activity of glucokinase by binding the enzyme non-covalently to form an inactive complex in the presence of fructose 6-phosphate [15–17]. The *GCKR* gene, located on chromosome 2p23.3–p23.2, consists of 19 exons and encodes a protein comprising 625 amino acids [10,11,16,18].

In a recent study, the rs780094 variant of the *GCKR* gene (minor allele frequency [MAF]: 38%) was found to be associated with elevated triglycerides and lower fasting glucose levels, decreased insulin resistance and a lower risk of T2D [19]; similar findings were also reported in a large Danish population [20]. The association of the *GCKR* rs780094 A minor allele with a decreased risk of T2D and obesity in an adult Han Chinese sample population has also been reported [21]. In addition, the French Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) study adult cohort revealed that carriers of the *GCKR* rs1260326 minor variant 446L have lower fasting glycaemia as well as insulin resistance and are protected against the development of T2D despite the raised triglyceride levels and risk of dyslipidaemia [22]. In a genome-wide study, the rs1260326 polymorphism in the *GCKR* gene was found to be associated with an extreme phenotype of abnormal plasma triglyceride. The results showed a significant accumulation of the rs1260326 446L variant (MAF: 52%) in subjects with hypertriglyceridaemia (HTG) [23]. No similar data have been reported in obese children, but an interaction effect of the rs780094 variant in *GCKR* and the rs1799884 variant in the *GCK* gene on metabolic traits was detected in a study of a healthy Chinese population, including both adults and adolescents [24].

The aim of our present study was to investigate the possible association of the *GCKR* gene variants rs780094, located in intron 16 of the gene, and rs1260326 in exon 15, with triglyceride and fasting glucose levels, and to study their allele distributions in obese Hungarian paediatric patients and controls.

2. Materials and methods

2.1. Study population

The present investigations were carried out in 221 obese children (122 boys, 99 girls; age: 13.5 ± 0.16 years; body mass index [BMI]: $31.5 \pm 0.32\,kg/m^2$) and in 115 healthy normal-weight children as controls (56 boys, 59 girls; age: 14.1 ± 0.21 years; BMI: $20.2 \pm 0.32\,kg/m^2$). Subjects were included in the study after the exclusion of chronic diseases, any endocrinological, nutritional, growth and renal diseases, and obesity syndromes. None of the children in either the obese or control groups were taking any kind of medication. Anthropometric measurements were carried out by the same investigator in the survey unit. Body height was measured to the nearest 0.1 cm by a Holtain stadiometer, while weight was obtained to the nearest 0.1 kg on a standard beam scale. BMI was calculated according to the formula: weight (kg) divided by squared height (m^2). Children were considered to be obese if their BMI was equivalent to a value above $25\,kg/m^2$ at the age of 18 years, as suggested by Cole et al. [25]. Blood samples were taken between 8:00 and 9:00 AM after an overnight fast. The blood specimens were centrifuged immediately after being collected. Triglyceride and total cholesterol parameters were measured by a modular automatic system (Hoffmann-La Roche Ltd, Basel, Switzerland), and HTG in the patients and controls was defined as a triglyceride level above or equal to 1.1 mmol/L [26].

The patients' DNA, together with the clinical dataset, was deposited at the local biobank. Patients also gave their informed consent for future genetic testing of the samples and for data analysis. The local biobank was established with the authorization of the National Ethics Committee.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes, using a standard desalting method. For polymerase chain reaction (PCR) amplification, the following primers were used: *GCKR* rs1260326: forward 5′-TGC AGA CTA TAG TGG AGC CG-3′ and reverse 5′-CAT CAC ATG GCC ACT GCT TT-3′; *GCKR* rs780094: forward 5′-GAT TGT CTC AGG CAA ACC TGG TAG-3′ and reverse 5′-CTA GGA GTG GTG GCA TAC ACC TG-3′. The amplifications were executed using an MJ Research PTC-200 thermal cycler (Bio-Rad, Hercules, CA, USA). PCR conditions for rs1260326 were the following: predenaturation at 96 °C for 2 min, followed by 35 cycles of denaturation at 96 °C for 20 s, annealing at 60 °C for 20 s, primer extension for 30 s at 72 °C and final extension at 72 °C for 5 min.