A common ancestor more than 10,000 years old for patients with R854Q-related type 2N von Willebrand's disease in Italy

Alessandra Casonato,[1] Viviana Daidone,[1] Giovanni Barbon,[1] Elena Pontara,[1] Irene Di Pasquale,[1] Lisa Gallinaro,[1] Letizia Marullo,[2] and Giorgio Bertorelle[2]

[1]Department of Cardiologic, Thoracic and Vascular Sciences, University of Padua Medical School, Padua; and [2]Department of Biology and Evolution, University of Ferrara, Italy

ABSTRACT

The impaired capacity of von Willebrand factor to carry factor VIII is identified as type 2N von Willebrand's disease. R854Q is the most common type 2N mutation, and almost the only one identified in Italy. This aim of this study was to ascertain whether R854Q mutations in a cohort of Italian patients with type 2N von Willebrand's disease originated from a single event or recurrent events. Thirteen unrelated Italian families were investigated, analyzing the von Willebrand factor gene haplotype associated with the R854Q mutation. A common haplotype emerged in all the families, extending from single nucleotide polymorphisms rs2166902 to rs216293 over 48.2 kb and including five intragenic markers. This haplotype is infrequent in the healthy Italian population (17% *versus* 100%, $P<0.0001$) and each genetic marker within the said haplotype is similarly rare. These data strongly suggest a founder effect, with a single R854Q mutation event being the cause of the type 2N von Willebrand's disease in our cohort of patients. Using DMLE+ software and the mathematical model of Bengtsson and Thomson, it was estimated that the R854Q mutation occurred from 10,000 to 40,000 years ago, which is consistent with the short dimension of the haplotype shared by our patients. Together with the fact that the R854Q mutation seems to be limited to Caucasian populations, these findings suggest that a single mutational event took place after human populations moved from Africa towards Europe.

 doi:10.3324/haematol.2012.066019

Introduction

von Willebrand factor (VWF) serves in blood coagulation as an adhesive protein mediating platelet adhesion to the subendothelium, and as a carrier of factor VIII (FVIII). Its binding to the latter protects the FVIII against inactivation by proteases, thus ensuring the survival of this clotting factor in the normal circulation.[1]

Deficiencies or anomalies of VWF cause von Willebrand's disease (VWD), one of the most common inherited bleeding disorders. One of the various subtypes of VWD is type 2N, a variant characterized by VWF that has abnormal FVIII binding capacity.[2,3] The first case of type 2N VWD was described in France; the 2N refers to Normandy, the French region where this first patient came from.[2,3]

Most cases of type 2N VWD have normal VWF levels and a normal VWF multimer pattern, but low FVIII levels, with a phenotype resembling that of hemophilia A, but the type 2N defect is transmitted as an autosomal trait. Type 2N VWD is easy to identify by means of a test that explores the FVIII binding capacity of VWF (VWF:FVIIIB) *in vitro*, using exogenous recombinant FVIII.[4,5]

The FVIII binding domain of VWF is located in the first 272 amino acids of the mature VWF.[6] This region is encoded by exons 18-23 of the *VWF* gene, but 85% of all mutations occur in exons 18-20.[7,8] Type 2N mutations usually occur within the hypermutable arginine codons (R816W and R854Q), and the R854Q mutation is the most common in absolute terms, being found in 73% of the type 2N VWD patients described so far.[5,9]

The correlations between type 2N mutations and type 2N VWD phenotypes particularly concern circulating FVIII levels, responsiveness to DDAVP, and bleeding symptoms. The most common mutation, R854Q, is associated with the mildest form of type 2N VWD, with modest bleeding occurring only in homozygotes. Other mutations, such as E787K, T791M and R816W, are associated with the lowest circulating FVIII levels and a more severe type 2N VWD phenotype.[10,11] In addition to the particular mutation involved, the presence of a compound type 2N and quantitative VWF defect may also modulate the severity of the type 2N phenotype.[12]

The prevalence of type 2N VWD among all VWD patients is reportedly 1.2-2%.[13] Studies conducted in healthy Dutch and Welsh populations have indicated that the type 2N VWF defect (mainly the R854Q mutation) may be found in up to 1% of the general population.[14-16] It remains to be seen whether this might be explained by a founder effect.

