Elevated Plasma Level of Soluble F11 Receptor/ Junctional Adhesion Molecule-A (F11R/JAM-A) in Hypertension

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BACKGROUND
The F11 receptor (F11R, also known as junctional adhesion molecule A (JAM-A)) plays a role in the development of hypertension in rat. Genetic variants in the human F11R gene were demonstrated to influence systolic blood pressure. In the present study, we investigated the relationship between F11R and hypertension by examining the levels of a circulating soluble form of F11R (sF11R) in hypertensive patients.

METHODS
Plasma sF11R was measured by enzyme-linked immunosorbent assay in 152 hypertensive and 166 normotensive subjects in whom seven tagging single-nucleotide polymorphisms (SNPs) in the F11R gene had been genotyped.

RESULTS
Plasma sF11R levels were significantly higher in hypertensive subjects than in normotensive subjects (median (interquartile) range): 162.8 (85.5–293.2) vs. 116.5 (74.1–194.8) pg/ml, P = 0.004), which remained significantly higher after adjusting for age, sex, body mass index (BMI), and homeostasis model assessment of insulin resistance (HOMA-IR) (P = 0.028). In stepwise multiple logistic regression, sF11R level (log-transformed) (P = 0.040), triglycerides (log-transformed) (P = 0.024), and HOMA-IR (log-transformed) (P < 0.001) were independently associated with hypertension. Plasma sF11R level correlated with systolic and diastolic blood pressures (r = 0.15, P < 0.001, and r = 0.13, P = 0.024, respectively). In stepwise multiple linear regression, hypertension (P = 0.013) and fibrinogen levels (P = 0.027) were significant independent predictors of sF11R level. A seven-locus haplotype, present in 2.1% of the subjects, was associated with higher sF11R level (P = 0.024).

CONCLUSIONS
These results further support a role of F11 receptor in the pathophysiology of human hypertension.

is triggered by inflammatory agents and as inflammatory cytokines are involved in the pathogenesis of hypertension,
we examined the association between circulating F11R and hypertension by measuring the levels of sF11R in hypertensive individuals.

**METHODS**

**Subjects.** The study included 318 unrelated Chinese subjects living in Hong Kong. The sample consisted of 152 hypertensive subjects and 166 normotensive controls drawn from the Hong Kong Cardiovascular Risk Factor Prevalence Study. They had been previously genotyped for seven tagging single-nucleotide polymorphisms (SNPs) in the F11R gene with the gene (position 159, 231, 608–159, 278, 358, GenBank accession number NC_000001). The study protocol was approved by the University of Hong Kong Faculty of Medicine Ethics Committee, and written informed consent was obtained from all participants. Hypertension was defined as blood pressure ≥140/90 mm Hg or taking antihypertensive medication. Blood pressure was measured in the right arm manually three times at 5-min intervals using a mercury sphygmomanometer. The first blood pressure measurement was performed to familiarize the patient with the procedure. The mean of the two subsequent readings was used for data analysis. Plasma fibrinogen level was measured using a photo-optical end-point clot detection method on a Cobas Fio instrument (Roche Diagnostics, Basle, Switzerland) with an interassay coefficient of variation of 4.7%. Other clinical characteristics and physical examinations had been described earlier.

**Blood sampling.** Subjects were studied in the morning after overnight fasting. After resting for at least 5 min in the sitting position, venous blood was taken from a forearm vein in the sitting position, placed in an ethylenediaminetetraacetic acid bottle, and immediately placed on ice. The blood was immediately centrifuged at 4°C, and the plasma was frozen at −70°C until the time of assay.

**sF11R level measurement.** The enzyme-linked immunosorbent assay for plasma sF11R was performed using recombinant F11R/Fc chimera (R&D Systems, Minneapolis, MN) as standard, M.Ab.F11 monoclonal antibody as capture antibody (BD Biosciences, San Jose, CA), and biotinylated antihuman F11R antibody (R&D Systems, Minneapolis, MN) as detection antibody according to the conditions described earlier. The sensitivity of the sF11R assay was 9.8 pg/ml, and the interassay coefficient of variation was <12%.

