Polymorphisms of the fibrinogen-beta gene are related to 2-hour glucose level after oral glucose tolerance test in Hong Kong Chinese

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Abstract. Fibrinogen, an acute phase protein, is an important inflammatory marker that is associated with cardiovascular diseases. We studied the association of three common human fibrinogen-\(\beta\) gene (\(FGB\)) variants, \(-455G>A\), \(-249C>T\), and \(-148C>T\) with glycemic parameters in 265 non-diabetic Hong Kong Chinese subjects. Both \(FGB\) variants, \(-455G>A\) and \(-148C>T\) were in complete linkage disequilibrium and were associated with higher levels of plasma fibrinogen and 2-h glucose after a 75-g oral glucose load (\(p<0.01\)). Carriers of \(FGB\) AC-haplotype, comprising the two nucleotide variants at positions \(-455\) and \(-249\), had higher fibrinogen level (2.64 ± 0.65 vs 2.42 ± 0.52 g/L, \(p=0.002\)) and 2-h glucose after a 75-g oral glucose load (5.87 ± 1.14 vs 5.47 ± 1.22 g/L, \(p=0.006\)). The associations were significant in men, but not women. In stepwise multiple regression analysis, AC-haplotype was independently associated with plasma fibrinogen level and 2-h glucose (\(p=0.002\) and 0.010 respectively). This suggests that fibrinogen may play a role in the development of impaired glucose tolerance.

Keywords: Fibrinogen, haplotype, impaired glucose tolerance, polymorphism

1. Introduction

Fibrinogen is an acute phase protein, in which its hepatic synthesis is mediated by interleukin-6 (IL-6) [5]. It is an important inflammatory marker that is associated with cardiovascular diseases [8]. Elevated fibrinogen level is associated with age, obesity, smoking, elevated low density lipoprotein cholesterol, elevated triglycerides, inflammation, diabetes mellitus, hypertension, and the metabolic syndrome [8,9,11,23]. A higher fibrinogen level is associated with glucose intolerance and severity of diabetes [16,21]. We previously reported that the IL-6 gene -572C>G polymorphism was associated with higher plasma fibrinogen level [28]. Fibrinogen consists of three polypeptides, \(\alpha\), \(\beta\), and \(\gamma\), in which the encoding genes are clustered together on the chromosome 4q23-32 [13]. The synthesis of the \(\beta\) chain is the rate-limiting step in the synthesis of fibrinogen [22]. Both \(-455 G>A\) and \(-148C>T\) polymorphism in fibrinogen-\(\beta\) gene (\(FGB\)) have been shown to be associated with increased fibrinogen level [23,24]. A recent study also demonstrates significant association of \(-455 G>A\) with increased fasting insulin level [18]. Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) in \(FGB\) may be associated with a higher risk for the metabolic syndrome, or at least its related glycemic component, like
insulin resistance, plasma glucose, and insulin level. We, therefore, studied three known SNPs in the promoter region of FGB, −455G>A (rs1800790), −249C>T (rs1800788), and −148C>T (rs1800787) with plasma glucose level among 265 non-diabetic subjects.

2. Subjects and methods

2.1. Subjects

265 unrelated non-diabetic Southern Chinese subjects of Han race living in Hong Kong were included in this study. The subjects were randomly selected from a previous cohort of subjects randomly recruited from the general population aged 25–74 years in 1995–1996 [12]. The study protocol has been approved by the Institutional Review Board, and written informed consent was obtained from all participants. All subjects were without diabetes, impaired fasting glucose, or impaired glucose tolerance according to the diagnostic criteria of the American Diabetes Association [1].

Physical examination (including measurement of height, weight, body mass index, waist circumference) and a full medical history (including past medical history, drug history, family history, smoking status) was obtained with a standard questionnaire. Subjects were defined as either current, former, or never smoker. Hypertension was defined as blood pressure \( \geq 140/90 \) mmHg or taking anti-hypertensive drug [6]. Blood pressure, triglyceride, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, 75-g oral glucose tolerance test (OGTT), fasting glucose, insulin, and homeostasis model assessment of insulin resistance index (HOMA-IR) were measured as described previously [16,28]. Fibrinogen (coefficient of variation 4.5% within run and 6.1% between runs) was measured in the Haematology Laboratory of the Department of Pathology, Queen Mary Hospital within 24 hours by the Clauss Method on the Cobas Fibro (Roche Diagnostics, Basle, Switzerland) [15,16]. Standardization was carried out using calibrated plasma for fibrinogen (Thrombosics Center, Whittington Hospital, Manchester, UK) which was calibrated against the First International Standard Plasma Fibrinogen (National Institute for Biological Standards and Control 89:644).

