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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Received 2 August 2010; received in revised form 7 February 2011; accepted 9 February 2011
Available online 20 April 2011

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.

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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 ± 0.32 kg/m²) and in 115 healthy normal-weight children as controls (56 boys, 59 girls; age: 14.1 ± 0.21 years; BMI: 20.2 ± 0.32 kg/m²). 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 (m²). Children were considered to be obese if their BMI was equivalent to a value above 25 kg/m² 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: pre-denaturation 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.
In the case of rs780094, the values of predenaturation, primer extension and final extension conditions were the same as for rs1260326. Predenaturation was followed by 30 cycles of denaturation at 96 °C for 20 s, annealing at 62 °C for 30 s, then primer extension and final extension. Amplification was in a final volume of 50 μL, including 5 μL reaction buffer (500 mM KCl, 14 mM MgCl₂, 10 mM Tris-HCl, pH 9.0), 1 μL 50 mM MgCl₂, 0.2 mM of each dNTP, 1 U of Taq polymerase, 0.2 mM of the adequate specific primer pairs and 1 μg DNA sample.

For the restriction fragment length polymorphism (RFLP) analysis, the target regions were amplified with specific primers that amplified a genomic region containing the polymorphism studied and an obligatory restriction site. This way, during restriction digestion, the enzymes cleaved the PCR product at the obligatory restriction site, regardless of the genotype-dependent cleavage site harbouring the polymorphism. The digested fragments were visualized by ultraviolet (UV) transillumination. For the digestion of rs1260326 and rs780094, the restriction endonuclease enzymes HpaII and PscI (Fermentas, Burlington, ON, Canada), respectively, were used. After the digestion of rs1260326 amplicons (231 bp), the normal (CC) genotype resulted in 18, 63 and 150 bp bands; in samples with a homozygous genotype (TT), 18 and 213 bp fragments appeared and, in samples with a heterozygous genotype, 18, 63, 150, and 213 bp digestion products were detected. The digestion of the rs780094 (427 bp) amplicon resulted in the following fragments: normal (GG) genotype: 62, 177 and 188 bp; homozygous genotype (AA): 62 and 365 bp; and heterozygous genotype: 62, 177, 188 and 365 bp fragments. In random samples, the PCR products were subjected to direct sequencing to test and confirm the genotyping results obtained by RFLP methods.

### 2.3. Statistical analysis

All data are presented as means ± SEM. The Mann–Whitney U test was used to compare quantitative data between obese patients and normal-weight controls. To compare metabolite data between individual GCKR polymorphisms, the one-way ANOVA test was applied. The chi-square test (χ²) was used to compare the allele frequencies in the patient vs control groups. Odds ratios (ORs) were calculated using logistic-regression analysis models to evaluate the possible associations of GCKR variants with obesity and HTG. A P value less than 0.05 was considered statistically significant. All statistical calculations were executed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

### 2.4. Linkage disequilibrium (LD) calculations

To evaluate the LD of the studied variants, single nucleotide polymorphism (SNP) genotype data were downloaded from the HapMap database (HapMap Data Rel 24/phase II Nov 08 on NCBI B36 assembly dbSNP b126), and the LD calculated using HaploView 4.2 software.

### 3. Results

The major clinical and laboratory parameters of the patients and controls are presented in Table 1. BMI, serum triglyceride, total cholesterol and fasting plasma glucose levels were significantly increased in the obese paediatric patients compared with the healthy normal-weight children (Table 1).

The associations of the individual GCKR variants with serum triglycerides, total serum cholesterol, fasting plasma glucose levels and BMI are shown in Table 2. An opposing effect was observed with both rs1260326 and rs780094 on triglyceride and fasting plasma glucose levels. The minor allele carriers of both SNPs showed raised triglycerides and lowered fasting plasma glucose concentrations compared with the non-carriers in the obese patients as well as in the controls. When the homozygous form of rs1260326 (TT) was compared with the heterozygous (CT) and carrier (CT + TT) forms in the obese patients, a stronger triglyceride-raising effect was observed. Total serum cholesterol levels were increased only in obese children with the TT genotype. For BMI, no variant-dependent changes could be detected (Table 2).

