Rapid Communication

Coagulation Factor VII, Serum-Triglycerides and the R/Q353 Polymorphism: Differences between Older Men and Women

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Summary

Coagulation factor VII activity (FVII:C) is a risk indicator for cardiovascular disease. It is related to serum-triglycerides and the R/Q353 polymorphism (alleles R and Q) in the gene coding for factor VII is strongly associated with factor VII. The association of serum-triglycerides with factor VII may differ between the genotypes, but the results of earlier studies were inconsistent and did not include older people. We studied FVII, triglycerides and the R/Q353 polymorphism in the Rotterdam Study.

In 1158 older subjects (489 men and 669 women) FVII:C, factor VII:Chr, serum-triglycerides and the R/Q353-genotype were determined.

In women triglycerides were positively associated with FVII:Chr and FVII:C (FVII:Chr: $\beta = 12.4$ % PP/mmol/L, CI: 10.3-14.5; FVII:C: $\beta = 13.1$ % PP/mmol/L, CI: 10.4-15.8). These associations varied by genotype (FVII:Chr: RR: $\beta = 11.7$, CI: 9.6-13.8, RQ/QQ: $\beta = 7.9$, CI: 4.6-11.2; FVII:C: RR: $\beta = 12.5$, CI: 9.5-15.5, RQ/QQ: $\beta = 6.4$, CI: 1.4-11.4).

In men, the associations of FVII:Chr and FVII:C with triglycerides were weaker (FVII:Chr: $\beta = 5.9$, CI: 4.1-7.7; FVII:C: $\beta = 8.7$, CI: 6.2-11.2). There was no difference between the genotype groups.

These results suggest that only in older women the strength of the association of factor VII with serum-triglycerides varies according to genotype of the R/Q353 polymorphism.

Introduction

Increased factor VII activity (FVII:C) is associated with an increased risk of heart disease (1, 2). Therefore, interest in determinants of factor VII has grown. The R/Q353 polymorphism in the gene coding for FVII is the result of a single base change in the codon for amino acid 353, leading to the replacement of Arginine (R) by Glutamine (Q) (3). Presence of the Q allele is related to lower levels of FVII:C and total factor VII (3).

In several studies serum-triglyceride concentration was positively associated with FVII:C (4, 5, 6, 7) and with total factor VII (8, 9). As the R/Q353 polymorphism has a substantial influence on the level of both FVII:C and total factor VII (10), the association of triglycerides with factor VII may differ between the genotypes. In a few studies this was investigated, however results are difficult to interpret, since subjects differed in ethnic background, disease status and gender (11, 12, 13, 14, 15). Furthermore, sample sizes were too small to account for the low frequency of the Q allele (0.099) (16). Although the thrombogenic potential of factor VII may be especially important in older people among whom atherosclerosis is common, none of these studies included elderly subjects. The Rotterdam Study, a population based study, gave the opportunity to evaluate factor VII, serum-triglycerides and the R/Q353 polymorphism in a large cohort of comparable numbers of Caucasian men and women, where adequate representation of the Q allele could be ensured by selecting the upper and lower quintile of the FVII:C distribution.

Methods

The Rotterdam Study is a population based study among 7,983 subjects of 55 years and over (17). Subjects with myocardial infarction in the year before the investigation, diabetes mellitus, use of anticoagulants or stasis during venepuncture were excluded, leaving 3005 subjects. None of the female subjects used hormone replacement therapy.

FVII:C was measured in non-fasting blood samples with a one-stage-clotting assay using human thromboplastin (Tromborel S, Behringwerke, Germany) and factor VII deficient plasma (Ortho Diagnostic System, Beersse, Belgium). The results are expressed as percentages of pooled plasma (%PP). Individuals in the extreme quintiles of the FVII:C distribution were selected ($n = 1158$), expecting an enrichment for the Q allele (i.e. subjects with the RQ or QQ genotype) in the lowest quintile. In plasma of these persons FVII:Chr (reflects total factor VII) was estimated by a two-stage amidolytic micro-titre assay (Chromogenix, Mölndal, Sweden) (18). Different pooled plasma's were used for the measurement of FVII:C and FVII:Chr. Serum-triglycerides were measured with a colorimetric assay using a Kodak Ektachem 250 Analyzer.

DNA polymorphisms were determined as described previously (16). Briefly, genomic DNA was amplified using PCR with oligonucleotide primers as described by Lane et al. (19). The reaction components were incubated at 94 °C for 4 min, followed by 32 cycles of 94 °C for 1 min, 59 °C for 1.5 min and 72 °C for 2 min. Ten μ l PCR product were digested with 5 units of MspI (Gibco BRL) at 37 °C. MspI digestion yielded a constant band of 40 base pair (bp). The common R allele gave bands of 205 bp and 67 bp and the Q allele gave a band of 282 bp as described previously (3).