2.2. Genotyping

Blood sample was taken and genomic DNA was extracted from the buffy coat as described previously [20, 28]. Genotyping of the three known SNPs in positions, −455, −249, and −148 of FGB were performed using the Sequenom MassARRAY system (Sequenom, San Diego CA) which utilizes Matrix Assisted Laser Desorption Ionization-Time Of Flight (MALDI-TOF) technology.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 13.0. Genotype frequencies for each SNP were tested for Hardy-Weinberg equilibrium. Haploview (ver. 3.2) was used to assess linkage disequilibrium for all possible SNP pairs by determining \( r^2 \) [2]. Haplotypes were predicted using the programme PHASE (ver. 2.1.1) [25, 26]. For haplotype analysis, subjects who carried a particular haplotype with a probability \( \geq 0.99 \) were included. Correction for multiple testing was performed by Bonferroni’s correction. Data were reported as mean ± standard deviation (SD), but the median (inter-quartile range) of fasting insulin, HOMA-IR, and triglyceride were given because of their skewed distribution. Comparisons of clinical characteristics were performed using unpaired student’s t test or Mann-Whitney U test where appropriate, and Fisher’s exact test was performed for categorical variables. Correlations of fibrinogen with other variables were analyzed by using Pearson correlations. Stepwise multiple linear regression was used to assess the independent association of FGB haplotype with plasma fibrinogen and 2-h post-OGTT glucose. A 2-tailed \( p \) value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

The clinical characteristics of the 265 subjects are shown in Table 1. In this study, all the subjects were not taking any cholesterol lowering medications and only 12 subjects were taking anti-hypertensive medications. Compared to women, men had significantly higher diastolic blood pressure, waist circumference, LDL cholesterol, triglyceride, fasting glucose, and percentage of smoking, but lower HDL cholesterol, 2-h glucose, fasting insulin, HOMA-IR, and fibrinogen level. Among all these subjects, fibrinogen level was strongly correlated positively with age, body mass index, 2-h glucose, fasting insulin, and HOMA-IR, but not fasting glucose (Table 2).
Table 1
Baseline clinical characteristics of 265 non-diabetic subjects

|                        | Men (n = 140) | Women (n = 125) | p     |
|------------------------|--------------|-----------------|-------|
| Age (years)            | 47.3 ± 11.6  | 45.9 ± 10.9     | 0.288 |
| Systolic blood pressure (mmHg) | 120.0 ± 14.6 | 118.6 ± 18.0    | 0.467 |
| Diastolic blood pressure (mmHg) | 76.1 ± 9.1   | 73.2 ± 10.6     | 0.016 |
| Hypertension (%)       | 14.3         | 12.8            | 0.858 |
| Current smoker (%)     | 39.3         | 4.0             | < 0.001|
| Body mass index (kg/m²) | 23.8 ± 3.2   | 24.0 ± 3.6      | 0.626 |
| Waist circumference (cm) | 81.2 ± 9.1   | 74.9 ± 8.0      | < 0.001|
| LDL cholesterol (mM)   | 3.3 ± 0.7    | 3.0 ± 0.7       | 0.001 |
| HDL cholesterol (mM)   | 1.2 ± 0.3    | 1.4 ± 0.3       | < 0.001|
| Triglyceride (mM)      | 1.00 (0.80–1.46) | 0.90 (0.66–1.20) | 0.007 |
| Fasting glucose (mM)   | 5.13 ± 0.42  | 4.98 ± 0.37     | 0.002 |
| 2-h glucose (mM)       | 5.35 ± 1.30  | 6.00 ± 0.97     | < 0.001|
| Fasting insulin (mIU/L)* | 4.4 (2.8–6.0) | 5.2 (3.5–7.5)  | 0.008 |
| HOMA-IR                | 0.98 (0.64–1.44) | 1.10 (0.76–1.80) | 0.027 |
| Fibrinogen (g/L)       | 2.42 ± 0.54  | 2.65 ± 0.62     | 0.001 |

LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance index.
Data are expressed as mean ± SD or median (inter-quartile range) unless otherwise stated.