**Statistical analysis.** Statistical analysis was performed using SPSS 15.0 for Windows (SPSS, Chicago, IL). Comparisons of clinical characteristics were performed using unpaired Student’s t-test or nonparametric Mann–Whitney U-test for continuous variables, where appropriate, and Fisher’s exact test was performed for categorical variables. Plasma sF11R level is presented as median and interquartile range due to its skewed distribution. The sF11R levels in normotensive, untreated hypertensive, and treated hypertensive subjects were compared using Kruskal–Wallis test. For bivariate correlations, Spearman correlation coefficients were calculated. Stepwise forward multiple logistic and linear regression analyses were performed with hypertension or sF11R level as the dependent variable, respectively. Variables with skewed distributions were log-transformed in the regression models. Haplotype analysis of SNPs with log-transformed sF11R level was performed using --hap--chop and --each-vs-others commands in the software program PLINK (version 1.0.3). A two-tailed P value <0.05 was considered statistically significant.

**RESULTS**

Our study consisted of 152 hypertensive and 166 normotensive subjects. The baseline characteristics of these subjects are shown in Table 1. Hypertensive subjects exhibited significantly higher blood pressure, body mass index (BMI), waist circumference, plasma triglycerides, fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), and HOMA-IR homeostasis model assessment of insulin resistance index. These differences were statistically significant using Student’s t-test or nonparametric Mann–Whitney U-test for continuous variables, where appropriate, and Fisher’s exact test was performed for categorical variables. Plasma sF11R level is presented as median and interquartile range due to its skewed distribution. The sF11R levels in normotensive, untreated hypertensive, and treated hypertensive subjects were compared using Kruskal–Wallis test. For bivariate correlations, Spearman correlation coefficients were calculated. Stepwise forward multiple logistic and linear regression analyses were performed with hypertension or sF11R level as the dependent variable, respectively. Variables with skewed distributions were log-transformed in the regression models. Haplotype analysis of SNPs with log-transformed sF11R level was performed using --hap--chop and --each-vs-others commands in the software program PLINK (version 1.0.3). A two-tailed P value <0.05 was considered statistically significant.
Plasma sF11R Level in Hypertension

Table 2 | Forward stepwise regression analysis for hypertension

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Odds ratio</th>
<th>P</th>
<th>Odds ratio</th>
<th>P</th>
<th>Odds ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td>Males</td>
<td></td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l, log-transformed)</td>
<td>4.50</td>
<td>0.024</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin (mIU/l, log-transformed)</td>
<td>NS</td>
<td>NS</td>
<td>14.36</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR (log-transformed)</td>
<td>11.92</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>45.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>NS</td>
<td>NS</td>
<td>1.76</td>
<td>0.036</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>sF11R level (pg/ml, log-transformed)</td>
<td>1.77</td>
<td>0.040</td>
<td>NS</td>
<td>NS</td>
<td>2.21</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Age, sex, body mass index, waist circumference, low density lipoprotein cholesterol, high density lipoprotein cholesterol, fasting glucose (log-transformed), current smoker (yes and no), and diabetes (with and without) were excluded from the stepwise model (P > 0.05).

HOMA-IR, homeostasis model assessment of insulin resistance index; NS, not significant (P > 0.05); sF11R, soluble form of F11 receptor.

(HOMA-IR), and significantly lower high-density lipoprotein cholesterol, as expected. We determined that both the median of the plasma sF11R level and mean of the log-transformed level were significantly higher in hypertensive subjects than in normotensive subjects (P = 0.004 and 0.027, respectively). The association between the log-transformed sF11R level and hypertension remained significant after adjusting for age, sex, BMI, and HOMA-IR (log-transformed) (P = 0.028). The association was even greater in strength when subjects within the upper (>502.8 pg/ml) and lower (<41.6 pg/ml) 10% of the plasma sF11R level were excluded (P = 0.004 after adjustment) so that the observed difference between sF11R level was not due to extreme outliers. Moreover, the association remained significant after excluding subjects on drug treatment for diabetes (P = 0.022 after adjustment).