In the present study, we sought to identify a founder effect among Italian type 2N VWD patients, and to calculate the prevalence of the R854Q mutation in the general population.

Design and Methods

All the subjects involved had previously given their written informed consent in accordance with the Helsinki Declaration and the study was approved by our institutional review board.

Manuscript received on March 12, 2012. Revised version arrived on June 4, 2012. Manuscript accepted on July 9, 2012.
Correspondence: Alessandra Casonato. E-mail: sandra.casonato@unipd.it

Patients and controls

Thirty-five patients belonging to 13 apparently not blood-related Italian families took part in the study. Ten families came from the region of Veneto, one from Emilia Romagna, one from Piedmont, and one from Sicily (Figure 1). Eighty normal subjects and their closest family members were also recruited as a control group for the analysis of *VWF* gene haplotypes. A further 322 consecutive normal blood donors were also investigated to calculate the prevalence of the R854Q mutation. Type 2N VWD was diagnosed at our center by means of hemostatic and genetic analyses.

Hemostatic analysis

Blood samples were anticoagulated using sodium citrate (3.8%, 1/10 v/v); platelet-poor plasma was obtained by centrifuging the samples at 800 *g* for 15 min; the plasma was then centrifuged at 12,000 *g* for 4 min to remove all cell fragments, and stored at -80°C until use (but for no more than 2 months). VWF antigen (VWF:Ag), its collagen binding capacity (VWF:CB), factor VIII activity (FVIII:C) and the VWF's FVIII binding capacity (VWF:FVIIIB) were assessed as reported elsewhere.[4,17] Briefly, for the VWF:FVIIIB assay, after coating microtiter plates with anti-VWF polyclonal antibody, plasma VWF was added and the plates were incubated for 1 h at room temperature. After washing, bound endogenous FVIII was removed with $CaCl_2$; 1 U/mL of recombinant FVIII (rFVIII) (Helixate; Aventis, Marburg, Germany) was added and the plates were incubated again for 1 h. After washing again, the amount of bound rFVIII was assessed with an anti-FVIII horseradish peroxidase-conjugated polyclonal antibody (Enzyme Research, South Bend, IN, USA).[4] The values were expressed in units per deciliter, assuming the optical density in the first dilution of normal pooled plasma as 100. A VWF:FVIII:B/VWF:Ag ratio (VWF:FVIIIB ratio) below 0.75 was indicative of a VWF with a potentially defective FVIII binding capacity.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp®DNA Blood Mini kit (Qiagen, Hilden, Germany). Exons 17, 18, 19 and 20 of the *VWF* gene were amplified and sequenced according to previously reported procedures[12] to identify the mutation responsible for the type 2N VWD. All polymerase chain reactions were performed using AmpliTaq Gold (Applied Biosystems, AB, Foster City, CA, USA) and a thermal cycler GeneAmp®PCR System 2700 (AB). The Big Dye Terminator Sequencing kit v.2.5 (Perkin Elmer, Wellesley, MA, USA) and an ABI 3100 Genetic Analyzer (AB) were used for DNA sequencing.

For haplotype analysis, nine polymorphic markers flanking the c.2561G>A mutation in the *VWF* gene were selected, i.e. six single nucleotide polymorphisms (rs1800379, rs216293, rs1063856, rs1063857, rs216311, rs216902) and three short tandem repeats (STRI, STRII, -2144GT_n) spanning approximately 140 Kb within the *VWF* gene (Figure 2). The single nucleotide polymorphisms and short tandem repeats were chosen for their highly polymorphic nature and because they were spread along the whole *VWF* gene. Single nucleotide polymorphisms were analyzed by direct sequencing, while short tandem repeat allele lengths were ascertained using fluorescently end-labeled polymerase chain reaction primers and an ABI 3100 Genetic Analyzer, as explained elsewhere.[18] All haplotypes were generated manually by inheritance.