Table 2
Correlation of fibrinogen with age and glycemic parameters

|                        | Fibrinogen (g/L) | r     | p     |
|------------------------|------------------|-------|-------|
| Age (years)            | 0.300            | < 0.001|
| Body mass index (kg/m²) | 0.160            | 0.009 |
| Fasting glucose (mM)   | 0.030            | 0.623 |
| 2-h glucose (mM)       | 0.197            | 0.001 |
| Fasting insulin (mIU/L)* | 0.152           | 0.019 |
| HOMA-IR                | 0.150            | 0.020 |

HOMA-IR, homeostasis model assessment of insulin resistance index; r, Pearson correlation coefficients.
*Data are logarithmically transformed.

3.2. SNP Genotyping

In FGB, −455G>A and −148C>T polymorphisms were in complete linkage disequilibrium ($r^2 = 1.0$). Therefore, only the variant −455G>A was tested and reported in subsequent analysis. The minor −249T allele was not detected in any individual carrying the minor allele of −455G>A polymorphism. Table 3 shows the results of genotyping of these two SNPs in FGB (−455G>A and −249C>T). For each SNP, the observed genotype frequencies showed no significant deviation from Hardy-Weinberg equilibrium. Characteristics of subjects who were homozygous for the minor allele or heterozygous were combined together into the same group in all remaining analyses to increase the sample size for comparison.

3.3. Association with plasma fibrinogen and 2-h glucose levels

The FGB variant, −455A was associated with higher plasma fibrinogen and 2-h glucose level, which remained significant after correction for multiple testing (Table 3). The most common two-locus haplotypes of FGB, comprising the two nucleotide variants at positions −455 and −249 were GT, AC, and GC. As expected, fibrinogen level and 2-h glucose level were significantly higher in AC-haplotype carriers than non-carriers, which remained significant after correction for multiple testing (Table 3). However, none of the single variants or haplotypes were associated with fasting glucose, fasting insulin, and HOMA-IR ($p > 0.05$). There were no significant differences in baseline clinical characteristics between AC-haplotype carriers and non-carriers, except that AC-haplotype carriers had lower HDL cholesterol level than non-carriers (1.33 ± 0.35 vs 1.24 ± 0.32 mM, $p = 0.031$). The association of 2-h glucose level with AC-haplotype remained significant after adjusting for age, sex, body mass index, fasting glucose, fasting insulin, and HDL cholesterol ($β = 0.155$, $p = 0.008$). Further adjustment for fibrinogen level did not affect the association significantly ($β = 0.155$, $p = 0.009$).

3.4. Sex-specific analysis

In sex-specific analysis (Table 4), the association of AC-haplotype with fibrinogen and 2-h glucose level was significant only in men, but not women. The AC-haplotype was associated with a higher fasting in-
Table 3
Association of FGB genotypes and haplotypes with plasma fibrinogen and 2-h glucose

| Genotypes / haplotypes | No. (%) of genotypes | Fibrinogen (g/L) | p       | 2-h glucose (mM) | p       |
|------------------------|----------------------|------------------|---------|------------------|---------|
| −455 (rs1800790) GG    | 139 (52.5)           | 2.42 ± 0.52      | 0.002   | 5.47 ± 1.22      | 0.006   |
| GA                     | 106 (40.0)           | 2.64 ± 0.6      |         | 5.87 ± 1.14      |         |
| AA                     | 20 (7.5)             |                  |         |                  |         |
| GA + AA                | 126 (47.5)           | 2.56 ± 0.54      | 0.624   | 5.65 ± 1.27      | 0.975   |
| CC                     | 70 (26.4)            |                  |         | 5.66 ± 1.17      |         |
| CT                     | 122 (46.0)           |                  |         |                  |         |
| TT                     | 73 (27.5)            |                  |         |                  |         |
| GT                     | 195 (73.6)           | 2.52 ± 0.62      |         |                  |         |
| Non-carrier            | 70 (26.4)            | 2.56 ± 0.54      | 0.624   | 5.65 ± 1.27      | 0.975   |
| Carrier (1 copy)       | 122 (46.0)           |                  |         | 5.66 ± 1.17      |         |
| Carrier (2 copies)     | 73 (27.5)            |                  |         |                  |         |
| Carrier (1 or 2 copies)| 195 (73.6)           | 2.52 ± 0.61      |         |                  |         |
| AC                     | 139 (52.5)           | 2.42 ± 0.52      | 0.002   | 5.47 ± 1.22      | 0.006   |
| Carrier (1 copy)       | 106 (40.0)           |                  |         | 5.87 ± 1.14      |         |
| Carrier (2 copies)     | 20 (7.5)             |                  |         |                  |         |
| Carrier (1 or 2 copies)| 126 (47.5)           | 2.64 ± 0.65      |         |                  |         |
| GC                     | 161 (60.8)           | 2.57 ± 0.60      | 0.120   | 5.76 ± 1.15      | 0.098   |
| Non-carrier            | 92 (34.7)            |                  |         | 5.51 ± 1.26      |         |
| Carrier (1 copy)       | 12 (4.5)             |                  |         |                  |         |
| Carrier (2 copies)     | 104 (39.2)           | 2.46 ± 0.59      |         |                  |         |