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Complete data were available for 669 women and 489 men. Differences between the lower and the upper quintile, and between genotype groups were compared with the Student T-test. The relationship of FVII:C and FVII:Chr with serum-triglycerides was examined using linear regression analyses, separately for men and women, with age and body mass index as possible confounders. Since adjustment of the regression models for belonging to the lowest or highest quintile did not change the results, the quintiles were combined. The same analyses were carried out separately for genotype groups. As the association of triglycerides with factor VII was in the same direction in individuals with the RQ and QQ genotype and because the group of individuals with the QQ genotype was too small ($n = 32$) for meaningful analyses, all individuals carrying the Q allele were combined in one group.

Results and Discussion

As the results for men and women were different and combined interpretation may not be appropriate, they are given separately.

Men

There was no difference between the lowest and the highest quintile of FVII:C in age (mean (SD): 67.3 (7.2) vs 65.8 (6.7) years). In the lowest quintile the frequency of the Q allele was 0.25 and triglyceride was 1.8 (0.8) mM, in the highest quintile this was 0.06 and 2.5 (1.4) mM ($p < 0.05$ for difference between quintiles). In the total group FVII:Chr was 48% PP and 88% PP for men with the QQ and RR genotype respectively, and FVII:C was 85% PP and 119% PP ($p < 0.05$ for difference between genotype groups) (Table 1A). FVII:Chr and FVII:C were both positively associated with serum-triglycerides (Table 2A). For FVII:Chr there was no difference between the genotype groups. For FVII:C there was a trend towards a stronger association with triglycerides in men carrying the Q allele.

Humphries et al. (11) reported in a group of Caucasian males a positive association between FVII:Ag and triglycerides in men homozygous for the R allele and a weaker association for men carrying the Q allele. Saha et al. (13) studied Dravidian Indians and observed a positive association in both genotype groups, the association being stronger in subjects carrying the Q allele. Several differences between these study populations, like ethnic background, diet and inclusion of women, may explain the difference in results. In our study among older men the association of FVII:Chr with triglycerides was confirmed both in those with the RR genotype and in those carrying the Q allele. We conclude that no major differences between the genotypes of the R/Q353 polymorphism in the association of FVII:Chr and triglycerides are present in Dutch elderly men.

Interpretation of results on FVII:C are more complicated, since it is increased immediately after fat intake and samples can be taken in fasting and postprandial state (20). In our study samples were taken throughout the morning and afternoon, but adjustment for time since last meal did not change the results, suggesting that postprandial effects of the Dutch light breakfast and lunch are limited. This may be different in other populations with different ethnic background and dietary habits. The relation between FVII:C and triglycerides did not differ between the genotype groups in our study. The same was observed in a study among Caucasian patients with non-insulin-dependent-diabetes mellitus, where fasting blood samples were taken (14). Humphries et al. (11) observed a positive association between non-fasting FVII:C and triglycerides in Caucasian males with the RR genotype, but not in those carrying the Q allele. Similar results were observed in Gujarati Indians by Lane et al. (12). In Dravidian Indians, however, the association between fasting FVII:C and triglycerides was only found in those

Table 1 FVII:C and FVII:Chr (means $\pm$ SE) per genotype for men (A) and women (B)

A. Men				
		Total group	Lowest quintile	Highest quintile
FVII:Chr*	RR	87.8 $\pm$ 1.1 (n=337)	68.8 $\pm$ 1.2 (n=122)	98.6 $\pm$ 1.1 (n=215)
	RQ	64.3 $\pm$ 1.6 (n=121)	57.6 $\pm$ 1.2 (n=93)	86.6 $\pm$ 2.8 (n=28)
	QQ	48.2 $\pm$ 1.8 (n=12)	47.5 $\pm$ 1.9 (n=11)	55.5 (n=1)
FVII:C*	RR	119.0 $\pm$ 1.7 (n=352)	79.7 $\pm$ 0.7 (n=127)	140.5 $\pm$ 1.0 (n=225)
	RQ	90.6 $\pm$ 2.5 (n=125)	76.7 $\pm$ 1.0 (n=96)	136.6 $\pm$ 2.5 (n=29)
	QQ	85.4 $\pm$ 1.2 (n=12)	73.6 $\pm$ 2.4 (n=11)	215.0 (n=1)