Dating the von Willebrand factor c.2561G>A mutation

DMLE+ software, rel. 2.3 (*www.dmle.org*), was used to estimate the original date of the c.2561G>A mutation in the *VWF* gene.[19] This program is based on a Markov chain Monte Carlo algorithm that enables a Bayesian inference of the age of the mutation. It uses the linkage disequilibrium (LD) between the disease mutation and the linked markers in patients and unrelated healthy controls. The software requires a knowledge of the patients' and healthy controls' haplotypes (or genotypes), an estimation of the population growth rate (*d*), and the proportion of disease-bearing chromosomes sampled (*f*).

A second mathematical approach, described by Bengtsson and Thomson, was used to confirm our findings. This is a moment method based on the linkage disequilibrium (δ), and the recombination frequency (θ), as expressed in the algorithm $\hat{g}=\log\delta/\log(1-\theta)$, where $\hat{g}$ is the age of the mutation expressed as the number of generations. The Luria-Delbrück correction was applied to this method to avoid the risk of underestimation.[20]

Statistical analysis

Differences in allele frequencies between patients and normal subjects were analyzed using the χ^2 test for the single markers investigated and for the whole c.2561A linked haplotype. The statistical threshold was set at 0.05.

Results

Type 2N VWD was diagnosed on the basis of a decrease in the FVIII/VWF:Ag ratio and, more importantly, in the VWF:FVIIIB assay. Instead of considering the absolute VWF:FVIIIB value for the diagnosis of type 2N VWD, however, we attributed more weight to the VWF:FVIIIB/VWF:Ag ratio (or VWF:FVIIIB ratio). This is especially useful for patients heterozygous for type 2N VWD, who may have FVIII levels, an FVIII/VWF:Ag ratio, and VWF:FVIIIB values within normal ranges, while their VWF:FVIIIB ratio is always pathological. When the VWF revealed an abnormal FVIII binding capacity, we explored the exons of the *VWF* gene encoding for the FVIII binding domain.

Thirteen families with type 2N VWD from different Italian regions were studied (Figure 1). The main pertinent hemostatic findings in the probands of these families are presented in Table 1.

Sequencing of exons 17-20 of the *VWF* gene revealed that all type 2N VWD patients carried the c.2561G>A mutation, which induces the substitution of an Arg with a Glu in position 854 (R854Q); 30 cases were heterozygous, three were homozygous, one was compound heterozygous for R854Q and R763C, and one was compound heterozygous for R854Q and c.2435delC. Finding a single mutation responsible for all our type 2N cases (whereas several causal mutations are described in the literature) prompted us to hypothesize a founder effect in our cohort of patients. A haplotype analysis was consequently performed to check whether our patients had a common c.2561 G>A-bearing haplotype (since a founder effect is expected to result in a common allele sequence in the vicinity of the mutation due to the existence of a single progenitor).

To verify this hypothesis all affected subjects (n=35) and their healthy relatives (n=21) were genotyped for the nine polymorphic markers selected, and the c.2561A associated haplotype was defined for each family (Figure 2). Ten different c.2561A-linked haplotypes came to light in the 13 families studied (Table 2), but all the different haplotypes shared the same core, i.e. the CA**A**CGC sequence (the bold A is the c.2561 mutation) extending from rs216902 to rs216293, over a total of 48.2 Kb (0.12 cM).