Plasma fibrinogen and 2-h glucose levels are expressed as mean ± SD.

* The most common two-locus haplotypes, comprising the two nucleotide variants of FGB at positions, −455 and −249.

Table 4
Sex-specific association of FGB AC-haplotypes with plasma fibrinogen and 2-h glucose

| Genotypes / haplotypes | Men (n = 140) | Women (n = 125) |
|------------------------|---------------|-----------------|
|                        | Non-carrier   | Carrier (1 or 2 copies) | p |
|                        | Non-carrier   | Carrier (1 or 2 copies) | p |
| n                      | 73            | 67               | 66  | 59               |
| Fibrinogen (g/L)       | 2.27 ± 0.44   | 2.57 ± 0.61      | 0.001 | 2.58 ± 0.55 | 2.73 ± 0.69 | 0.202   |
| Fasting glucose (mM)   | 5.10 ± 0.46   | 5.17 ± 0.37      | 0.353 | 5.03 ± 0.36 | 4.93 ± 0.37 | 0.337   |
| 2-h glucose (mM)       | 5.05 ± 1.26   | 5.67 ± 1.28      | 0.005 | 5.92 ± 1.01 | 6.09 ± 0.92 | 0.337   |
| Fasting insulin (mIU/L)| 4.0 (2.7–5.7) | 4.7 (3.0–7.5)    | 0.041 | 6.4 (0.4–8.6) | 4.4 (3.1–6.6) | 0.049   |
| HOMA-IR                | 0.86 (0.56–1.32)| 1.12 (0.69–1.84) | 0.040 | 1.44 (0.83–1.94)| 0.98 (0.67–1.41)| 0.045   |

Data are expressed as mean ± SD or median (inter-quartile range) unless otherwise stated.

sul and HOMA-IR in men, but lower fasting insulin and HOMA-IR in women, although such association in men and women was not significant after correction for multiple testing.

3.5. Multiple linear regression analysis

As shown in Table 5, in a stepwise multiple linear regression analysis which includes the FGB AC-haplotype and other potential confounding variables in the model, the AC-haplotype was an independent predictor of plasma fibrinogen and 2-h glucose levels.

4. Discussion

Fibrinogen has been recognized as an important inflammatory risk factor for cardiovascular events, and

