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Evidence of a Founder Effect for the Protein C Gene 3363 Inserted C Mutation in Thrombophilic Pedigrees of French Origin

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Keywords

Protein C deficiency, thrombophilia, genetics

Summary

We have previously reported that the 3363 inserted (Ins) C mutation in exon 6 of the protein C gene was present in four unrelated French patients and in four French Canadian families with type I protein C deficiency as well as in a large Vermont protein C deficient kindred of French Canadian origin. The present study was designed to investigate the likelihood of the existence of a founder effect for this mutation in protein C deficient individuals of French origin living in France, Québec and Vermont. In order to demonstrate a possible founder effect for the 3363 InsC mutation, we have previously constructed a high-resolution genetic map to locate several highly polymorphic markers close to the protein C locus. Thereafter, the markers D2S347, D2S2339, D2S383, D2S2271 and D2S2215 were genotyped in 117 heterozygotes from France (n = 7), Québec (n = 36) or Vermont (n = 74). The allelic frequency distribution of these five markers was also determined in fifty control French Canadian subjects and thirty-two unaffected members of the Vermont kindred with normal protein C levels and compared with their frequency in our cohort of heterozygotes. Our data suggest that patients from Québec and Vermont carry a common haplotype at the protein C locus. Moreover, in order to study the evolutionary history of the 3363 InsC mutation, we traced back the ascending genealogy of one proband in each of the families with this mutation. These results showed that the 3363 InsC mutation was most probably introduced in North America by a couple of French settlers who established themselves in 1669 on Isle d'Orleans located near Québec City. All heterozygotes for the 3363 InsC mutation living in North America are related to these founders within 10 generations. Thus, these families afford a unique opportunity to evaluate the role of the protein C system in thrombophilia due to the high degree of linkage disequilibrium at the protein C gene, which in essence holds that variable more constant than in a more heterogeneous population.

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Introduction

Protein C is a vitamin K-dependent glycoprotein and zymogen of a serine-protease which plays an important role in the regulation of thrombin activity. Following proteolytic activation by thrombomodulin-bound thrombin, activated protein C downregulates the coagulation cascade by selective proteolytic inactivation of the procoagulant factors Va and VIIIa in the presence of calcium and phospholipids (1, 2). It also enhances fibrinolysis in part by neutralizing plasminogen activator inhibitor-1 by way of the thrombin-activatable fibrinolysis inhibitor (TAFI). The protein C gene is located on chromosome 2, at position 2q13-q21 (3-8), and comprises 9 exons encompassing 11 kb genomic DNA and encoding a 1795-bp mRNA (9, 10).

Protein C deficiency is caused by mutation in the protein C gene and is inherited as an autosomal codominant trait. More than 190 germline mutations in the protein C have been described around the world (11). The clinical expression of protein C deficiency is highly variable and the diagnosis based on plasma measurements of protein C is often difficult because of the significant overlap between heterozygotes and non-carriers. Heterozygous subjects with plasma protein C levels between 30% and 70% of normal values possess an increased risk of developing venous thrombosis (12). The prevalence of asymptomatic heterozygotes for protein C deficiency in the general population ranges from 1 in 200 to 1 in 500 (13, 14) while the prevalence of symptomatic heterozygotes ranges from 1 in 16,000 to 1 in 32,000 (15, 16). Homozygous or compound heterozygous subjects with plasma protein C levels < 1% are at risk of neonatal purpura fulminans and massive venous thrombosis (17). When protein C levels are measurable, thrombotic manifestations start later during childhood or early adult life.

We have previously reported that the 3363 inserted (Ins) C mutation in exon 6 of the protein C gene was present in four unrelated French patients (18) and in four French Canadian families with type I protein C deficiency (8) as well as in a large Vermont protein C deficient kindred of French Canadian origin (19). Thus, the present study was designed to investigate the likelihood of the existence of a founder effect for this mutation in protein C deficient individuals of French origin living in France, Québec and Vermont. Specifically, the demonstration of a founder effect for this mutation would provide a great opportunity to study the genetic and environmental factors modulating clinical expression of protein C deficiency.