B. Women				
		Total group	Lowest quintile	Highest quintile
FVII:Chr*	RR	101.1 $\pm$ 1.2 (n=492)	78.8 $\pm$ 1.0 (n=203)	116.7 $\pm$ 1.2 (n=289)
	RQ	71.5 $\pm$ 1.5 (n=141)	66.6 $\pm$ 1.1 (n=120)	99.5 $\pm$ 1.9 (n=21)
	QQ	50.5 $\pm$ 2.8 (n=19)	50.5 $\pm$ 2.8 (n=19)	
FVII:C*	RR	129.9 $\pm$ 1.5 (n=506)	91.5 $\pm$ 0.8 (n=207)	156.3 $\pm$ 0.8 (n=299)
	RQ	97.4 $\pm$ 2.3 (n=143)	87.6 $\pm$ 1.2 (n=122)	154.4 $\pm$ 2.2 (n=21)
	QQ	77.7 $\pm$ 4.0 (n=20)	77.7 $\pm$ 4.0 (n=20)	

* FVII:C and FVII:Chr are presented in %PP, different pooled plasma's were used

Table 2 Regression coefficients and 95% confidence intervals of triglycerides on FVII:C and FVII:Chr by genotype for men (A) and woman (B)

A. Men				
	FVII:Chr		FVII:C	
	B	95% CI	B	95% CI
Total group	5.9	4.1,7.7	8.7	6.2,11.2
RR	5.5	3.7,7.3	7.3	4.6,10.0
RQ/QQ	5.7	2.7,8.7	10.8	5.8,15.7

B. Women				
	FVII:Chr		FVII:C	
	B	95% CI	B	95% CI
Total group	12.4	10.3,14.5	13.1	10.4,15.8
RR	11.7	9.6,13.8	12.5	9.5,15.5
RQ/QQ	7.9	4.6,11.2	6.4	1.4,11.4

* All models adjusted for age and body mass index. The regression coefficients give the change in FVII:Chr and FVII:C in percent pooled plasma per unit change in triglycerides (% PP/mmol/L)

carrying the Q allele. The discrepancies in these results may be ascribed to the different prandial states and the differences in dietary habits between the study populations. Furthermore, if the relationship between FVII:C and triglycerides depends on the amount of activated factor VII in the sample, different results will be expected between essays for FVII:C which differ in sensitivity towards activated factor VII (21). This could thus be another explanation for the discrepancy in findings between the studies described. Further analysis in larger groups and with specific attention to the prandial state and the essay used to measure factor VII is required.

Women

There was no difference between the lowest and the highest quintile of FVII:C in age (mean (SD): 66.8 (8.2) vs 66.6 (7.3) years). In the lowest quintile the frequency of the Q allele was 0.23 and triglyceride 1.7 (0.7) mM, in the highest quintile this was 0.03 and 2.4 (1.1) mM ($p < 0.05$ for difference between quintiles). In the total group FVII:Chr was 51% PP and 101% PP for women with the QQ and RR genotype respectively, and FVII:C was 78% PP and 130% PP ($p < 0.05$ for difference between genotypes) (Table 1B). The triglyceride concentration was positively associated with FVII:Chr and FVII:C (Table 2B). The regression coefficients were almost twice as high in women with the RR genotype, compared to women carrying the Q allele ($p < 0.05$ for interaction) and they were larger compared to those found in men.

No substantial data on the relation of triglycerides with factor VII between genotype groups in women are available. In our study, we observed a stronger relationship of both FVII:Chr and FVII:C with triglycerides in RR homozygotes, while these associations in Q allele carriers was similar to that in men. These results suggest that the regulation of the factor VII metabolism by the R allele is different in women compared to men. Support for this view is given by Meilahn et al. (15), who observed a post-menopausal increase in FVII:C only in women homozygous for the R allele and not in those carrying the Q allele. This may explain why they did not find a genotype-triglycerides interaction in the total group of pre- and postmenopausal women. In conclusion, our findings suggest, that at least in postmenopausal women the triglyceride status may contribute in those with the RR genotype to an increased risk for myocardial infarction, provided that the risk observed in epidemiological studies in men also applies to women.

Conclusion

Humphries et al. (11) previously discussed the impact of the interaction of triglyceride status and the R/Q353 polymorphism in relation to the risk of myocardial infarction. Our present data strongly suggest to pay specific attention to gender differences. The impact of genetic variation in factor VII may obviously be quite distinct in populations which differ in life style. The possibility that both genetically susceptible groups and life style elements can be identified as risk determinants may open possibilities for targeted prevention.