Table 3 shows the allele frequencies and results of the multivariate logistic-regression analysis. Allele frequencies of the rs1260326 and rs780094 variants were in Hardy–Weinberg equilibrium in both cohorts. The LD between GCKR rs1260326 and rs780094 variants in this paediatric population was strong (r² = 0.94; Fig. 1). There were no differences observed in allele frequencies between obese patients and controls, although the prevalence of carriers (homozygous and homozygous subjects together) of each variant was significantly decreased in the obese children compared with the healthy lean children. The results of the logistic-regression models revealed that being a carrier of both the GCKR rs780094 (GA + AA) and rs1260326 (CT + TT) minor alleles may be protective against obesity in children after adjusting the regression analysis for age, gender, triglycerides, cholesterol and fasting glucose (Table 3). When all subjects and the obese children were analyzed separately for HTG, a higher frequency of the minor allele was observed, as well as an increased prevalence of carriers of two GCKR variants in the HTG groups vs normal triglyceride (NTG) groups in all subjects and in obese children. The results of the logistic-regression analysis showed a strong correlation between carriers (CT + TT rs1260326, GA + AA rs780094) and HTG in both the obese group and all study subjects compared with the NTG sample.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Obese children (n = 221)</th>
<th>Healthy children (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (males/females, n/n)</td>
<td>122/99</td>
<td>56/59</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.5 ± 0.16</td>
<td>14.1 ± 0.21</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.5 ± 0.32</td>
<td>20.2 ± 0.32</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1.43 ± 0.03</td>
<td>1.00 ± 0.03</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/L)</td>
<td>4.51 ± 0.06</td>
<td>4.00 ± 0.06</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>4.71 ± 0.03</td>
<td>4.17 ± 0.03</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM.  

*P < 0.05 vs healthy lean children, Mann–Whitney U test.

### Table 3

<table>
<thead>
<tr>
<th>SNP</th>
<th>95% CI of OR (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1260326 (CT + TT)</td>
<td>0.32a (0.16)</td>
</tr>
<tr>
<td>rs780094 (GA + AA)</td>
<td>0.21 (0.03a)</td>
</tr>
</tbody>
</table>

rs1260326, GA + AA rs780094 and CT + TT rs1260326, GA + AA rs780094 are significantly decreased in the obese children compared with the healthy lean children (P < 0.05).
Table 2
Serum triglyceride, cholesterol and fasting plasma glucose levels, and BMI in obese children and control subjects, stratified by individual genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Obeze children</th>
<th>Healthy lean children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>Serum triglycerides (mmol/L)</td>
</tr>
<tr>
<td><strong>GCKR rs1260326</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (n=66)</td>
<td>31.2 ± 0.61</td>
<td>1.25 ± 0.04</td>
</tr>
<tr>
<td>CT + TT (n=122 + 33)</td>
<td>31.7 ± 0.37</td>
<td>1.50 ± 0.04</td>
</tr>
<tr>
<td>CT (n=122)</td>
<td>31.6 ± 0.42</td>
<td>1.43 ± 0.03</td>
</tr>
<tr>
<td>TT (n=33)</td>
<td>31.8 ± 0.78</td>
<td>1.77 ± 0.13</td>
</tr>
<tr>
<td>GG (n=73)</td>
<td>31.4 ± 0.58</td>
<td>1.27 ± 0.04</td>
</tr>
<tr>
<td>GA + AA (n=110 + 38)</td>
<td>31.6 ± 0.38</td>
<td>1.51 ± 0.04</td>
</tr>
<tr>
<td><strong>GCKR rs780094</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (n=21)</td>
<td>19.1 ± 0.59</td>
<td>1.04 ± 0.03</td>
</tr>
<tr>
<td>CT + TT (n=79 + 15)</td>
<td>20.5 ± 0.37</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>CT (n=79)</td>
<td>20.6 ± 0.42</td>
<td>1.06 ± 0.08</td>
</tr>
<tr>
<td>TT (n=15)</td>
<td>19.6 ± 0.60</td>
<td>0.83 ± 0.06</td>
</tr>
<tr>
<td>GG (n=22)</td>
<td>19.2 ± 0.61</td>
<td>1.04 ± 0.03</td>
</tr>
<tr>
<td>GA + AA (n=79 + 14)</td>
<td>20.4 ± 0.36</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td><strong>GA (n=110)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AA (n=38)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 vs CC with GCKR rs1260326; vs GG with GCKR rs780094

