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Association of biochemical parameters and RAGE gene polymorphisms in healthy infants and their mothers

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ABSTRACT

Background: The receptor for advanced glycation end-products (RAGEs) and its gene polymorphisms are implicated in the pathogenesis of different chronic diseases including diabetes and its complications. Infant formulas contain high amounts of advanced glycation end-products (AGEs) — the ligands of RAGE.
Methods: In this cross-sectional study, we examined the impact of G82S and −374 A/T polymorphisms in the gene encoding RAGE on standard blood chemistry, soluble (s)RAGE and inflammatory markers in 244 healthy infants (3–16 months of age) and in 119 healthy mothers. Children were subdivided according to age (younger and older than 8 months) and for the −374 A/T polymorphism according to the feeding regimen (breast-fed vs. infant formula-fed).
Results: Minor allele of the RAGE gene polymorphism G82S was associated with reduced plasma sRAGE in all age groups and with increased sICAM-1 in older children and mothers. Minor allele carrying mothers had decreased insulin sensitivity and HDL. The A allele of the RAGE gene promoter polymorphism −374 A/T was associated with higher indices of insulin resistance in young infant formula-fed, but not breast-fed children. In older, formerly infant formula-fed children signs of insulin resistance diminished, while formerly breast-fed children with A allele were more insulin sensitive.
Conclusions: The phenotype of minor allele carriers in G82S is associated with reduced levels of protective sRAGE in healthy infants. With increasing age sICAM-1 levels increased and insulin resistance developed. In early childhood the phenotype of the −374 A/T polymorphism was diet-dependently associated with changes in glucose metabolism, which diminished with increasing age.

1. Introduction

Advanced glycation end-products (AGEs) are heterogenous compounds formed *in vivo* by non-enzymatic glycation of proteins and lipoproteins. AGEs are implicated in the development and progression of various diseases, e.g. diabetes and its complications, atherosclerosis, chronic kidney disease etc. [1]. Some of the pathological effects of AGEs are mediated by their receptors, of which RAGE (receptor for AGEs) is the best described. RAGE is a 35-kD cell-surface protein of the immunoglobulin superfamily which acts as a multiligand pattern recognition receptor. Interaction between RAGE and its ligands results in activation of pro-inflammatory and pro-atherogenic genes [2], e.g. resulting in upregulation of vascular cell adhesion molecule-1 (VCAM-1) [3,4]. Truncated form of RAGE — the soluble RAGE (sRAGE) — serves as a decoy, thus is thought to be protective by trapping toxic AGEs or pro-inflammatory ligands [1,5].

AGEs analogues are formed on foods, especially during heat processing (>100 °C). Food-derived AGEs are partially absorbed from the digestive tract to the circulation [6]. Studies in rodents and in humans suggest that an exaggerated consumption of AGEs-rich diet may exert negative health effects [6]. We have shown that infant formulas contain up to 70-fold higher amounts of AGEs in comparison to mother milk, and infants fed with formulas have higher plasma AGEs levels and urinary AGE excretion (1.5 and 60-fold, respectively) [7].

AGER — the gene encoding RAGE — is highly polymorphic [1,8]. Two of the best described single nucleotide polymorphisms (SNPs) are the Gly82Ser (G82S; dbSNP rs2070600) and the −374 promoter polymorphism T>A (−374 A/T; dbSNP rs1800624), both associated with diabetes and its complications in adults [8–16].

In this study we asked whether the RAGE gene polymorphisms G82S and −374 A/T are associated with phenotypic changes

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(alterations of blood chemistry parameters, mainly related to glucose metabolism and inflammation) already in healthy infants. Furthermore, we hypothesized that the diet derived AGEs might have an impact on such phenotypic alterations even without affecting the levels of circulating AGEs [17]. Thus we employed a nutrigenomic approach to reveal the impact of feeding regimen of infants (low-AGE diet: breast milk versus high-AGE diet: infant formula) on these associations [18]. The same panel of parameters was also analyzed in healthy mothers of the studied infants.

2. Materials and methods

2.1. Study design and population

The study was carried out according to Declaration of Helsinki, after the approval of the study protocol by the Ethics Board of the Slovak Medical University (Bratislava, Slovak Republic) and after obtaining written informed consent from mothers/legal guardians of the children.

This cross-sectional study was carried out in collaboration with pediatricians of primary care, two foster homes and professional families (recruitment of 32 exclusively infant formula-fed children, 13 of them younger than 8 months) from Bratislava and surroundings, and the Pediatric Departments of Children's Hospital in Bratislava, from March 2006 to December 2008. Blood samples were collected from 262 infants aged 3 to 16 months and 122 mothers of recruited children. Blood samples from mothers were not obtained when the infant appeared with a different legal guardian, the mother refused blood sampling, or met the exclusion criteria (age bellow 18 years, pregnancy, any ongoing disease and/or medication apart from food supplements, excessive smoking and alcohol consumption). After exclusion of 3 mothers (one with newly diagnosed diabetes, one due to proteinuria, one due to elevated inflammatory markers), 119 healthy mothers were included into the evaluation (Table 1). Exclusion criteria for infants were: pathological findings in general physical examination, elevated inflammatory markers, anamnestic data of acute/recurrent inflammatory or chronic diseases, and positivity of antibodies against HCV/HIV. After exclusion (18 children), 244 healthy infants were evaluated (Table 1). To assess the potential impact of age and infant feeding regimen, children were divided into two subgroups — younger, and older than 8 months. To evaluate the effect of low- versus high-AGE diet (i.e. breast-feeding vs. infant formula feeding) in early childhood, the group of younger children was further divided to those exclusively breast-fed till 6 months of age followed by total breast-feeding till age of 8 months (breast-fed group), and infant formula-fed group. Children older than 8 months were allocated to formerly exclusively/totally breast-fed (formerly breast-fed group), and formerly infant formula-fed group. Final groups yielded comparable numbers of children (Table 1). Subgroups based on different diet intake were not analyzed in the G82S polymorphisms due to very low number of subjects with the minor A allele.