Finding that all patients in our cohort shared the same allele sequence in the vicinity of the c.2561G>A mutation

pointed to a common origin. To support this hypothesis, the frequency of the CA_CGC haplotype (where the underscore sign indicates the site of the R854Q mutation) was assessed in the 102 haplotypes obtained by genotyping 80 unrelated normal Italian subjects and their closest family members (Table 3). The frequency of the common CA_CGC haplotype in the sample of normal controls proved to be statistically lower than that in the patients with type 2N VWD, i.e. 17% *versus* 100% ($P<0.0001$). A significant difference also emerged when the allele frequency of each marker in the interval between rs216902 to rs216293 was compared between the normal population and the patients with type 2N VWD ($P<0.0001$) (Table 3). All these findings are consistent with the existence of a founding ancestor for the c.2561 G>A type 2N mutation in our cohort of patients with type 2N VWD.

Dating the c.2561G>A mutation

Assuming the existence of a common ancestral mutation event, an attempt was made to estimate when this VWF c.2561 G>A mutation occurred. Two different approaches were used: DMLE+ software and the mathematical model of Bengtsson and Thomsom, based on linkage disequilibrium analysis. The DMLE+ software requires an estimation of the population growth rate (d) and the proportion of disease-bearing chromosomes (f): d was initially set at 0.025,[21] while f was calculated by estimating the frequency of the c.2561G>A mutation in the healthy Italian population. For this purpose, exon 20 of the *VWF* gene was sequenced in 322 consecutive normal subjects: two heterozygous c.2561G>A carriers were identified, giving a mutation frequency of 0.621% (2/322), which corresponds to an f of $3.98x10^{-5}$.

Assuming that d=0.025[22] and f=3.98^10^{-5}, the DMLE+ estimated the allele's age to be 387 generations (95% CI: 323-480). Considering generations of 25 years,[22] these data indicate that the c.2561 G>A mutation could have occurred around 9,675 years ago (95% CI: 8,075 - 12,000), i.e. several thousand years before Christ (BC). Since a d of 0.025 is attributed in the period after Christ (AD), this parameter seemed to be unable to describe the real situation. Although no data are available on the growth rate of the ancient Italic populations BC, but bearing in mind that d values are known to be lower than 0.025 in times BC,[22,23] a set of growth rates in the range from less than 0.025 to 0.005 was considered. Depending on the oscillation of the d values, the age of the c.2561G>A mutation was calculated to span from 387 to 1605 generations (9,675 to 40,125 years), the older the age, the lower the growth rate considered (Figure 3).

When the mathematical algorithm $\hat{g}=\log\delta/\log(1-\theta)$ was used, the age of the c.2561G>A mutation was estimated to be from 469 to 1,422 generations, i.e. it could have occurred from 11,725 to 35,550 years ago (assuming generations lasting 25 years). Applying the Luria-Delbrück correction to this method, assuming that d=0.025, the age of the mutation increased to 586–1,571 generations, i.e. from 14,650 to 39,275 years ago.

Discussion

About 20 years ago, a French patient from Normandy was reported to have a missense mutation impairing the capacity of VWF to bind FVIII, leading to the discovery of type 2N VWD. Cases of type 2N were subsequently identified all over the world. The first type 2N patient was found to carry the T791M VWF mutation, while a number of type 2N mutations were described in other patients, including C788Y, Y795C, C804F, R816W, R854Q, C858F D879N and R763C.[24-26]

Despite the considerable number of type 2N mutations reported to date in different countries, the R854Q mutation was almost the only one to be found in our cohort of type 2N VWD patients, be they homozygotes, compound heterozygotes or heterozygotes with no bleeding symptoms. The only exception was the R763C mutation in exon 17,