*P < 0.05 vs CT genotype with GCKR rs1260326; ANOVA test and Mann–Whitney U test (means ± SEM)
Table 3
Multiple logistic-regression analysis models of GCKR rs1260326 and GCKR rs780094 single nucleotide polymorphisms (SNPs) for obesity and hypertriglyceridaemia (HTG).

<table>
<thead>
<tr>
<th></th>
<th>Logistic regression for obesity</th>
<th>Logistic regression for HTG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy lean children (n = 115)</td>
<td>Obese children (n = 221)</td>
</tr>
<tr>
<td></td>
<td>χ²</td>
<td>P</td>
</tr>
<tr>
<td>GCKR rs1260326 T (MAF %)</td>
<td>0.47</td>
<td>0.43</td>
</tr>
<tr>
<td>CT + TT carrier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model for carrier T: OR, (95% CI), P value</td>
<td>0.81</td>
<td>0.56, (0.34–1.01)</td>
</tr>
<tr>
<td>Adjusted model for carrier T: OR, (95% CI), P value</td>
<td>0.46a, (0.25–0.83)</td>
<td>0.010</td>
</tr>
<tr>
<td>GCKR rs780094 (MAF %)</td>
<td>0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>GA + AA carrier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model for carrier A: OR, (95% CI), P value</td>
<td>0.81</td>
<td>0.49, (0.28–0.84)</td>
</tr>
<tr>
<td>Adjusted model for carrier A: OR, (95% CI), P value</td>
<td>0.41a, (0.23–0.74)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

χ² test: for comparing minor allele frequencies (MAF %) of SNPs between different groups; logistic-regression analysis: for association of SNPs with obesity and HTG
a Adjusted for age, gender, triglycerides, cholesterol and fasting plasma glucose.
b P < 0.05 vs healthy normal-weight children.
c adjusted for age, gender, BMI, cholesterol and fasting plasma glucose.
d P < 0.005 vs normal triglyceride (NTG) samples in the mixed and obese groups; HTG: hypertriglyceridaemia samples.
although it appears that the correlation was stronger in the obese (Table 3).

4. Discussion

The incidence of T2D is increasing worldwide. The disease results from the interactions between genetic predisposing factors and behavioural as well as environmental risk factors [27]. There is also strong evidence that obesity is one of the main non-genetic determinants of the disease [28–31]. Impaired glucose tolerance is an intermediate category between normal glucose tolerance and overt diabetes [32], and several studies have demonstrated that subjects with impaired glucose tolerance have an increased risk of T2D [33].

While investigation of the functional genomics of the glucokinase–GCKR axis is still new in the literature, it has recently become clear that this complex plays an essential role in diabetes susceptibility. Glucokinase is one of four hexokinase isoenzymes that catalyze the phosphorylation of glucose, and is regulated by the glucokinase regulatory protein [8,11,17]. The association of glucokinase with diabetes has been evidenced by the identification of specific rare mutations in the glucokinase gene as the cause of subsets of mature-onset diabetes of the young [34] and permanent neonatal diabetes mellitus (PNDM) [35]. Adenoviral-mediated overexpression of GCKR in mouse liver can increase glucokinase activity and lower fasting blood glucose [36], while overexpression of glucokinase in liver resulted in lowered blood glucose and increased triglyceride concentrations [37,38].