exerts significant effects on endothelial function and blood viscosity [14]. Higher level of fibrinogen indicates that hypofibrinolysis and increased vascular inflammation might be present in subjects with hypertension and the metabolic syndrome. As elevated fibrinogen level is associated with diabetes mellitus and the metabolic syndrome [8,9,11,23], it would be expected that FGB variants such as −455G>A and −148C>T, which are associated with higher fibrinogen level, may also be associated with elevated glucose level among healthy non-diabetic subjects. To our knowledge, this is the first study investigating the association of the genetic polymorphisms in FGB with 2-h glucose during OGTT. This suggests that SNPs in FGB may be associated with impaired glucose tolerance in non-diabetic Hong Kong Chinese.
In this study, carriers of the AC-haplotype had significantly higher fibrinogen and 2-h glucose level than those who did not carry the haplotype, especially among men. The associations were confirmed by multivariate regression analyses even after adjusting for other potential confounding factors. The −455G>A polymorphism in FGB has been shown to be associated with increased fibrinogen level [23,24] and our study has confirmed such association in the Hong Kong Chinese population. In a study of healthy Caucasian adults, the −455G>A polymorphism was associated with higher fasting insulin level in men, but lower level in women [18]. Our study is the first report of similar findings in a different population, although the association with fasting insulin was not significant after correction for multiple testing. Moreover, we further reveal a much stronger association of −455G>A polymorphism with plasma 2-h glucose level. Such association is independent of fibrinogen level as adjustment for fibrinogen level did not reduce the association significantly. In fact, the −455G>A polymorphism is located in an IL-6 responsive HNF1 element and can alter the binding of a transactivation protein complex, resulting in greater luciferase reporter gene activity [3,10]. The other variant, −148C>T, is almost in complete linkage disequilibrium with −455G>A in our population. The −148C>T polymorphism is located in a putative transforming growth factor-β responsive element and is near to an IL-6 responsive element [27]. In a cohort of Italian Caucasians, plasma IL-6 and fibrinogen were elevated in subjects with impaired glucose tolerance, but not in those with impaired fasting glucose [4]. Thus, these variants may affect the cytokine and/or growth factor mediated transcription of FGB. Increased fibrinogen level may cause insulin secretion through endothelial dysfunction and vascular damage, leading to insulin resistance [18].

The independent association of AC-haplotype with 2-h glucose during OGTT, especially in men, is not surprising. Plasma fibrinogen level was correlated with 2-h glucose level, but not fasting glucose level. This is consistent with other reports, which show a positive relationship between fibrinogen level and the degree of glucose intolerance or severity of diabetes in men [16,21]. The relationship of fibrinogen and diabetes in the Scottish Heart Health Study was significant only in men, but not women [17]. It was suggested that fibrinogen can indicate the degree of glucose intolerance, but not the development of diabetes in future [7]. In fact, the 2-h glucose level is a better predictor than fasting glucose level in the prediction of all-cause and cardiovascular mortality [7,19]. In this study, the AC-haplotype was independently associated with higher 2-h glucose in non-diabetic subjects, and such association was stronger than the fibrinogen level itself.

In conclusion, the FGB haplotype AC was a major determinant of 2-h glucose levels in Hong Kong Chinese with normal glucose tolerance. This suggests that fibrinogen may play a role in the development of impaired glucose tolerance. Further studies are needed to confirm such association in a different setting or population with larger sample size.

In the stepwise multiple regression analyses, independent variables in the model include age, sex, smoking (never, former and current smokers), body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride (logarithmically transformed), fasting glucose, 2-h glucose, fasting insulin (logarithmically transformed), homeostasis model assessment of insulin resistance index (logarithmically transformed), fibrinogen level, and the FGB AC-haplotype. \( R^2 \) was 0.213 and 0.221 for fibrinogen level and 2-h glucose respectively. "-" indicates those variables which were insignificant and excluded from the stepwise regression model.

Table 5
Independent association of FGB AC-haplotype with fibrinogen level and 2-h glucose: results of stepwise multiple regression analyses

| Independent variables                  | Fibrinogen (g/L) | 2-h glucose (mM) |
|----------------------------------------|------------------|-----------------|
|                                        | \( \beta \)     | \( p \)         | \( \beta \)     | \( p \)         |
| ACT-haplotype (referent: non-carrier)  | 0.176            | 0.002           | 0.151           | 0.010           |
| Age (years)                            | 0.326            | < 0.001         | –               | –               |
| Body mass index (kg/m\(^2\))          | 0.295            | < 0.001         | 0.355           | < 0.001         |
| Sex (referent: male)                   | –                | –               | 0.198           | 0.001           |
| Fasting glucose (mM)                   | –                | –               | –               | –               |
| Fasting insulin (mIU/L)                | 0.205            | 0.001           | –               | –               |
| HDL cholesterol (mM)                   | –                | –               | –0.157          | 0.013           |
| Smoking (referent: never smoker)       | 0.176            | 0.011           | –               | –               |

HDL, high density lipoprotein; \( \beta \), standardized regression coefficient.
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Conflict of interest

None.

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