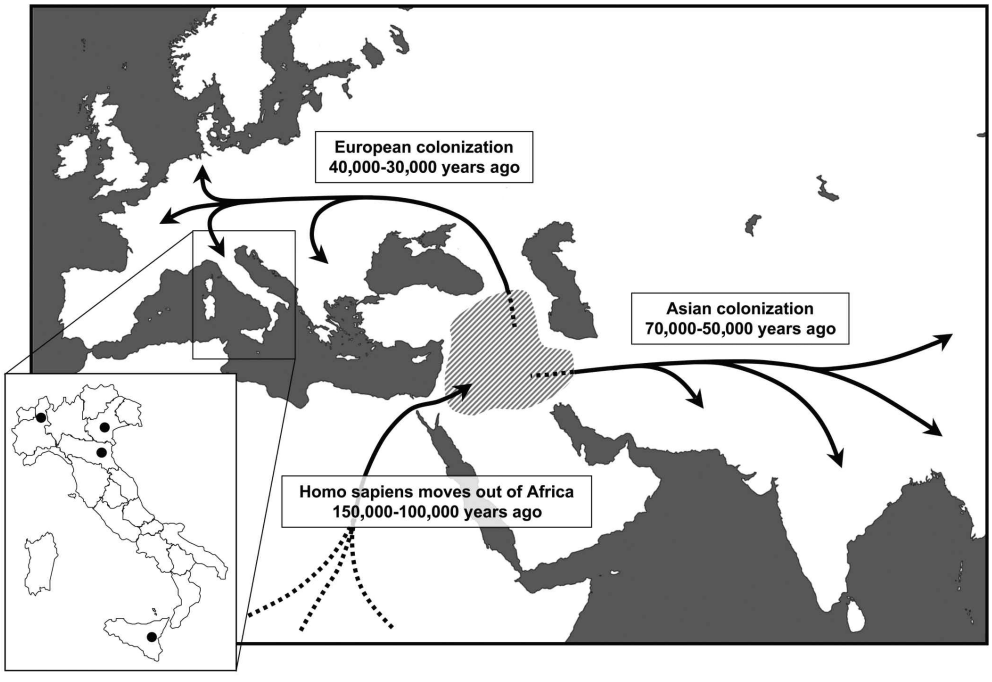

Figure 1. Geographic distribution of the type 2N VWD families studied in Italy and migration fluxes from Africa to Asia and Europe in the last 100,000 years.

Table 1. Main hemostatic parameters and geographical provenance of the type 2N VWD cases investigated.

Propositus	Geographical provenance	Sex	Age	Blood group	aPTT (sec)	VWF:Ag (U/dL)	FVIII:C (U/dL)	FVIII/VWF:Ag ratio	VWF:FVIIIB (U/dL)	VWF:FVIIIB ratio	Mutation
BS	Veneto	F	14	A	37.8	58.5	46.2	0.79	29.7	0.51	c.2561 G>A heterozygous
PR	Veneto	M	31	O	37.8	41.7	41.8	1.00	15.4	0.37	c.2561 G>A+ c.2278C>T
FM	Veneto	F	68	B	41.7	46.6	19.8	0.42	7.7	0.17	c.2561G>A+ c.2435delC
BG	Veneto	F	66	A	30.1	119.4	92	0.77	74	0.62	c.2561G>A heterozygous
PL	Piemonte	M	37	O	39.3	56.6	45	0.80	30.5	0.54	c.2561G>A heterozygous
AA	Sicilia	M	-	A	-	-	-	-	-	-	c.2561G>A homozygous
RM	Veneto	F	18	O	36.1	47.4	56.8	1.20	29	0.61	c.2561G>A heterozygous
GS	Veneto	F	42	O	-	170.9	110	0.64	97.3	0.57	c.2561G>A heterozygous
AS	Emilia Romagna	F	25	A	33	163.3	102	0.62	112.9	0.69	c.2561G>A heterozygous
DA	Veneto	M	62	O	30.7	75.2	74.4	0.99	47	0.63	c.2561G>A heterozygous
TA	Veneto	M	11	O	34.3	82.5	66.5	0.81	47.6	0.58	c.2561G>A heterozygous
PF	Veneto	F	34	O	42.6	69.4	25.5	0.37	30.7	0.44	c.2561G>A homozygous
DM	Veneto	M	70	O	43.2	71.9	24.1	0.34	14.9	0.21	c.2561G>A homozygous
Normal range					**30-40**	**60-160**	**60-160**	**>0.75**	**65-150**	**>0.75**	-

