JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
SHORT REPORT

Low serum level of high-sensitivity C-reactive protein in a Japanese patient with maturity-onset diabetes of the young type 3 (MODY3)

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Keywords
Hepatocyte nuclear factor 1α, High-sensitivity C-reactive protein, Maturity-onset diabetes of the young type 3

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J Diabetes Invest 2014; 5: 513–516

doi: 10.1111/jdi.12237

ABSTRACT

High-sensitivity C-reactive protein (hs-CRP) levels in European populations are lower in patients with maturity-onset diabetes of the young type 3 (MODY3) than in those with type 2 diabetes. hs-CRP levels have been suggested to be useful for discriminating MODY3 from type 2 diabetes. As hs-CRP levels are influenced by various factors including race and body mass index, it is worthwhile to examine whether hs-CRP can serve as a biomarker for MODY3 in Japanese. Here we describe the case of a Japanese MODY3 patient with a nonsense mutation in the *HNF1A* gene. Two measurements showed consistently lower hs-CRP levels (<0.05 and 0.09 mg/L) than in Japanese patients with type 1 and type 2 diabetes. Hepatic expression of *Crp* messenger ribonucleic acid was significantly decreased in *Hnf1a* knockout mice. The hs-CRP level might be a useful biomarker for MODY3 in both Japanese and European populations.

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus characterized by autosomal dominant inheritance and early onset. We previously reported that heterozygous mutations of the hepatocyte nuclear factor 1α (*HNF1A*) gene cause MODY3[1]. We and others have shown that HNF1α controls β-cell function by regulating *Slc2a2*, *Tmem27*, *Hgfac* and *Hnf4a*[2–5].

Genetic testing, such as deoxyribonucleic acid (DNA) sequencing, is necessary for the diagnosis of MODY3. Selection of patients for genetic testing of MODY3 is based mainly on clinical features, such as family history and age of onset, but merely fulfilling clinical features does not provide effective selection criteria for genetic testing of MODY3[6]. The C-reactive protein (*CRP*) gene has two HNF1α binding sites in its promoter region, and HNF1α activates gene expression by binding to these sites[7]. Furthermore, common variants in the *HNF1A* gene are associated with circulating high-sensitivity CRP (hs-CRP) levels[8]. Recent studies have shown that hs-CRP levels are lower in patients with MODY3 than in those with type 2 diabetes in European populations, and hs-CRP has been suggested to be a useful prescreening tool for identifying patients for genetic testing[9–12]. However, hs-CRP levels are influenced by various factors including race and body mass index (BMI), and hs-CRP levels in Japanese people are notably lower than those in Western populations[13–15]. Therefore, it is unclear whether hs-CRP has the potential to serve as a biomarker for Japanese MODY3.

Here we describe the case of a Japanese MODY3 patient where, interestingly, the patient's serum hs-CRP level was markedly reduced. The hs-CRP level could be a useful biomarker for MODY3 in both Japanese and European populations.

MATERIALS AND METHODS

Participants

A 35-year-old man was diagnosed with diabetes at 8 years-of-age. He was first treated with diet therapy and started nateglinide at 22 years-of-age. Insulin therapy was started at Nissay Hospital at 33 years-of-age as a result of poor glycemic control. Fasting plasma C-peptide immunoreactivity (CPR) level was 1.09 ng/mL, and antibody to glutamic acid decarboxylase was negative. His younger sister had also been diagnosed as having

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Received 21 July 2013; revised 12 November 2013; accepted 27 November 2013

early-onset diabetes, and his mother had been diagnosed as having gestational diabetes. He was not taking any medications, such as statins, aspirin, antihypertensive drugs or glucocorticoids.

The patient's serum hs-CRP levels were compared with those of 65 Japanese patients with type 2 diabetes reported previously[15] and 41 Japanese patients with type 1 diabetes measured in the present study (Table 1). Further information on the type 1 diabetes patients is provided in the Supporting Information.

Screening of HNF1α Gene Mutations

A detailed description of the DNA sequencing to detect HNF1α gene mutations is provided in the Supporting Information.

Biochemical Analysis

Biochemical data were measured by standard laboratory assays. Glycated hemoglobin (HbA1c) levels (National Glycohemoglobin Standardization Program) were calculated from HbA1c (Japan Diabetes Society) levels as described previously[16]. The serum hs-CRP level was measured by latex-enhanced immunonephelometrics on a BN II Analyzer (Dade Behring, Marburg, Germany)[17]. The range of determinants was 0.05–10 mg/L.