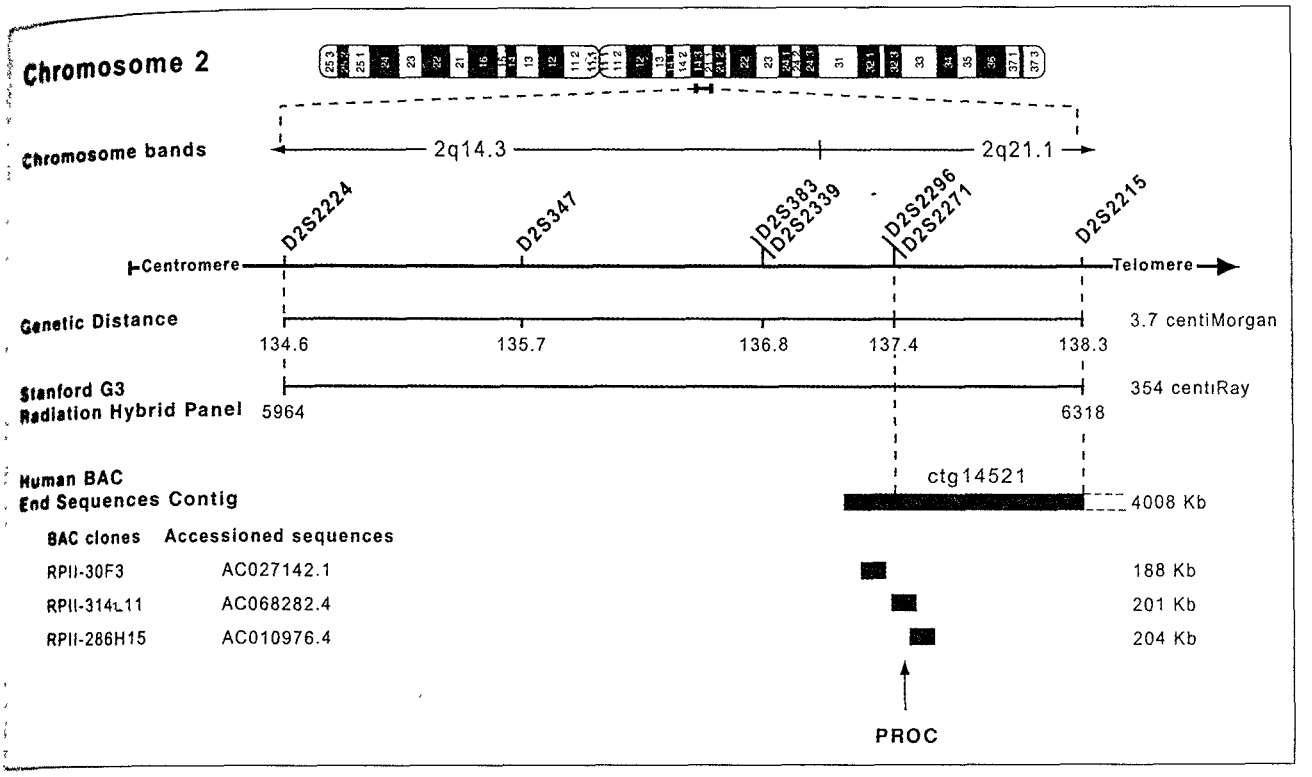

Fig. 1 Fine chromosomal localization of Protein C gene. Location in centiMorgan of each markers was determined according to the genetic linkage map of Genethon and position of the external markers in CentiRay was obtained onto the Stanford G3 radiation hybrid map (resolution = 41.5 cR10000 per megabase) The human BAC end sequences contig was obtained by using the Human Genome Project Working Draft of the Washington University Genome Sequencing Center. St. Louis, MO, USA (http://genome.ucsc.edu/goldenPath/hgTracks.html). The clones come from the Pieter J. de Jong RPCI-11 librairies (Children's Hospital Oakland – BACPAC Resources Oakland, CA, USA)

Subjects, Materials and Methods

Subjects

One hundred and seventeen heterozygotes for the 3363 InsC mutation from France (n = 7), Québec (n = 36) or Vermont (n = 74) with type I protein C deficiency were analyzed. The diagnosis of type I protein C deficiency was based on the presence of plasma levels of protein C antigen below 69% of normal and low levels of activity by functional assay. The study participants from Vermont were drawn from two large related kindreds that consisted of more than 500 members over six generations (19) while heterozygotes from Québec belonged to four large kindreds unrelated to the first or second degree (8). The majority of these individuals resided in northern Vermont and the Québec City area. Heterozygotes from France belonged to three small unrelated nuclear families. All available family members were contacted and blood sample was taken for protein C measurement and mutation analysis. Fifty control French Canadian subjects and 32 unaffected members of the Vermont kindreds with normal protein C levels were also analyzed. All subjects were of French origin. This study was approved by the Human Experimentation Ethics Committee of participating hospitals and informed consent was obtained from all participants.

Mutation Detection

Symmetric PCR was performed as previously described (8) using the primer pair 6c 5'-[illegible]GACAACGGCGGCTGCAGG-3' and 6d 5'-CAGG[illegible]'GTATT-3' for the 3363 InsC mutation. The PCR products were digested with the enzyme HaeIII, as recommended by the supplier (New England Biolabs, Beverly, MA) and the resulting fragments were size-separated by electrophoresis on an 1.5% agarose gel.

Microsatellite Marker Analyses

Five microsatellite markers close to the protein C locus: D2S347, D2S2339, D2S383, D2S2271 and D2S2215 were genotyped in 117 subjects heterozygous for the 3363 InsC mutation. The allelic frequency distribution of these five markers were also determined in 82 control subjects with normal protein C levels and compared with their frequency in our cohort of heterozygotes. Fig. 1 gives the fine chromosomal localization of protein C gene. The location in centiMorgan of each markers was determined according to the genetic linkage map of Genethon and position of the external markers in CentiRay was obtained onto the Stanford G3 radiation hybrid map (resolution = 41.5 cR_{10000} per megabase). The human BAC end sequences contig was obtained by using the Human Genome Project Working Draft of the Washington University Genome Sequencing Center . St. Louis, MO, USA (http://genome.ucsc.edu/golden-Path/hgTracks.html). The clones come from the Pieter J. de Jong RPCI-11 librairies (Children's Hospital Oakland – BACPAC Resources Oakland, CA, USA). The microsatellites were analyzed using PCR and PCR products were size-separated by electrophoresis on an 6% polyacrylamide gel. The alleles are given as base pairs.

