Gene 567 (2015) 159–163

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Examination of the brain natriuretic peptide rs198389 single-nucleotide polymorphism on type 2 diabetes mellitus and related phenotypes in an Algerian population

Djabaria Naïma Meroufel [a], Hadjira Ouhaïbi-Djellouli [a,b], Sounnia Mediene-Benchekor [a,b], Xavier Hermant [c], Benjamin Grenier-Boley [c], Sarah Aïcha Lardjam-Hetraf [a], Houssam Boulenouar [a], Imane Hamani-Medjaoui [a,d], Nadhira Saïdi-Mehtar [a], Philippe Amouyel [c], Leïla Houti [a,e,f], Louisa Goumidi [c], Aline Meirhaeghe [c,*]

[a] Laboratoire de Génétique Moléculaire et Cellulaire, Université des Sciences et de Technologie d'Oran Mohamed Boudiaf, BP 1505, El M'Naouer, 31000 Oran, Algeria
[b] Département de Biotechnologie, Faculté des Sciences de la Nature et de la Vie, Université d'Oran, BP 1524, El M'Naouer, 31000 Oran, Algeria
[c] INSERM, U1167, Institut Pasteur de Lille, Université de Lille, 1 rue du Pr. Calmette, BP 245, F-59019 Lille cedex, France
[d] Caisse Nationale des Assurances Sociales des travailleurs salariés, Clinique Spécialisée en Orthopédie et Rééducation des Victimes des Accidents de Travail, Hai Bouamama (El Hassi) Oran, Algeria
[e] Faculté de Médecine, Université Djillali Liabes de Sidi Bel Abbes, BP 89, 22000 Sidi-Bel-Abbès, Algeria
[f] Laboratoire des Systèmes d'Information en Santé, Université d'Oran, BP 1524, El M'Naouer, 31000 Oran, Algeria

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 14 April 2015
Accepted 27 April 2015
Available online 28 April 2015

Keywords:
NPPB gene
BNP
Polymorphism
ISOR study
Insulin

ABSTRACT

Background: In European populations, the *NPPB* rs198389 single nucleotide polymorphism (SNP) is associated with a reduced risk of type 2 diabetes mellitus (T2DM). We investigated the putative associations between *NPPB* rs198389, the T2DM risk and quantitative metabolic traits in an Algerian population.
Methods: The association analysis was performed as a T2DM case–control study (with 78 cases and 645 controls) nested into the ISOR population-based study.
Results: The *NPPB* rs198389 SNP was not associated with T2DM (odds ratio (OR) [95% confidence interval (CI)] = 0.73 [0.51–1.04], $p = 0.08$). However, the C allele was associated with lower fasting plasma insulin levels ($p = 0.05$) and a lower homeostatic model assessment insulin resistance index ($p = 0.05$) in non-diabetic individuals.
Conclusion: The *NPPB* rs198389 SNP might modulate fasting insulin levels in an Algerian population.

1. Introduction

In recent decades, diabetes mellitus has become a major public health issue worldwide. In Algeria, the prevalence of type 2 diabetes mellitus (T2DM) was 10.5% in 2007 (Zaoui et al., 2007). Type 2 diabetes mellitus is a complex disorder caused by both decreased insulin sensitivity and impaired insulin secretion due to pancreatic β-cell defects (Stumvoll et al., 2005). Although the mechanisms responsible for disease onset are not fully understood, T2DM is thought to result from the interaction of environmental/lifestyle risk factors with genetic risk variants in predisposed individuals (Permutt et al., 2005; O'Rahilly et al., 2005).

A number of candidate gene studies have revealed an association between the natriuretic peptide precursor B (*NPPB*) gene (encoding brain natriuretic peptide (BNP)) and the risk of T2DM. Meirhaeghe et al. showed that the C allele of the rs198389 SNP (also referred as T-381C, and located in the *NPPB* promoter region) was associated with a lower risk of T2DM in populations in France and in the United Kingdom (Meirhaeghe et al., 2007). Accordingly, a meta-analysis of seven European case–control studies confirmed that the rs198389 CC genotype had a protective effect on the T2DM risk (Choquet et al., 2009). Although this association has not been reported in genome-wide association studies (GWASs) (Scott et al., 2012) (probably due to their highly stringent significance threshold), a Mendelian randomization analysis