Quantitative Reverse Transcription Polymerase Chain Reaction

A detailed description of the quantitative reverse transcription polymerase chain reaction is provided in the Supporting Information.

Statistical Analysis

Significance was assessed with the unpaired *t*-test at $P < 0.05$.

RESULTS

Identification of *HNF1A* Gene Mutation

A nonsense mutation in codon 229, CGA (Arg) to TGA (stop; R229X, c.685C>T), was identified (Figure 1). This R229X mutation, which results in no transactivation domain, has previously been identified in other MODY patients[18–21], and has been shown to be a loss-of-function mutation by reporter gene assay[18]. Other family members were not available for genetic testing.

Table 1 | Clinical characteristics of type 1 diabetic participants

Variables	Mean ± SD
n	41
Sex (men/women)	20/21
Age (years)	38.6 ± 18.8
Duration of diabetes (years)	7.1 ± 7.5
BMI (kg/m^2)	20.7 ± 3.4
Hypertension (yes/no)	2/39
Smoking (yes/no)	12/29
Insulin dose (IU/kg/day)	0.67 ± 0.32
HbA1c (NGSP%)	10.5 ± 2.9
Total cholesterol (mmol/L)	4.61 ± 1.30
Triglycerides (mmol/L)	1.04 ± 0.41
Creatinine (μmol/L)	52.8 ± 17.2
hs-CRP (mg/L)	0.26 (0.11–0.60)*

BMI, body mass index; HbA1c, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; NGSP, National Glycohemoglobin Standardization Program; SD, standard deviation. *Median (range).

Clinical and Biochemical Profiles of the Patient with *HNF1A* R229X Mutation

The patient's BMI was 22.1 kg/m^2 and his HbA1c level was 6.1% (National Glycohemoglobin Standardization Program). He had simple diabetic retinopathy and microalbuminuria, and no previous history of cardiovascular disease. B-mode ultrasound showed no thickening of the carotid arteries (maximum carotid intima-media thickness of 0.65 mm). Brachial-ankle pulse wave velocity was 1192 cm/s. Biochemical tests for liver function and kidney function were normal. Serum levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride were also normal. Two measurements showed notably decreased serum hs-CRP levels (<0.05, and 0.09 mg/L) and decreased plasma fibrinogen levels (184 and 215 mg/dL; normal, 220–435 mg/dL).

Serum Hs-CRP Levels in Japanese Type 1 Diabetic Patients

The median hs-CRP concentration in the 41 Japanese patients with type 1 diabetes was 0.26 mg/L (interquartile range 0.11–0.60 mg/L).

Expression of CRP and Fibrinogen Genes in the Liver of *Hnf1a* Knockout Mice

The expression levels of *Hnf1a* and *Crp* messenger ribonucleic acid (mRNA) in the liver of adult *Hnf1a*$^{+/-}$ mice were normal (Figure S1). *Hnf1a*$^{-/-}$ mice show severe liver dysfunction and die around the time of weaning[22]. As liver failure impairs CRP production[23], we investigated the hepatic *Crp* mRNA expression in *Hnf1a*$^{-/-}$ mice on embryonic day 18.5. Expression of *Pah*, a known target gene of HNF1α[22], was significantly decreased in the liver of *Hnf1a*$^{-/-}$ mice (Figure 2), and expression of *Crp* was also significantly decreased in the HNF1α knockout mice to 35.1% of the control level ($P < 0.001$). These results show the importance of HNF1α in the transcriptional regulation of the *CRP* gene *in vivo*. *Fga* mRNA expression was decreased in the liver of *Hnf1a*$^{-/-}$ mice to 56.4% of the control level, but the difference was not significant ($P = 0.076$).

DISCUSSION

We previously reported that the median concentration of hs-CRP levels in 65 Japanese type 2 diabetic patients (BMI 24.4 ± 3.2 kg/m^2) was 0.49 mg/L (interquartile range 0.26–0.87 mg/L)[15]. In contrast, the serum hs-CRP level in the present Japanese MODY3 patient with the R229X mutation was notably lower (<0.05 and 0.09 mg/L). The patient was treated with insulin, but not with medications, such as statins, aspirin or steroids, which are known to reduce hs-CRP levels[15,24,25]. These results suggest that hs-CRP can be used as a marker for discriminating MODY3 from type 2 diabetes in