Genealogical Studies

Information on the pedigrees of the protein C deficient families was first obtained by questioning family members. Then, the ascending genealogies were reconstructed at the University of Québec in Chicoutimi using the BALZAC population register and the RETRO database. The BALZAC population register was initially constructed by computerizing and linking the Catholic parish registers of baptisms, marriages and burials of the entire Saguenay region (Québec, Canada) from 1840 to 1971 (20, 21). BALZAC allows one to automatically reconstruct the genealogies of the Saguenay population over this period. It is now being expanded to the entire province of Québec for the 19th and 20th centuries. The RETRO database (22) is a satellite database of the BALZAC population register. It was developed through various research projects involving genealogical analyses. At present, RETRO contains linked information on close to 134 000 marriages going back as far as the 16th century. The genealogies were also completed using the University of Montréal research

program in historical demography database, numerous published marriage repositories, genealogical dictionaries, etc. The genealogies were analyzed using various software programs developed at the University of Québec in Chicoutimi (23). The genetic contribution of each ancestor and the kinship coefficient between probands were calculated as previously described (24). The genetic contribution of an ancestor represents the sum of transmission probabilities of one gene to each individual of a given group and is calculated for each ancestor as:

$$\sum_{k}\sum_{p}(1/2)^{g}$$

where k is the number of individuals in a given group genealogically related to the ancestor; p is the number of genealogical paths between the ancestor and the individual; and g is the number of generations separating the ancestor from the individual, for each path. For example, the genetic contribution of a grandfather to his 10 grandchildren is 2.5. The mean genetic contribution per individual is the genetic contribution divided by the number of individuals. On the other hand, the kinship coefficient between two individuals (B_1 and B_2) represents the probability that one allele from individual B_1 is identical by descent to an allele from individual B_2 picked at random at the same locus and is calculated as:

$$\sum_{A}\sum_{C}(1/2)^{n(A,C)+n(A,C)+1}(1+F(A))$$

where A is a common ancestor of B_1 and B_2; C is the number of genealogical paths between B_1 and B_2; $n(A, C)$ is the number of generations separating the founder A from the individual B_1, for each path; $n(A, C)$ is the number of generations separating the founder A from the individual B_2, for each path; and $F(A)$ is the inbreeding coefficient of the founder A. For example, the kinship coefficient between a grandfather and his grandson is 0.125. The mean kinship coefficient for a group of individuals is the kinship coefficient divided by the total number of pairs of individuals.

Statistical Analysis

The allele frequencies were compared using the chi-squared test or Fisher's exact test where appropriate. These analyses were performed using the JMP statistical software (release 4.0.1, SAS Institute Inc).

Results

Genetic Basis of the Founder Effect for the 3363 InsC Mutation

In order to investigate the origin of the frameshift mutation 3363 InsC present in Vermont, Québec and France, we examined allele frequencies of the five microsatellite markers D2S347, D2S2339, D2S383, D2S2271 and D2S2215 which surround and are within 2.6 centiMorgans (cM) of the protein C locus in 117 heterozygotes from Vermont (n = 74), Québec (n = 36) or France (n = 7). The allelic frequency distribution of these five markers was also determined in fifty control French Canadian subjects and thirty-two unaffected members of the Vermont kindred with normal protein C levels and compared with their frequency in our cohort of heterozygotes (Table 1). These results indicated the presence of a common putative haplotype linked to the 3363 InsC mutation in patients from Vermont and Québec. The common putative haplotype included the alleles 286 (D2S347), 208 (D2S2339), 180 (D2S383), 160 (D2S2271), and 153 (D2S2215). Moreover, segregation analyses in protein C deficient families strongly suggested that 3363 InsC heterozygotes from Vermont and Québec shared a common mutant allele (Fig. 2). In protein C deficient families from France, however, only the two microsatellite markers (D2S2271, D2S2215) closest to the protein C locus exhibited identical alleles, most probably because genetic recombinations occurred at the other markers.

Table 1 Allelic distribution frequencies of microsatellite markers in individuals of French origin living in Québec and Vermont

D2S347

Allele	Québec		Vermont	
	N n=50	3363 n=36	N n=32	3363 n=74
264	00	00	.02	00
266	00	00	.35	.21
276	.36	.19	00	.01
278	.04	00	00	00
280	00	00	.02	00
282	00	00	.02	.01
284	.08	00	00	.03
286	.28	.63*	.30	.56*
288	.24	.18	.02	.01
290	00	00	.25	.14
292	00	00	.02	.03

D2S2339

Allele	Québec		Vermont	
	N n=50	3363 n=36	N n=32	3363 n=74
202	.18	.06	.14	.06
204	.44	.16	.31	.18
206	.18	.16	.28	.15
208	.20	.60*	.27	.60*
210	00	00	00	.01
212	00	.02	00	00

D2S383

Allele	Québec		Vermont	
	N n=50	3363 n=36	N n=32	3363 n=74
168	00	00	.03	.06
172	.06	.13	.20	.15
174	.04	00	.25	.11
176	.20	.11	.13	.04
178	.22	.11	.06	.04
180	.40	.63*	.31	.56*
182	.08	.02	00	.03
184	00	00	.02	.01