Abbreviations: SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus; NPPA, natriuretic peptide precursor A; NPPB, natriuretic peptide precursor B; OR, odds ratio; CI, confidence interval; BMI, body mass index; BNP, brain natriuretic peptide; GWAS, genome-wide association studies; ANP, atrial natriuretic peptide; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-B, homeostasis model assessment of beta-cell function; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* Corresponding author at: INSERM U1167, Institut Pasteur de Lille, 1 rue du Pr. Calmette, BP 245, F-59019 Lille cedex, France.
E-mail addresses: meroufeln@yahoo.fr (D.N. Meroufel), djellouli.ouhaibi@gmail.com (H. Ouhaïbi-Djellouli), sonia_mediene@yahoo.fr (S. Mediene-Benchekor), Xavier.Hermant@pasteur-lille.fr (X. Hermant), Benjamin.Grenier-Boley@pasteur-lille.fr (B. Grenier-Boley), lardjamhetraf_sarah@yahoo.com (S.A. Lardjam-Hetraf), boulenouar.houssam@gmail.com (H. Boulenouar), amanim20002000@yahoo.fr (I. Hamani-Medjaoui), nmehtar2002@yahoo.fr (N. Saïdi-Mehtar), Philippe.Amouyel@pasteur-lille.fr (P. Amouyel), leilahouti@yahoo.fr (L. Houti), louisa.goumidi@pasteur-lille.fr (L. Goumidi), Aline.Meirhaeghe@pasteur-lille.fr (A. Meirhaeghe).

http://dx.doi.org/10.1016/j.gene.2015.04.073
0378-1119/

of eight case–control studies in Caucasians provided evidence for a potential causal, protective role of the BNP hormone system in the etiology of T2DM (Pfister et al., 2011). Besides the effect of the *NPPB* rs198389 SNP on T2DM, several studies have shown an association between the C allele of this SNP and higher plasma levels of BNP and N-terminal probrain natriuretic peptide (NT-proBNP) (Meirhaeghe et al., 2007; Pfister et al., 2011; Lanfear et al., 2007; Lajer et al., 2007; Ellis et al., 2011; Costello-Boerrigter et al., 2011); this may explain the association with T2DM.

The brain natriuretic peptide is a member of a family that also includes the atrial natriuretic peptide (ANP, encoded by the *NPPA* gene) and the C-type natriuretic peptide. Both ANP and BNP are circulating hormones and are secreted predominantly by the cardiac atria and ventricle, respectively. The *NPPA* and *NPPB* genes are tightly linked on human chromosome 1p36 and share a common ancestry (Arden et al., 1995). Brain natriuretic peptide has an important role in regulating blood pressure and body fluid volume (Takeishi et al., 2007).

Because initial and replication studies have been mainly reported in populations of European descent, the challenge remains to extend the studies to other populations. To the best of our knowledge, there is no published data on the impact of the *NPPB* rs198389 SNP on diabetes-related parameters in North African populations. We therefore decided to evaluate the association between this SNP and (i) the risk of T2DM and (ii) quantitative metabolic traits in an Algerian population sample (the ISOR study).

2. Materials and methods

2.1. Subjects from the InSulino resistance in Oran (ISOR) study

The ISOR study was performed between 2007 and 2009. The study's objectives and procedures were approved by the independent ethics committee at the Algerian National Agency for the Development of Health Research (since renamed as the Thematic Agency of Research in Health Sciences). The datasets were anonymized and the subjects' names, initials or hospital identification numbers were deleted. All subjects gave their written, informed consent to participation (Boulenouar et al., 2013; Lardjam-Hetraf et al., 2014; Ouhaibi-Djellouli et al., 2014; Badsi et al., 2015). The ISOR study is a population-based, cross-sectional study of a representative sample of 787 subjects originating from north-western Algeria (378 men and 409 women, aged between 30 and 64) recruited from within the city of Oran. Subjects were selected at random from social security rolls. Details of the studies have been described elsewhere (Boulenouar et al., 2013; Lardjam-Hetraf et al., 2014; Ouhaibi-Djellouli et al., 2014; Badsi et al., 2015). A questionnaire on lifestyle (physical activity, tobacco use and alcohol intake), personal and family medical histories, current medication and socio-economic and educational levels was completed during a face-to-face interview. Anthropometric data were also recorded.

The level of physical activity was defined (in quartiles) as “none”, “low”, “medium” and “high”, after summing exercise scores for sporting activities, walking, housework and physical activity at work.