References

1. Meade TW, Brozović M, Chakrabarti RR, Haines AP, Imeson JD, Mellows S, Miller GJ, North WRS, Stirling Y, Thompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *The Lancet* 1986; 6: 533-7.
2. Assmann G, Cullen P, Heinrich J, Schulte H. Hemostatic variables in the prediction of coronary risk: results of the 8 year follow-up of healthy men in the Münster heart study (PROCAM). *Isr J Med Sci* 1996; 32: 364-70.
3. Green F, Kelleher C, Wilkes H, Temple A, Meade T, Humphries S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. *Arterioscler Thromb* 1991; 11: 540-6.
4. Folsom AR, Wu KK, Davis CE, Conlan MG, Sorlie PD, Szklo M. Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. *Atherosclerosis* 1991; 91: 191-205.
5. Kario K, Matsuo T, Kario T, Kobayashi H. Sex-related differences in the relationship between plasma factor VII levels and serum lipids, lipoproteins and apolipoproteins. *Thromb Res* 1991; 64: 69-73.
6. Tervahauta M, Pekkanen J, Kivinen P, Stengård J, Jauhiainen M, Ehnholm C, Nissinen A. Prevalence of coronary heart disease and associated risk factors among elderly Finnish men in the Seven Countries Study. *Atherosclerosis* 1993; 104: 47-59.
7. Marckmann P, Sandström B, Jespersen J. The variability of and associations between measures of blood coagulation, fibrinolysis and blood lipids. *Atherosclerosis* 1992; 96: 235-44.
8. Donders SHJ, Lustermaas FAT, van Wersch JWJ. Coagulation factors and lipid composition of the blood in treated and untreated hypertensive patients. *Scand J Clin Lab Invest* 1993; 53: 179-86.
9. Donders SHJ, Lustermaas FAT, van Wersch JWJ. Glycometabolic control, lipids, and coagulation parameters in patients with non-insulin-dependent diabetes mellitus. *Int J Clin Lab Res* 1993; 23: 155-9.
10. Bernardi F, Marchetti G, Pinotti M, Arcieri P, Baroncini C, Papacchini M, Zeponi E, Ursicino N, Chiarotti F, Mariani G. Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. *Arterioscler Thromb Vasc Biol* 1996; 16: 72-6.
11. Humphries SE, Lane A, Green FR, Cooper J, Miller GJ. Factor VII coagulant activity and antigen levels in healthy men are determined by interaction between factor VII genotype and plasma triglyceride concentration. *Arterioscler Thromb* 1994; 14: 193-8.
12. Lane A, Cruickshank JK, Mitchell J, Henderson A, Humphries S, Green F. Genetic and environmental determinants of factor VII coagulant activity in ethnic groups at differing risk of coronary heart disease. *Atherosclerosis* 1992; 94: 43-50.
13. Saha N, Liu Y, Heng CK, Hong S, Low PS, Tay JSH. Association of factor VII genotype with plasma factor VII activity and antigen levels in healthy Indian adults and interaction with triglycerides. *Arterioscler Thromb* 1994; 14: 1923-7.
14. Heywood DM, Mansfield MW, Grant PJ. Factor VII gene polymorphisms, factor VII:C levels and features of insulin resistance in non-insulin-dependent-diabetes mellitus. *Thromb Haemost* 1996; 75: 401-6.
15. Meilahn E, Ferrell R, Kiss J, Temple A, Green F, Humphries S, Kuller L. Genetic determination of coagulation factor VIIc levels among healthy middle-aged women. *Thromb Haemost* 1995; 73: 623-5.
16. de Maat MPM, Green F, de Knijff P, Jespersen J, Kluft C. Factor VII polymorphisms in populations with different risk of cardiovascular disease. *Arterioscler Thromb Vasc Biol* (in press).
17. Hofman A, Grobbee DE, de Jong PTVM, van den Ouweland FA. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; 7: 403-22.
18. Avvisati G. Evaluation of a new chromogenic assay for factor VII and its application in patients on oral anticoagulant treatment. *Br J Haematol* 1980; 45: 343-52.
19. Lane A, Cruickshank JK, Mitchell J, Henderson A, Humphries S, Green F. Genetic and environmental determinants of factor VII coagulant activity in ethnic groups at differing risk of coronary heart disease. *Atherosclerosis* 1992; 94: 43-50.
20. Humphries S, Temple A, Lane A, Green F, Cooper J, Miller G. Low plasma levels of factor VIIc and antigen are more strongly associated with the 10 base pair promoter (-323) insertion than the glutamine 353 variant. *Thromb Haemost* 1996; 75: 567-72.
21. Miller GJ, Stirling Y, Esnouf MP, Heinrich J, van de Loo J, Kienast J, Wu KK, Morrissey JH, Meade TW, Martin JC, Imeson JD, Cooper JA, Finch A. Factor VII-deficient substrate plasmas depleted of protein C raise the sensitivity of the factor VII bio-assay to activated factor VII: an international study. *Thromb Haemost* 1994; 71: 38-48.

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