D2S2271

Allele	Québec		Vermont	
	N n=50	3363 n=36	N n=32	3363 n=74
140	00	00	.20	.18
142	.26	.15	.14	.02
144	.02	00	00	.01
146	.02	.05	00	00
148	00	00	.02	00
150	.04	.03	.20	.07
152	.16	.06	.11	.08
154	.04	.08	.18	.05
156	.28	.03	.11	.07
158	.12	.10	.02	.02
160	.04	.50*	.02	.50*
162	.02	00	00	00

D2S2215

Allele	Québec		Vermont	
	N n=50	3363 n=36	N n=32	3363 n=74
139	.04	.06	.03	.01
143	.04	.03	00	.01
145	.12	.05	.31	.11
147	.12	.10	.19	.13
149	.12	.08	.19	.06
151	.12	.13	.03	.01
153	.16	.47*	.05	.52*
155	.12	.05	00	.01
157	.12	.03	.14	.09
159	.04	00	.06	.05

Significance levels for comparison between normal subjects (N) and heterozygotes calculated by the chi-square test in a pairwise manner are indicated as *P*-values: * $P < 0.001$.

Fig 1 Comparison of ha... segregating with ... 3363 InsC mutation in ... C gene in families ... Quebec, Vermont and ... France

Origin of families	Family's number	Number of 3363 InsC carriers	Haplotypes				
			Microsatellite markers (Cen→Tel) (D2S .)				
			347	2339	383	2271	2215
Quebec	Q01	4	286	208	180	160	153
		1	286	208	180	160	151/151
	Q02	10	286	208	180	160	153
	Q03	10	286	208	180	160	153
	Q04	5	286	208	180	160	153
Vermont	V02	2	286	208	180	160	153
		1	290/284	208	180	160	153
	V01	56	286	208	180	160	153
		1	286	208	180	160	157/147
		1	286	208	180	160	157/149
		1	286	208	180	160	159/147
		1	286	208	180	160	149/145
		1	286	208	180	158/140	157/147
		1	290/266	206/206	180	160	153
		1	292/266	208	180	160	153
		1	286	208	178/176	160	153
		1	286	208	186/172	160	153
		1	290/266	206/204	172/172	160	153
		1	290/288	208	176/172	160	153
		2	292/266	208	182/172	160	153
France	F01	1	266/286	202/208	180/182	140/160	147/153
	F02	4	268/284	204	170	160	153
	F03	2	288	204/210	176	160	153

Genealogy of the 3363 InsC Mutation in North America

In order to study the evolutionnary history of the 3363 InsC mutation in North America, we traced back the ascending genealogy of one proband in each of the six families with this mutation We found ten ancestors (four married couples and two individuals) common to all six protein C deficient families and we hypothesized that each of them could be at the origin of the 3363 InsC mutation in the families Then, in order to determine which couple or individual most likely represented the common ancestor to each proband heterozygous for the 3363 InsC mutation, we determined the genetic contribution of those ten ancestors in the six case and four control genealogies (Table 2) The control subjects were the spouses of the four French Canadian cases Although all 10 ancestors could be at the origin of the mutation subjects 1 and 2 (couple A) have the highest genetic contribution and therefore the highest probability of having introduced the mutation in the French

Table 2 Mean genetic contribution of the common ancestors in the six case and four control genealogies

Ancestors					Cases		Controls*		
ID	Couple	Date of marriage	Place of marriage	Gender	Mean genetic contribution per subject (a) ($X10^{-4}$)	Proportion** (%)	Mean genetic contribution per subject (b) ($X10^{-4}$)	Proportion** (%)	DELTA (a) - (b) ($X10^{-4}$)
1	A	1669	Quebec	F	40 69	100	0 00	0	40 69
2	A	1669	Quebec	M	40 69	100	0 00	0	40 69
3		1640	Quebec	F	36 21	100	13 43	75	22 79
4	B	1631	France	M	34 99	100	25 63	75	11 19
5	B	1631	France	F	34 99	100	25 63	100	11 19
6	C	unknown	France	M	19 73	100	8 54	75	11 19
7	C	unknown	France	F	19 73	100	8 54	75	11 19
8		1627	France	F	17 09	100	14 65	75	2 44
9	D	1628	France	F	28 48	100	36 62	100	-8 14
10	D	1628	France	M	28 89	100	40 89	100	-12 00

*The control subjects were the spouses of the four first French Canadian cases
**Proportion of case or control genealogies where the ancestor is found