Among the 152 hypertensive subjects, 81 (53.3%) of the individuals were on antihypertensive medications, and 35 (43.2%) of these individuals had a blood pressure <140/90 mm Hg. There was no significant difference in sF11R level between treated and untreated hypertensive subjects (154.5 (83.7–279.2) vs. 184.5 (86.7–350.6) pg/ml, P = 0.54). The plasma sF11R level was elevated in both untreated and treated hypertensive subjects (P = 0.011 and 0.032, respectively, vs. normotensive controls, using the Mann-Whitney U test and P = 0.014 using the Kruskal-Wallis test). Among the 81 treated hypertensive subjects, plasma sF11R level did not differ significantly between subjects whose blood pressure was <140/90 mm Hg and those with inadequate control (145.9 (72.8–271.1) vs. 166.4 (91.8–304.3) pg/ml, P = 0.47). Of the 81 subjects treated with antihypertensive medications, 73 had reported the names of the antihypertensive drugs used: angiotensin II receptor antagonists (n = 3), angiotensin-converting enzyme inhibitors (n = 11), beta-blockers (n = 31), calcium antagonists (n = 25), diuretics (n = 18), other hypertensive drugs (n = 13), and 22 individuals reported use of more than one class of antihypertensive drugs. Plasma sF11R level did not differ significantly between subjects taking or not taking a specific type of drug (P > 0.05), although subjects taking more than one class of antihypertensive drugs had significantly higher plasma sF11R levels than those taking only one class of hypertensive drugs (254.6 (105.3–419.3) vs. 139.0 (79.2–186.0) pg/ml, P = 0.033).

Among all subjects, plasma sF11R level was not related to sex, smoking, or diabetes (P > 0.05 using Mann-Whitney U-tests). In univariate Spearman correlation analysis, plasma sF11R level correlated with both systolic and diastolic blood pressures (r = 0.15, P = 0.006, and r = 0.13, P = 0.024, respectively) as well as plasma triglycerides (r = 0.11, P = 0.044). The correlation remained significant for systolic blood pressure after exclusion of hypertensive subjects on drug treatment (r = 0.15,

Table 3 | Forward stepwise regression analysis for plasma sF11R

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>n</th>
<th>sF11R level (pg/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs790056</td>
<td>TT</td>
<td>230</td>
<td>130.1 (81.9–260.0)</td>
</tr>
<tr>
<td></td>
<td>TC + CC</td>
<td>88</td>
<td>134.3 (75.5–195.6)</td>
</tr>
<tr>
<td>rs2481084</td>
<td>TT</td>
<td>219</td>
<td>130.2 (77.4–248.5)</td>
</tr>
<tr>
<td></td>
<td>TC + CC</td>
<td>99</td>
<td>141.4 (78.0–249.4)</td>
</tr>
<tr>
<td>rs6695707</td>
<td>AA</td>
<td>191</td>
<td>136.5 (73.3–242.2)</td>
</tr>
<tr>
<td></td>
<td>AC + CC</td>
<td>127</td>
<td>124.0 (85.6–249.4)</td>
</tr>
<tr>
<td>rs7546890</td>
<td>TT</td>
<td>145</td>
<td>127.3 (81.7–292.6)</td>
</tr>
<tr>
<td></td>
<td>TC + CC</td>
<td>173</td>
<td>135.9 (76.4–228.9)</td>
</tr>
<tr>
<td>rs11576837</td>
<td>AA</td>
<td>139</td>
<td>132.6 (79.2–256.7)</td>
</tr>
<tr>
<td></td>
<td>AG + GG</td>
<td>179</td>
<td>131.5 (77.3–247.4)</td>
</tr>
<tr>
<td>rs3737787</td>
<td>GG</td>
<td>220</td>
<td>129.4 (77.3–254.0)</td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>98</td>
<td>142.9 (78.0–247.2)</td>
</tr>
<tr>
<td>rs2774276</td>
<td>CC</td>
<td>228</td>
<td>122.7 (74.0–248.3)</td>
</tr>
<tr>
<td></td>
<td>CG + GG</td>
<td>90</td>
<td>146.7 (80.1–258.6)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range). Subjects homozygous for the minor allele were grouped with heterozygotes for comparison with those homozygous for the major allele to increase the sample size for comparison, assuming a dominant inheritance model. sF11R, soluble form of F11 receptor.