Table 1
Participants' characteristics.

Children	All ($n=244$)	<8 months ($n=124$)	>8 months ($n=120$)
Age [months]	8.2 ± 3.4	5.6 ± 1.2	10.9 ± 2.7
Gender [male/female]	115/129	58/66	57/63
Weight [g]	8107 ± 1536	7222 ± 1119	8900 ± 1392
Gestational age [weeks]	38.3 ± 2.7	38.3 ± 2.8	38.2 ± 2.7
Birth weight [g]	3106 ± 702	3133 ± 715	3092 ± 701
Birth length [cm]	48.7 ± 3.1	48.9 ± 3.2	48.5 ± 3.2
Mothers ($n=119$)	Age [years]	BMI	Weight gain during pregnancy [kg]
	29.5 ± 4.7	23.3 ± 4.4	14.1 ± 4.9

2.2. Gathered data and analyses

Pediatricians/educated nurses and mothers/legal guardians of the infants cooperated in filling out the questionnaires focusing on infant's feeding regimen. These data determined the division of the children into either exclusively breast-fed during the first 6 months of age, or infant formula-fed groups. In the infants we recorded age, gender, gestational age, weight and length at birth, the actual body weight and intake of food supplements (e.g. vitamins D, K and C, iron, calcium, etc.). In the mothers we recorded age, body weight, height and weight gain during pregnancy. According to anamnestic data, none of the included mothers was tested positive for gestational diabetes. Venous blood was taken into Li-heparin (blood chemistry) and K_2-EDTA (blood counts, DNA isolation) tubes. In infants, the total amount of withdrawn blood varied between 3.9 and 4.7 ml, according to type of containers used. Blood counts (Sysmex K-21 Analyzer, Kobe, Japan) and standard blood chemistry (glucose, albumin, uric acid, creatinine, urea, triacylglycerols, cholesterol, HDL-cholesterol; bilirubin, sodium, potassium, calcium, phosphate, and iron concentrations; and aspartate–aminotransferase, alanine–aminotransferase, glutamyl-transferase and alkaline phosphatase activities; Vitros 250 Analyzer, J&J, Rochester, USA) were determined within 3 h after sampling. VLDL- and LDL-cholesterol concentrations were calculated according to the Friedewald formula. Plasma and suspension of erythrocytes with buffy-coat were stored at −80 °C until analyses. The concentration of immunoreactive insulin in plasma was measured by commercially available radioimmunoassay (Immunotech, Prague, Czech Republic). The homeostasis model assessment (HOMA) index was calculated as fasting plasma insulin level (μU/ml) × fasting plasma glucose level (mmol/L)/22.5. Concentrations of interleukin-6 (IL-6), soluble intracellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1, ELISA, all Bender MedSystems, Vienna, Austria), soluble receptor for advanced glycation end-products (sRAGE, ELISA, R&D, Minneapolis, MN, USA) and high-sensitivity C-reactive protein (hsCRP, ELISA, Immun Diagnostik, Bensheim, Germany) were determined with commercially available kits according to manufacturers' instructions. Total N^{ε}-carboxymethyllysine (CML) was determined in plasma by a specific ELISA (Microcoat, Bernried, Germany) after release of protein/peptide bound CML by pretreatment of samples with Proteinase K (Roche, Mannheim, Germany) according to manufacturers' instructions as described previously [19].

2.3. DNA isolation and analyses of RAGE gene polymorphisms

DNA was isolated from EDTA blood samples (200 μl) using QIAamp DNA Blood Mini Kit on the QIAcube platform (Qiagen, Hilden, Germany). Quality and concentration of DNA was checked spectrophotometrically (Nanodrop ND-1000, Nanodrop Technologies, Willington, USA). PCR was run with 5-Prime Mastermix (5-Prime, Hamburg, Germany) on the Veriti 96-well thermal cycler (AppliedBiosystems, Foster City, USA) using the following primers — Fw-5′-CTTCATGATGCAGGCCCAAT-3′ and Rev-5′-CTCATCCTGGATCCCGACAG-3′. Specificity of the PCR products was verified by gel electrophoresis and the PCR products were cleaned using ExoSAP-IT (USB, Cleveland, USA). Genotyping was performed by bidirectional sequencing on an ABI PRISM 3130xl, and the sequences were analyzed using the SeqScape v2.5 software (AppliedBiosystems, Foster City, USA). The Hardy–Weinberg equilibrium of the genotypes was controlled using XLGenetics (http://www.deakin.edu.au/~rodneyc/xlgene.htm).

2.4. Statistical analyses

Data are presented as mean ± SEM. Genotype distribution differences from Hardy–Weinberg equilibrium and frequencies of