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A genome search for genetic determinants of markers of protein C activation

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PS Gla domain may contain functionally important structural determinants that are not present in prothrombin and possibly in other vitamin K-dependent proteins.

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A genome search for genetic determinants of markers of protein C activation

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Activated protein C (APC) is inhibited primarily by the serine proteinase inhibitors α_1 -antitrypsin (α_1 AT) [1], protein C inhibitor (PCI) [1], and α_2 -macroglobulin [2,3]. We recently demonstrated in a large French–Canadian kindred that over half of the variance in the APC– α_1 AT complex and APC–PCI complex plasma concentrations could be attributed to genetic influences [4]. As this thrombophilic kindred has protein C deficiency and these complexes reflect the level of APC, we used

variance component linkage analysis to identify quantitative trait loci that might explain the inherited component of the variance in APC– α_1 AT and APC–PCI plasma concentrations.

The study subjects were members of the French–Canadian kindred. The ascertainment, evaluation, and blood collection of all family members were described previously [5], as well as the methods for measuring plasma concentrations of APC– α_1 AT complex, APC–PCI complex, protein C activity, and prothrombin activation fragment 1.2 (F1.2) [4]. α_1 AT plasma concentrations were measured by the Beckman–Coulter Image system, and PCI plasma concentrations were measured using a kit from Enzyme Research Laboratories (South Bend, IN, USA). This study was approved by the Human Experimentation Committee of the University of Vermont College of Medicine, Burlington, Vermont, USA. All participating subjects gave informed consent. For the current analysis, we included individuals for whom we had plasma concentrations

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Table 1 Results of the variance component linkage analysis on plasma concentrations of APC- α_1 AT and APC-PCI for locations showing initially a LOD score above 1.9

	APC- α_1 AT (cM)		[APC-PCI (cM)] chromosome 11
	Chromosome 13	Chromosome 19	
Initial LOD score* (location [†])	2.0 (56)	3.1 (35)	2.2 (141)
Removal effect thrombosis (location)	1.6 (56)	2.4 (35)	2.9 (141)
Removal effect PC status (location)	1.9 (57)	3.0 (35)	2.1 (141)
LOD score after finemapping* (location)	N/a	2.2 (29)	1.8 (140)
Removal effect thrombosis (location)	N/a	1.9 (27)	2.3 (140)
Removal effect PC status (location)	N/a	2.2 (29)	2.3 (140)

APC, activated protein C; α_1 AT, α_1 -antitrypsin; PCI, protein C inhibitor; PC, protein C mutation; N/a, not applicable.

*With removal of the effect of age, sex, and the use of female hormones.

[†]Location in cM on the Marshfield map.

of APC- α_1 AT and APC-PCI complex, and genotyping data ($n = 148$). We excluded individuals on coumarin derivatives ($n = 9$), women who were pregnant at the time of the blood draw ($n = 2$), and women for whom we had no information on the use of female hormones ($n = 2$). Genotyping was performed with 375 autosomal markers as described by Hasstedt *et al.* [6]. The probability of identity by descent (IBD) was estimated using the linkage analysis package Loki [7]. Additional markers for fine mapping were genotyped using an ABI310 or 3100 at the Vermont Cancer Center DNA analysis facility, and selected markers were run by Decode Genetics Inc. (Reykjavik, Iceland). Using Sequential Oligogenic Linkage Analysis Routines (SOLAR) [8] version 2.1.4, we performed variance component linkage analysis. All (transformed) levels were assumed to be distributed as a multivariate normal density. The parameters for heritability (h^2), household effect (c^2), and heritability that was contributed to a specific genomic location (q^2) as well as the effects of the covariates were estimated simultaneously using maximum likelihood analysis. LOD scores were computed as the \log_{10} likelihood for q^2 estimated to $q^2 = 0$. As proposed by Lander and Kruglyak [9], we defined significant linkage as statistical evidence expected to occur 0.05 times in a whole genome scan (probability 5%; LOD score ≥ 3.3) and suggestive linkage as statistical evidence that would be expected to occur one time at random in a whole genome scan (LOD score between 1.9–3.3). The 95% confidence interval (CI) was determined by the points on the curve defined by dropping the lod score by one unit.