Table 4 | Relationship of sF11R level with genetic variants

**Figure 1** illustrates the relationship of sF11R level with genetic variants.
Plasma sF11R Level in Hypertension

P = 0.026) but not for diastolic blood pressure (r = 0.11, P = 0.10). No significant correlation was found for the other variables as shown in Table 1.

In forward stepwise logistic regression analysis with hypertension as a dichotomous dependent variable and all the other 14 variables except blood pressure from Table 1 as independent variables, sF11R level, plasma triglycerides, and HOMA-IR were independent predictors, accounting for 21.6% of the total variance among all subjects (Table 2). However, in sex-specific analysis, sF11R level was an independent predictor of hypertension in women but not in men.

In forward stepwise linear regression analysis with sF11R level (log-transformed) as the dependent variable and all the other 16 variables (including hypertension) shown in Table 1 as independent variables, only hypertension (β = 0.14, P = 0.013) and fibrinogen levels (β = –0.13, P = 0.027) were significant independent predictors among all subjects (Table 3). However, this stepwise model could only account for 2.5% of the variance in sF11R level, suggesting that there were unidentified factors influencing sF11R levels. The independent association of hypertension with sF11R (log-transformed) remained significant after adjusting for age, sex, BMI, plasma triglycerides, fibrinogen, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fasting glucose, fasting insulin, HOMA-IR, diabetes, smoking, and antihypertensive drug treatment (P = 0.041), whereas replacement of BMI with waist circumference in the regression model did not appreciably affect the P value (P = 0.038). Fibrinogen was significantly associated with sF11R level in the subgroup of normotenive subjects (Table 3).

We further investigated the effects of seven tagging SNPs. Individually, these seven SNPs were not significantly associated with sF11R level (all P > 0.05) (Table 4). Haplotype analysis suggested a nominally significant association of the seven-locus haplotype TTATAGG, comprising all the seven SNPs, rs790056, rs2481084, rs6695707, rs7546890, rs11576837, rs3737787, and rs2774276, with sF11R level (regression coefficient = 0.25, P = 0.024, haplotype frequency = 2.1%). The association remained significant after adjusting for age, sex, fibrinogen, and hypertension (P = 0.040).

**DISCUSSION**

The sF11R was first found in circulation by Salifu et al.8 who demonstrated an elevation in sF11R level in the sera of hemodialysis patients that was positively correlated with inflammatory cytokines, such as tumor necrosis factor-α, interleukin-10, and interleukin-6, in these patients. In another study, Cavusoglu et al.9 demonstrated increased plasma sF11R levels in the circulation of CAD patients to levels that suggested that circulating sF11R can serve as a potential marker of human atherosclerosis. Here, we report for the first time that the level of plasma sF11R is elevated in hypertension and correlates positively with blood pressures and more strongly with systolic blood pressure. This finding is consistent with the in vivo experiment in rats showing increased systolic blood pressure after overexpression of F11R in the brain.5 Individually, none of the SNPs in the F11R gene was significantly associated with sF11R level. However, there was a nominal association of sF11R level with the TTATAGG haplotype. This is an uncommon haplotype found in 2% of the subjects. Our finding needs to be confirmed in a larger sample in another population with different ethnic groups.

Plasma sF11R level does not appear to correlate with other traditional cardiovascular risk factors, such as age, BMI, insulin resistance, and lipid profile. In a study of CAD patients, the plasma sF11R level did not relate to diabetes, obesity, or hyperlipidemia, but it correlated positively with fasting insulin and was significantly lower in current smokers.9 Interestingly, plasma sF11R level did not differ significantly between CAD patients with or without hypertension.9 In this study, we did not find any gender difference in the plasma sF11R level. However, the association of sF11R level with hypertension was significant in women but not in men in the stepwise regression analysis. Sex hormones have been suggested to contribute to the gender difference in blood pressure regulation.14 The expression level of F11R has been shown to be affected by sex hormones, at least in the female genital tracts in mice.15