APTT: activated partial thromboplastin time.

which interferes with the cleavage of the VWF propeptide, the persistence of which sterically hinders factor VIII binding to the VWF monomer.[26] No Italian type 2N VWD patients carrying mutations other than R854Q mutation have been reported to date, prompting us to hypothesize a founder effect in our type 2N VWD patients. To test this hypothesis, 13 unrelated families with type 2N VWD were studied (including both affected and unaffected members) to seek a common haplotype associated with the disorder.

All the families, which came from the north-east, north-west and south of Italy, shared the same CA**A**CGC haplotype in their *VWF* gene. This common haplotype extended over 48.2 Kb within the *VWF* gene and was found significantly less frequently in a sample of the general Italian population (only 17% of the healthy subjects investigated had the CA_CGC haplotype). This difference was confirmed when single genetic markers within the common genetic region were analyzed, since their frequency in the normal population was significantly lower than that in the patients with type 2N VWD. These findings strongly support the existence of a common ancestor whose descendants (our type 2N VWD patients) carry R854Q mutations. The region of the *VWF* gene that our patients have in common is very small, which would point to the R854Q mutation deriving from a very ancient event. In fact, dating the R854Q mutation suggests that it occurred in the human population from 10,000 to 40,000 years ago, which is consistent with the short dimension of the region shared by our patients (the older the mutation, the shorter the genomic region affected).

This estimated age would mean that the R854Q mutation happened after human populations left Africa (around 110,000 years ago) to colonize the Middle East and then move on towards Asia (around 70,000 years ago) and Europe (around 50,000 years ago) (Figure 1). This hypothesis is supported by the fact that R854Q seems to be extremely rare in Africa: no data are available on populations of Sub-Saharan Africa, but information can be gleaned from the *NHLBI Exome Sequencing Project* (*http://evs.gs.washington.edu/EVS*), which involved genomic analyses on African Americans (including DNA variations in thousands of exomes from various well-phenotyped US cohorts,

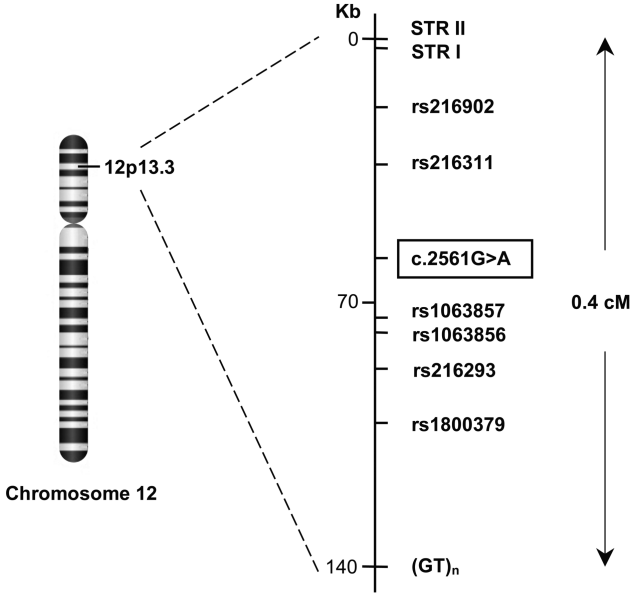

Figure 2. Schematic map of the nine markers selected (6 single nucleotide polymorphisms and 3 short tandem repeats) flanking the c.2561G>A mutation. The physical position of each marker on chromosome 12 was derived from the UCSC Genome Browser (February 2009 assembly).