Genome-wide association studies have revealed that rs780094 and rs1260326 polymorphisms at the GCKR locus are associated with decreased fasting glucose and insulin concentrations, and elevated triglyceride levels [19,39,40]. The Diabetes Genetics Initiative (DGI) genome-wide association study for T2D and quantitative metabolic traits found that the rs780094 SNP had a trend towards association with lower fasting glycaemia, less insulin resistance and a lower chance of the development of T2D [19]. Previously, rs780094 was shown to be in strong LD with the other non-synonymous GCKR variant, rs1260326 [22], a finding we could also confirm in our studied paediatric population (LD: $r^2=0.94$). Recently, a large population-based study of 12 independent cohorts representing several ancestral groups provided strong evidence that functional variants of GCKR are associated with elevated fasting triglycerides and decreased glucose concentrations, and also confer modest protection against T2D [41]. In addition, the rs780094 A allele was shown to be associated with a lower risk of obesity in an adult Han Chinese population [21]. Data from Vaxillaire et al. [22] supported the idea that the minor allele (T) of the rs1260326 variant of the GCKR gene could protect against T2D. The minor T allele of rs1260326 was strongly associated with lower fasting glucose rates, a decreased risk of hyperglycaemia and higher triglyceride levels [22].

Based on mice, rat and human studies [15,36,37], the GCKR rs780094 polymorphism, or another variant with which it is in strong LD, can influence GCKR expression and result in enhanced activity of GCKR [20]. It was also suggested that the decrease in serum insulin release in GCKR rs780094 A carriers might induce a secondary increase in serum triacylglycerol by increasing hepatic fatty-acid oxidation [20]. Beer et al. [42] demonstrated that P446L-GCKR (rs1260326) reduced regulation with physiological concentrations of fructose 6-phosphate, indirectly resulting in enhanced glucokinase activity. This is thought to increase glycolytic flux, thereby promoting hepatic glucose metabolism and enhancing concentrations of malonyl-CoA (CoA) thus providing a mutational mechanism for the reported association of this variant with raised triglycerides and lower glucose levels [42]. Based on these findings, we hypothesize that the rs780094 SNP, which is in strong LD with P446L-GCKR (rs1260326; Fig. 1), could have a similar effect on both hepatic triglyceride and glucose metabolism. Our present results confirm the inverse effect of both rs780094 and rs1260326 functional GCKR gene variants on serum triglyceride and plasma glucose levels. The carriers of both minor allele functional variants had increased serum triglycerides, with decreased plasma glucose concentrations, compared with non-carriers in both patients and controls. The differences with the metabolites studied here were moderate, especially for fasting plasma glucose, which is similar to the metabolic traits studied in recent genome-wide association studies [43]. The prevalence of carriers of both SNPs was significantly decreased in obese paediatric patients compared with healthy lean children, although the MAFs were not significantly different in the patient vs control groups.

In the present study using logistic-regression models, the carrier form of both GCKR variants was found to confer a lower risk for the development of obesity in the studied population, and was partly in agreement with the results of a Chinese study evaluating the rs780094 polymorphism in adults [21].

Based on the association of paediatric obesity and the risk for the development of T2D, our findings suggest that the functional minor alleles of the GCKR gene protect against the development of paediatric obesity and, as a consequence, are also protective against the development of T2D and the metabolic syndrome in adults.
Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

This work was supported by a grant from the Hungarian Science Foundation (OTKA T 49589 to B.M.) and grants from the Ministry of Health (ETT 497/2006 to B.M. and 273/2006 to D.M.). Noemi Polgar was supported by the Bolyai Foundation of the Hungarian Academy of Sciences.

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