The causative role of sF11R in hypertension of humans is unknown at present. Rodent models may shed light on mechanisms by which F11R may elevate blood pressure. Expression of F11R in the nucleus tractus solitarii of the rat brain stem was shown to increase prior to the onset of hypertension, and overexpression of F11R increased systolic blood pressure with the effect lasting for more than 14 days.7 It has been demonstrated that F11R serves to promote the adherence of leukocytes to the endothelium resulting in vascular inflammation16 and thus may be responsible in part for the increase in the total peripheral resistance observed in spontaneously hypertensive rats.17 Moreover, the mediation by F11R in the arrest and transendothelial migration of T cells may further suggest a role for the involvement of F11R in the pathogenesis of hypertension.18,19 However, the relationship between brain F11R with plasma sF11R is unknown. Our study did not find a significant difference in sF11R level between treated hypertensive subjects with or without normalized blood pressure, suggesting that sF11R may not be the cause of elevation in blood pressure, although the lack of a significant difference could also be due to an insufficiently large sample size.

It has been suggested that the soluble form(s) of F11R, detected in circulating plasma at the pg/ml level,9 may arise by shedding from endothelial cells and/or platelets by a protease20 or other mechanisms21 resulting in the release of the extracellular domain of F11R into the circulation as sF11R. In addition to endothelial cells and platelets, F11R is also expressed in erythrocytes, monocytes, lymphocytes, neutrophils, and antigen-presenting cells.18,22 Thus, the concentration of sF11R in the circulation would be dependent on the level of expression of the native, transmembrane F11R and on the rate of the release of its extracellular domain into the circulation. Interestingly, in spontaneously hypertensive rats, enhanced proteolytic activity in endothelium and in plasma increases cleavage of cell membrane receptors, including
insulin receptors and CD18 membrane adhesion molecules. Whether the low level of circulating (pg/ml range) sF11R has active biological functions or is an inactive degradation product is still unknown. Pharmacological concentrations of a recombinant soluble extracellular portion of rsF11R, at the μg/ml level, were shown to inhibit platelet aggregation induced by the stimulatory monoclonal antibody, M.Ab.F11, and the adhesion of platelets to immobilized rsF11R. Such high concentrations of rsF11R were also shown to inhibit mononuclear cell recruitment on inflamed or atherosclerotic endothelium. Whether the generated level of sF11R may provide a negative feedback mechanism that regulates the activity of the native F11R is speculative at the present time. Further study using F11R antagonists in animal models may be needed to validate the functional relevance of sF11R to hypertension.

Our finding of an association of sF11R with hypertension, a major risk factor of cardiovascular diseases, is consistent with a previous report showing an association with CAD. However, the sF11R level in normotensive subjects measured in the present study is significantly higher than that measured in normal subjects in a previous report. The cause of this difference lies in the study population itself, the sites, or sample handling, although stringent adherence to the protocols was followed. However, this difference between studies in normotensive levels does not detract from the final conclusion of the present study regarding the significant correlation with sF11R levels in people with hypertension. Further prospective studies are needed to investigate the ability of sF11R to predict the development of hypertension and hence its causative role.

The independent association of sF11R level with fibrinogen, an acute phase protein, in stepwise multiple linear regression analysis is consistent with its correlation with inflammatory cytokines such as tumor necrosis factor-α and its role in inflammation. The sF11R could be another inflammatory cytokine involved in the pathogenesis of hypertension. It is interesting that the direction of association between sF11R level and fibrinogen is negative. Besides fibrinogen, fibronectin is another molecule that mediates platelet aggregation. Soluble fibronectin can attenuate the effects of tumor necrosis factor-α involved in the pathogenesis of hypertension. It is interesting that the direction of association between sF11R level and fibrinogen is negative. Besides fibrinogen, fibronectin is another molecule that mediates platelet aggregation. In conclusion, plasma sF11R is elevated in hypertension and is related to fibrinogen level. Its use as a novel biomarker of hypertension and other related cardiovascular risk merits further study.

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