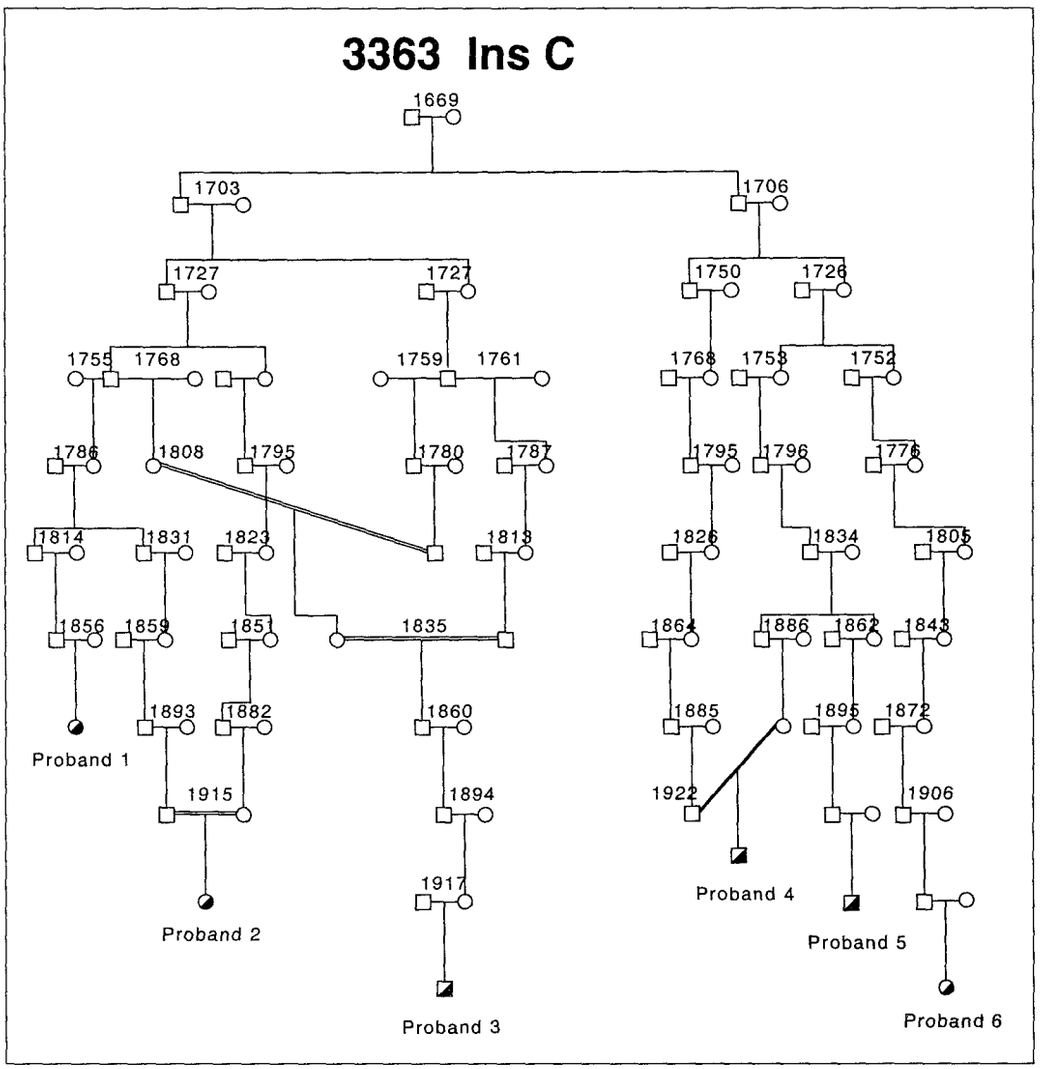

Fig. 3 Genealogy of the 3363 InsC mutation in North America with year of marriage for most couples Probands 1 and 3 reside in Vermont and probands 2, 4, 5 and 6 reside in the Québec City area

Canadian population. Moreover, these two ancestors are present in all 6 genealogies of cases but they are absent from those of controls. In fact, ancestors 1 and 2 have the most specific genetic contribution to cases based on the calculation of the difference in genetic contribution to both groups.

The mean kinship coefficient among the six index cases was also determined at various genealogical depths (3, 6, 9 or total number of generations) and compared with that of controls. Table 3 shows that the mean kinship coefficient is greater among index cases than in controls and indicates that the probability for index individuals to share an allele identical by descent at the same locus is greater than that of controls These results showed that the 3363 InsC mutation was most probably introduced in North America by a couple of French settlers who established themselves in 1669 on Isle d'Orleans located near Québec City. All heterozygotes for the 3363 InsC mutation living in North America are related to these founders within ten generations (Fig. 3).

Table 3 Mean kinship coefficients of the six cases and four controls at various genealogical depths

		Kinship coefficient ($X10^{-4}$)			
Number of generations		3	6	9	Total
Cases	6	5.2083	8.6263	12.1494	12.7213
Controls*	4	0	0	2.346	3.0896

*The control subjects were the spouses of the four French Canadian cases.

Discussion

The 3363 InsC mutation which was present in four large French Canadian families has previously been reported in French patients with homozygous type I protein C deficiency and in a large Vermont kindred with a high incidence of venous thrombosis (19, 25). In the present study, five highly polymorphic microsatellite markers flanking the protein C locus were used to trace back the origin of the 3363 InsC mutation present in Québec, France and Vermont. The association and segregation analyses presented herein indicate that the allelic distribution of the five microsatellite markers differs between control subjects and homozygous patients for the 3363 InsC mutation suggesting that patients from Québec, Vermont and possibly from France carry a common mutant allele at the protein C locus. The microsatellite marker analyses together with the genealogical studies indicate that the 3363 InsC mutation must have a single origin, most likely in France. The possibility of recurrent independent mutations is not supported by the findings of this study. Thus, the 3363 InsC mutation would have been attached to the French Canadian population as a result of a founder effect which is defined as "the establishment of a new population by few original founders who carry only a small fraction of the total genetic variation of the parental population" (26). Some evidence for founder effect in protein C deficiency has already been reported in other populations, namely Dutch (27) and Finns (28).