In terms of tobacco use, individuals were categorized as either smokers (i.e. individuals reporting at least one cigarette per day) or non-smokers. In the study questionnaire, subjects were asked to report their weekly consumption of wine, beer, and spirits. As there were very few drinkers (n = 25, 3.2%), this variable was not taken into account in the final analysis.

The anthropometric measurements included height, body weight, waist circumference and hip circumference while the subject was barefoot and lightly dressed. The BMI was calculated according to the Quetelet equation. Systolic and diastolic blood pressure values (SBP and DBP, respectively) were measured on the right arm with the subject in the sitting position, using a standard mercury sphygmomanometer. Measurements were made before and after completion of the questionnaire, with an interval of at least 10 min. The mean value of the two blood pressure readings was considered in the final analysis.

Venous blood samples were collected in the morning after 12 h overnight fast for measurements of plasma insulin, glucose, lipids and lipoproteins. Plasma insulin levels were measured in a microparticle enzyme immune assay running on an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL, USA). A multichannel analyzer and dedicated kits (Humastar®, HUMAN Diagnostics, Wiesbaden, Germany) were used for the colorimetric enzymatic measurement of cholesterol (kit: monotest cholesterol with cholesterol esterase, cholesterol oxidase and peroxidase), triglycerides (kit: peridochrom triglyceride with glycerol phosphate oxidase and peroxidase) and glucose (kit: glucose, glucose oxidase and peroxidase). For participants with triglyceride levels <4.56 mM, plasma LDL-cholesterol levels were calculated according to the Friedewald equation. High-density lipoprotein cholesterol levels were measured after sodium phosphotungstate/magnesium chloride precipitation of chylomicrons, VLDL and LDL-cholesterol and then centrifugation. The homeostasis model assessment insulin resistance (HOMA-IR) index was calculated as (fasting plasma glucose [mmol/L] × fasting plasma insulin [mIU/L]/22.5). The homeostasis model assessment of beta-cell function (HOMA-B) was calculated as (20 × fasting plasma insulin [mIU/L]/fasting plasma glucose [mmol/L]-3.5) (Matthews et al., 1985). Of the 787 individuals included in the ISOR study, 80 had T2DM according to the WHO definition, .i.e. fasting plasma glucose ≥7.0 mmol/L and/or treatment for diabetes included diet and/or oral antidiabetic drugs and/or insulin to achieve glycemic control (World Health Organization, 1999). Control subjects were defined as having fasting plasma glucose ≤6.1 mmol/L and no treatment for diabetes. Genomic DNA was extracted from white blood cells (by using a Stratagene® kit (Agilent Technologies, Les Ulis, France) according to the manufacturer's protocol) and stored at −20 °C.

2.2. Genotyping

The *NPPB* rs198389 polymorphism was genotyped using KASPar technology (KBioscience, Hoddesdon, UK), a competitive allele-specific polymerase chain reaction incorporating a fluorescent resonance energy transfer quencher cassette (for more information see http://www.kbioscience.co.uk/reagents/KASP.html). The KASPar assay was designed using the Primer Picker software available at http://www.kbioscience.co.uk/primer-picker.htm (KBioscience). Genotyping assays was carried out with a Hydrocycler (Applied Biosystems, Foster City, CA) in a final volume of 2 μl containing 4× Reaction Mix (KBioscience), 120 nM of each allele-specific primer and 300 nM of common primer, 1.5 μl of Master mix (KBioscience) and 5 ng of genomic DNA. The following thermal profile was used for reaction: 15 min at 94 °C; 20 cycles of 10 s at 94 °C, 5 s at 57 °C and 10 s at 72 °C; and 18 cycles of 10 s at 94 °C, 20 s at 57 °C and 40 s at 72 °C. Appropriate negative control samples were used.

The genotyping success rate was 99.6%. Around 9% of the participants of the ISOR study was double genotyped, with a 100% concordance rate.

2.3. Statistical analysis

Statistical analyses were performed with SAS software (version 9.1, SAS Institute Inc. Cary. NC, USA). The Hardy–Weinberg equilibrium was tested using a χ^2 test with one degree of freedom.

Odds ratios (ORs) and 95% confidence intervals (CI) were calculated in multivariate logistic regression analyses (adjusted for age, gender, smoking status, level of physical activity and, when indicated, BMI) using an additive genetic model.

Intergroup comparisons of means were performed with a general linear model using an additive genetic model. In order to obtain normal data distributions, log-transformation was used for data on fasting glucose, insulin and triglyceride levels and HOMA-IR and HOMA-B. The adjustment variables were age, gender, smoking status, level of physical