A total of 135 subjects were included in the analysis. Subjects were from 92 households with a mean of 1.4 individuals per household (range 1–4 individuals). The mean age at the blood draw was 28 years (range 1–75 years), 75 participants were women (56%), 28 (21%) carried the protein C 3363C insertion, 28 (21%) carried the prothrombin G20210A mutation (one was homozygous) and eight (6%) had a history of confirmed venous thrombosis. Variance component linkage analysis on plasma concentrations of APC- α_1 AT complex revealed LOD scores above one for chromosomes 13, 14, and 19 (adding age, sex, and use of female hormones as covariates to the model). LOD scores suggestive of linkage were found on chromosome 19p13.2 (LOD score 3.1 at 35 cM; 95% CI 25–40 cM, nearest marker D19S586) and on chromosome 13q14 (LOD score 2.0

at 56 cM; 95% CI 49–68 cM, nearest marker D13S800) (Table 1). However, adding markers in the area under the peak at chromosome 19 decreased the LOD score to 2.2 (Table 1). Eliminating levels of individuals with a confirmed history of thrombosis decreased the LOD score on chromosome 13 to 1.6 and on chromosome 19 to 1.9 (Table 1). Inclusion of protein C status as a covariate in the model did not alter the lod scores (Table 1). For plasma concentrations of APC-PCI complex LOD scores above one were found on chromosomes four and 11 (adding age, sex, and use of female hormones as covariates in the model). The highest LOD score was found on chromosome 11q25 (LOD score 2.2 at 141 cM; 95% CI 126–147, nearest marker D11S1304) (Table 1). The LOD score increased to 2.9 after eliminating the levels of individuals with a confirmed history of venous thrombosis (Table 1). Adding markers in the area under the peak decreased the LOD score on chromosome 11 to 2.3 (Table 1). The LOD score remained 2.3 after removal of the effect of protein C status in the model (Table 1). No evidence for linkage was observed for protein C, F1.2, α_1 AT, and PCI plasma concentrations at the identified fine-mapped peaks on chromosomes 11 and 19.

We have presented suggestive evidence for quantitative trait loci linked to the variance in plasma concentrations of APC-PCI and APC- α_1 AT complexes, which reflect the activity of the protein C system. In the regions showing suggestive linkage evidence, no specific candidate genes were identified. Thus, it appears that yet-to-be-determined novel genes may play a role in modulating APC activity. The only other report of quantitative trait loci associated with the activity of the protein C system was that of Soria *et al.* [10], which reported a locus on chromosome 18 that influences variation in the APC resistance phenotype.

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More on: clinical experience with retrievable vena cava filters – results of a prospective observational multicenter study

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See also Hull RD. Changes in the technology of inferior vena cava filters promise improved benefits to the patient with less harm, but a paucity of evidence exists. *J Thromb Haemost* 2005; **4**: 1368–9; Imberti D, Bianchi M, Farina A, Siragusa S, Silingardi M, Ageno W. Clinical experience with retrievable vena cava filters: results of a prospective observational multicenter study. *J Thromb Haemost* 2005; **4**: 1370–5.

We read with interest the paper by Imberti *et al.* [1] and agree with their views regarding the need for documentation of the length of implantation time for retrievable vena cava filters. The authors report an average implantation time of 123 days with a maximum of 345 days.

In our own recent experience (21 filters implanted and only 12 already extracted at the moment), the longest implantation time was 485 days in a 35-year-old man in which filter implantation was indicated for proximal deep vein thrombosis in a context of acute alcoholic pancreatitis with abdominal bleeding. The patient was momentarily lost for follow-up after hospital discharge but came back to our institution 15 months

later. Decision to make an attempt at removing the filter was taken despite this long delay because of his poor compliance for any kind of follow-up and the absence of permanent risk factor for thromboembolic disease. Correct position of filter and permeability of vena cava were confirmed by computed tomographic scan prior to the procedure. The filter was easily extracted using the procedure recommended by the manufacturer without any mechanical damage to the venous wall. The examination of the filter *ex vivo* revealed only minor clots in the legs. The implantation time in our patient is, to our knowledge, the longest described in the literature [1,2].

As with other authors [3], we believe that the interest of using such temporary filters increases when the expected period of implantation time can be counted in weeks or months rather than in days, and agree that further scientific evidence is needed [4] before such long implantation durations, as in the case we report, should be encouraged. In the mean time, we feel that such individual experience driven by unusual clinical situations should be reported in order to make the information available. A large multicenter register could be the most efficient way for this purpose.

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