Several demographic factors could have contributed to enhance the prevalence of the 3363 InsC mutation in the early days of French settlement in Canada and during expansion of the new population. The onset of colonization in North America coincided with the peak of mercantilism in France and in other parts of Europe (29). At that time, French emigration to the colonies was not supported and population growth was encouraged to fulfill the needs of a trading state for welfare of its inhabitants. The existence of colonies was perceived as advantageous to achieve prosperity by providing primary resources and raw materials. This policy led to the establishment of measures to restrict emigration to "La Nouvelle-France" and a series of local incentives in the colony supporting early marriages and high birth rate. The immigration was selective and designed to meet the demands of this policy. Most of the immigrants were skilled artisans sent for specific periods with promise of enhanced status on their return to France; they were also soldiers encouraged to settle in Canada. The number of French immigrants to Canada in the 17th century has been estimated to be around 8000 but many of them did not survive long enough to procreate, many returned to France alone or with their families, and some were members of religious orders. Thus, it was estimated that between 1608 and 1680 which is the period corresponding to the peak of French immigration in "La Nouvelle-France", approximately 3380 pioneers settled permanently in the St-Lawrence River Valley. This rather limited number of founders constitute the basis for the the French Canadian population of today. At that time and following, the birth rate was very high and the growth of the population was rapid (29). Samuel de Champlain founded Québec City with 30 men in 1608 and, in the first census of the French colony in 1666, there were 3215 people in 538 families. It has been estimated that a little more than 1500 women married within the first 50 years of the settlement, contributing half a century later to > 50000 descendants.

The French settlers and their descendants lived in relative isolation because of conditions imposed by climate, geography, religion, language, socioeconomic status, political contraints and a rural life-style (30). They tended to establish small, stable and self-sufficient communities. These factors favored endogamy. Since the economy was based mostly on seasonal agriculture and wood industry, population growth quickly exceeded the employment opportunities and between 1840 and 1930, nearly one million French Canadians emigrated to United States, mainly to Vermont, New Hampshire, Maine and Massachusetts (31). This would explain the presence of the 3363 InsC mutation in the protein C gene and other "French Canadian" mutations responsible for various genetic diseases in New England states (32). Moreover, the early introduction of the 3363 InsC mutation in the Québec settlement process could imply that this mutation has a high prevalence in the French Canadian population. Epidemiological studies are needed to address this question since the prevalence of protein C deficiency is unknown in the Province of Québec.

Major advantages may be derived from the knowledge of a founder effect for the 3363 InsC mutation in the New England states and the Province of Québec, as is the case for several genetic disorders including familial hypercholesterolemia (33, 34), phenylketonuria (35) and familial hyperchylomicronemia (36) in Québec. It facilitates screening, genetic counseling, and treatment. It also provides a great opportunity to study (i) gene-gene interactions involved in the pathogenesis of thromboembolic disease, (ii) genetic and environmental factors modulating phenotypic expression of protein C gene mutations and (iii) the geographic distribution of this mutation as well as population movements.

Addendum

We would like to specify the role each author played in the study. Couture, Bovill, Simard, Long, Aiach, Jomphe and Rosendaal were the researchers involved in the design, execution and analysis of the study. Demers and Delage were responsible for the study's conception and design. During the performance of the study, Scott, Valliere and Callas were responsible for daily supervision of the researchers.

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GYNAECOLOGIE/OBSTETRIE

Wat zijn de huidige adviezen ten aanzien van het gebruik van orale anticonceptiva en het risico op trombose?

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Inleiding

Kort na de introductie van orale anticonceptie werd in 1961 door Jordan in de Lancet melding gemaakt van het optreden van veneuze trombose bij een pilgebruikster (1). Hierop volgden meer meldingen en, in de jaren zestig en zeventig, ook grote vergelijkende studies waaruit bleek dat het hier inderdaad om een bijwerking van de pil ging. Deze onderzoeken toonden aan dat orale anticonceptiva niet alleen de kans op veneuze trombose verhoogden, maar ook op arteriële aandoeningen (2). Recent onderzoek heeft aangetoond dat deze trombotische bijwerkingen van orale anticonceptiva nog steeds bestaan (3).

Trombose

Veneuze trombose treedt doorgaans op in de diepe vaten van het been; de trombose ontstaat meestal in een kuitvene en breidt zich uit tot voorbij de knie of zelfs tot in de lies. Regelmatig (bij circa 20%) treedt embolisering op, waarbij een stolsel via het hart naar de longen wordt vervoerd en aanleiding geeft tot de acute verschijnselen van een longembolie.
Veneuze trombose is een ernstige aandoening: niet alleen is er een letaliteit van 1-2%, maar daarnaast houdt ongeveer de helft van patiënten na een trombosebeen last van chronische klachten van het been, het zogenaamde 'posttrombotisch syndroom'.
Bij een kwart van de patiënten is dit invaliderend door pijnklachten of ulceratie.