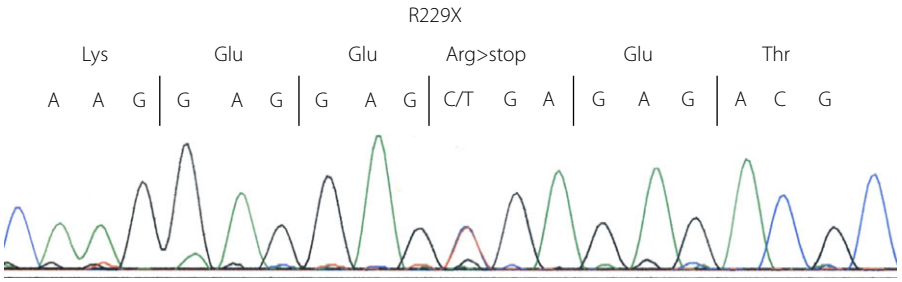

Figure 1 | R229X mutation in the *HNF1A* gene.

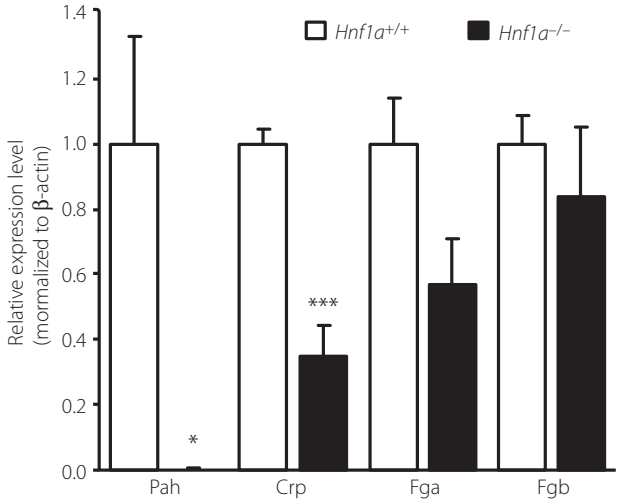

Figure 2 | Gene expression of *Pah*, *Crp*, *Fga,* and *Fgb* messenger ribonucleic acid in the liver of $Hnf1a^{+/+}$ ($n = 4$) and $Hnf1a^{-/-}$ ($n = 4$) mice on embryonic day 18.5. Expression is normalized to that of β-actin. Data are expressed as means ± standard error (*$P < 0.05$, ***$P < 0.001$).

Japanese patients. Using the same assay as ours, Thanabalasingham *et al.*[12] reported that the cut-off value of hs-CRP for discriminating MODY3 from type 2 diabetes is 0.5 mg/L. However, this cut-off value was similar to the median value of hs-CRP in the Japanese patients with type 2 diabetes. Therefore, a further large cohort study is necessary to identify the appropriate hs-CRP cut-off levels in Japanese MODY3 patients. In the present study, we also measured the serum hs-CRP levels in type 1 diabetic patients (BMI 20.7 ± 3.4 kg/m^2, median 0.26 mg/L [interquartile range 0.11–0.60 mg/L]). The lack of glutamic acid decarboxylase antibodies has been reported as a useful criterion for discriminating MODY3 from type 1 diabetes[26]. The preset results results suggest that hs-CRP might also be beneficial for distinguishing between MODY3 and type 1 diabetes.

Crp expression was significantly decreased to 35.1% of that of the controls in the liver of $Hnf1a^{-/-}$ mice, which is consistent with a previous DNA microarray analysis using *Hnf1a* knockout mice[27]. These findings suggest that HNF1α plays an important role in the expression of CRP *in vivo*. It has been reported that HNF1 is required for the optimal promoter function of the genes encoding the α and β chains of fibrinogen[28,29]. Although a previous small-scale study found no significant difference in plasma fibrinogen concentration between MODY3 and MODY1[30], it is interesting that the plasma fibrinogen level was lower in our patient with MODY3. There is also a tendency for decreased *Fga* mRNA expression in the liver of $Hnf1a^{-/-}$ mice. Therefore, a combination of hs-CRP and fibrinogen levels might serve as a useful biomarker for identifying MODY3 in the Japanese population.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research (S), a Grant-in-Aid for Scientific Research (B), and grants from the Suzuken Memorial Foundation and Daiichi Sankyo Foundation of Life Science. The authors declare that there is no conflict of interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1 | Materials and methods.

Figure S1 | Gene expression of *Hnf1a* and *Crp* in the liver of 16-week-old female $Hnfla^{+/+}$ (n = 3) and $Hnfla^{+/-}$ (n = 3) mice.