Veneuze trombose

De oorzaken van trombose worden doorgaans verdeeld in verworven en genetische oorzaken (4). Genetische afwijkingen die tot een tromboseneiging leiden (trombofilie) zijn ten eerste erfelijke deficiënties aan de natuurlijke stollingsremmers proteïne C, proteïne S of antitrombine. Deze afwijkingen komen voor bij minder dan 1% van de bevolking. De tweede groep genetische afwijkingen leiden tot een toegenomen procoagulante activiteit. Bij factor V Leiden is stollingsfactor V ongevoelig voor inactivatie door geactiveerd proteïne C (APC); men spreekt van APC-resistentie (5). Bij de factor II mutatie (protrombine 20210A) zijn de plasmaspiegels van factor II verhoogd. Factor V Leiden en factor II mutatie komen voor bij respectievelijk 5% en 2% van de bevolking (4).
Recent zijn overigens nog een aantal protrombotische afwijkingen gevonden die, naar alle waarschijnlijkheid, van gemengd genetische en verworven oorsprong zijn. Dit zijn hoge niveaus aan stollingsfactor VIII, IX en XI en hyperhomocysteïnemie (4).
Het risico van veneuze trombose is vooral verhoogd bij operaties en immobilisatie. Andere verworven situaties met een verhoogd risico zijn kanker, zwangerschap, kraambed en het gebruik van vrouwelijke hormonen, zoals in orale contraceptiva en postmenopausale hormoonsuppletie.
Veneuze trombose komt jaarlijks voor bij 1 op de 1000 mensen. Er is een sterke leeftijdsgradiënt, waarbij het risico voor het 40e levensjaar circa 1 per 10.000 per jaar, en na het 80e jaar ongeveer 1 per 100 per jaar is.
Bij trombofilie, zowel door deficiënties van proteïne C, proteïne S of antitrombine, als door factor V Leiden of protrombine 20210A, is dit risico 5-10 maal hoger (4).
Orale anticonceptiva verhogen de kans op veneuze trombose ongeveer vier maal. Gegeven de relatief jonge leeftijd van pilgebruiksters, betekent dit dat de

kans op veneuze trombose stijgt van 1 per 10.000 vrouwen per jaar naar 4 per 10.000 vrouwen per jaar. Vanwege de sterke leeftijdstrend van trombose zal het risico wat lager zijn bij de jonge pilgebruikster, en wat hoger bij de oudere pilgebruikster. Enerzijds is dit een in absolute zin bijzonder laag risico, anderzijds is de pil, omdat grote groepen vrouwen deze gebruiken, de belangrijkste oorzaak van veneuze trombose bij jonge vrouwen. Bij vrouwen met erfelijke protrombotische stollingsafwijkingen bestaat een synergistisch effect. Zo is bij pilgebruiksters met factor V Leiden het risico 30 keer hoger (6).

Risico op arteriële trombose

Orale anticonceptiva verhogen eveneens de kans op een arteriële aandoening, zoals een hartinfarct en herseninfarct (2, 3). Het risico is vooral verhoogd bij vrouwen die ook andere risicofactoren voor arteriële aandoeningen hebben, waarvan de belangrijkste zijn roken, diabetes mellitus, hypertensie en hypercholesterolemie. Vooral voor roken is een synergistisch effect aangetoond. Deze factoren zijn risicofactoren voor atherosclerose, en spelen daarom geen rol in de etiologie van veneuze trombose. De protrombotische stollingsfactorafwijkingen die het risico van veneuze trombose zo sterk verhogen, hebben slechts een gering effect op het optreden van arteriële aandoeningen.
Hoewel het hartinfarct een grotere letaliteit heeft dan veneuze trombose (10% versus 2%), is de incidentie bij jonge vrouwen aanzienlijk lager dan van veneuze trombose, zo zeer zelfs dat veneuze trombose tot meer sterfgevallen bij jonge vrouwen leidt dan arteriële trombose. Zo rond het 30-35e jaar draait dit om.

Omgaan met kleine risico's

Bij het omgaan met risico's spelen twee elementen een belangrijke rol: ten eerste informatieverschaffing en ten tweede de afweging tegen mogelijke nadelen en tegen de voor- en nadelen van alternatieven. Bij de afweging over wel versus geen pilgebruik wordt de effectiviteit en het gemak afgezet tegen de kans op bijwerkingen. Dit is enigszins een afweging tussen onvergelijkbare grootheden en daarom individueel en subjectief. Hier is een goede informatie aan de gebruikster het belangrijkst. De afgelopen decennia hebben veel vrouwen deze afweging door laten slaan ten faveure van pilgebruik. Veel eenvoudiger is de keuze tussen verschillende types monofasische orale anticonceptiva: aange-zien er geen verschillen in effectiviteit zijn, dient het preparaat met de laagste frequentie van bijwerkingen te worden gekozen.

Soorten orale anticonceptiva

De monofasische combinatiepreparaten worden verreweg het meest gebruikt. We zullen ons hier beperken tot deze combinatiepreparaten. Er zijn geen goed gedocumenteerde voordelen wat betreft werking en bijwerkingen voor de andere combinatiepiltypen, zoals bifasische en trifasische preparaten en middelen die uitsluitend progestageen bevatten ('progestin-only').
De eerste anticonceptiva die op de markt kwamen begin jaren 60 bevatten een hoge dosis oestrogeen, 100 tot 150 microgram (meestal ethinyloestradiol) en een eerste generatie progestageen (zoals lynestrol). In het streven naar veiliger anticonceptiva zijn er twee ontwikkelingen geweest. Ten eerste is de dosis oestrogeen verlaagd, eerst naar 50 microgram, toen 30 microgram en thans zijn er anticonceptiva op de markt met 20 of minder microgram oestrogeen. Aan de oestrogene component zelf is niet veel veranderd, dit is ethinyloestradiol gebleven. Wat betreft de progestagene component zijn er marginale veranderingen in de dosis geweest. De belangrijkste verandering was de introductie van nieuwe progestagenen. Deze worden aangeduid met 'generaties'. In de jaren 70 kwam de tweede generatie progestagenen, waartoe levonorgestrel behoort. Sinds midden jaren tachtig zijn er orale anticonceptiva met een derde generatie progestageen, dit zijn de stoffen desogestrel en gestodeen. Op het ogenblik zijn de meest voorgeschreven orale anticonceptiva die met 30 microgram ethinyloestradiol, met een ongeveer gelijke verdeling over tweede- en derde generatie progestagenen.

Effect van dosisverlaging aan oestrogenen

Het is aannemelijk dat de oorspronkelijke orale anticonceptiva die 100 microgram ethinyloestradiol bevatten, meer trombogeen waren dan de latere middelen met 50 of minder microgram ethinyloestradiol. Of de verdere verlaging van de dosis tot een verdere afname van het risico van trombose heeft geleid, is echter zeer de vraag. Er zijn slechts een gering aantal studies die anticonceptiva met 50 en 30 microgram vergeleken, met tegenstrijdige resultaten (3). Hoewel anticonceptiva met minder dan 30 microgram ethinyloestradiol worden aangeprezen als veiliger, zijn er geen gegevens die deze bewering ondersteunen (3). Aangezien de cycluscontrole vaak minder is met deze zeer laag ge-

doseerde anticonceptiva, lijkt er weinig reden te zijn om orale anticonceptiva met minder dan 30 microgram ethinyloestradiol voor te schrijven.

Effect van verandering van de progestagene component

In 1995 bleek in een aantal studies, waaronder een Nederlandse, dat orale anticonceptiva met een derde generatie progestageen het risico van veneuze trombose verhoogden en zelfs tweemaal vaker trombose veroorzaakten dan orale anticonceptiva met een tweede generatie progestageen (7, 8). Het gaat hierbij om anticonceptiva die desogestrel of gestodeen bevatten. Recent bleek in een prospectieve farmaco-epidemiologische studie uit Utrecht dat het risico vooral was verhoogd, tot zeven maal, bij de jongste gebruikers (9). Deze risico's zijn gesuperponeerd op het viervoudig verhoogde risico van tweede generatie contraceptiva. Omdat deze bevindingen onverwacht waren, gaven zij aanleiding tot enige controverse. Onderzoek uit Maastricht hielp de verhoogde risico's te verklaren, aangezien bleek dat anticonceptiva met een derde generatie progestageen de bloedstolling ernstiger verstoorden dan anticonceptiva met een tweede generatie progestageen (10).
De derde generatie progestagenen waren echter ontwikkeld om het risico van arteriële ziekten te verminderen. Inderdaad bleken zij bij gezonde gebruiksters tot een verbetering van het lipidenprofiel te leiden, met een verhoging van het HDL-cholesterol. De vraag is of dit zich vertaalt in een verminderde kans op een hartinfarct. Een groot Engels onderzoek toonde aan dat er geen gunstig effect was op het optreden van een hartinfarct (11).

Anticonceptie bij vrouwen met risicofactoren

Bij vrouwen met risicofactoren voor zowel arteriële als veneuze trombose draagt het gebruik van orale anticonceptie bij aan verdere verhoging van het risico. Voor veneuze trombose betreft dit ten eerste vrouwen met een voorgeschiedenis van veneuze trombose, en ten tweede vrouwen met een mogelijke erfelijke tromboseneiging, dat wil zeggen met factor V Leiden of een positieve familiegeschiedenis voor trombose. Het risico van arteriële trombose stijgt vanaf het 30-35e jaar en daarom is het van belang de combinatie pilgebruik en roken te vermijden bij vrouwen boven deze leeftijd. Ongecontroleerde hypertensie, hypercholesterolemie en diabetes zijn contra-indicaties voor orale anticonceptie. Geen van deze contra-indicaties is absoluut omdat er soms geen adequate alternatieven zijn. De belangrijkste contra-indicatie is een eerdere trombose, aangezien de recidiefkans na een eerste trombose enige procenten per jaar is. Voor vrouwen met risicofactoren zullen sociale omstandigheden mede bepalend zijn of toch voor een oraal anticonceptivum wordt gekozen. Hierbij moet men ook naar de leeftijd van de vrouw kijken: bij jonge vrouwen is de kans op trombose in absolute termen zeer klein, en blijft klein zelfs wanneer verscheidene risicofactoren aanwezig zijn. Nederland is overigens een van de weinige landen waar grote aantallen vrouwen boven de 35 jaar orale anticonceptiva gebruiken.

Samenvatting

Er zijn geen aanwijsbare voordelen verbonden aan het gebruik van orale anticonceptiva met minder dan 30

microgram ethinyloestradiol. Preparaten met een derde generatie progestageen (desogestrel, gestodeen) verhogen de kans op veneuze trombose, zonder een verminderde kans op arteriële trombose, en dienen daarom te worden vermeden, zeker bij vrouwen die met de pil beginnen. Het eerder doorgemaakt hebben van een veneuze of arteriële trombose is een sterke contra-indicatie voor orale anticonceptie. Aanwezigheid van risicofactoren voor trombose, vooral een erfelijke tromboseneiging (factor V Leiden, deficiënties van proteïne C, proteïne S of antitrombine) vormen een contra-indicatie voor orale anticonceptie.

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