divided by African or European ethnicity) and revealed that the incidence of the R854Q mutation in the Afro-American exome sample is 0.1% (2 of 3,738 alleles sampled), whereas the incidence of the mutation among Caucasian Americans is around 1.2%, a figure fairly similar to those observed in British and Dutch populations.[15,16] The small number of R854Q mutations detected among African Americans would seem to confirm indirectly that this mutation is rare (if not absent) in Africa. On the other hand, while R854Q mutations were found only rarely in the African Americans' exomes (probably the result of 5 centuries of cohabitation with Caucasian Americans), the same sample revealed a high incidence of the type 2N H817Q mutation (with an

allele frequency of about 11%), demonstrating that other mutations occur in the FVIII binding domain of VWF.[27]

If the R854Q mutation occurred before the migrant human populations separated to head for Europe or Asia, we could expect to find it in both continents, but this does not seem to be the case. The mutation has been repeatedly reported in Europe, while there is no mention in the literature of its occurrence in Asia, although other type 2N mutations have been registered in the Sheffield data bank for these countries.

That the R854Q mutation has been around for such a long time is confirmation of its mild effect on bleeding. The widespread distribution of the R854Q mutation in different countries enables us to conclude that it does not shorten the

Table 2. c.2561 G>A linked haplotypes in type 2N VWD patients.

	STR II	STR I	rs216902	rs216311	c.2561G>A	rs1063857	rs1063856	rs216293	rs1800379	(GT)n	Frequency
H1	239	106	C	A	**A**	C	G	C	C	234	3/15
H2	235	102	C	A	**A**	C	G	C	C	238	2/15
H3	235	102	C	A	**A**	C	G	C	T	238	2/15
H4	235	102	C	A	**A**	C	G	C	C	234	2/15
H5	239	102	C	A	**A**	C	G	C	T	234	1/15
H6	239	102	C	A	**A**	C	G	C	T	238	1/15
H7	231	102	C	A	**A**	C	G	C	C	234	1/15
H8	235	102	C	A	**A**	C	G	C	C	236	1/15
H9	235	102	C	A	**A**	C	G	C	T	234	1/15
H10	231	102	C	A	**A**	C	G	C	C	240	1/15

*The CA**A**CGC haplotype sequence (highlighted in gray) is common to all type 2N VWD patients. It ranges from marker rs216902 to rs216293 for a total of 48.2 Kb (0.12 cM).*

Table 3. Linkage disequilibrium analysis and age calculation for the c.2561 G>A mutation.

Linkage disequilibrium analysis						Age calculation					
Marker	Physical position (bases)	Ancestral allele	Ancestral allele frequency		*P* values	δ^	Distance (θ)°	g^1	years*	g_0^2	years*
			Patients	Normals							
STRII	6092407	235	0.53	0.57	0.796	-0.082	0.001341				
STRI	6092919	102	0.80	0.57	0.088	0.536	0.001328	468.937	11,723.425	586.359	14,658.969
rs216902	6105387	C	1.00	0.72	0.017	1.000	0.001003				
rs216311	6128443	A	1.00	0.31	<0.0001	1.000	0.000404				
c.2561G>A	6143978										
rs1063857	6153514	C	1.00	0.37	<0.0001	1.000	0.000248				
rs1063856	6153534	G	1.00	0.37	<0.0001	1.000	0.000248				
rs216293	6153659	C	1.00	0.41	<0.0001	1.000	0.000252				
rs1800379	6167196	C	0.67	0.42	0.075	0.424	0.000604	1,421.978	35,549.45	1,570.922	39,273.049
(GT)n	6234469	234	0.53	0.37	0.234	0.256	0.002353	578.043	14,451.075	672.574	16,814.353

*^δ =(Pd–Pn)/(1–Pn); °Assuming 2.6 cM/Mb; [1]g=logδ/log(1–θ); [2]Luria-Delbrück correction: g_o=–(1/d)ln(θfd), assuming d =0.025 and fd=1/d. * Assuming generations lasting 25 years.*

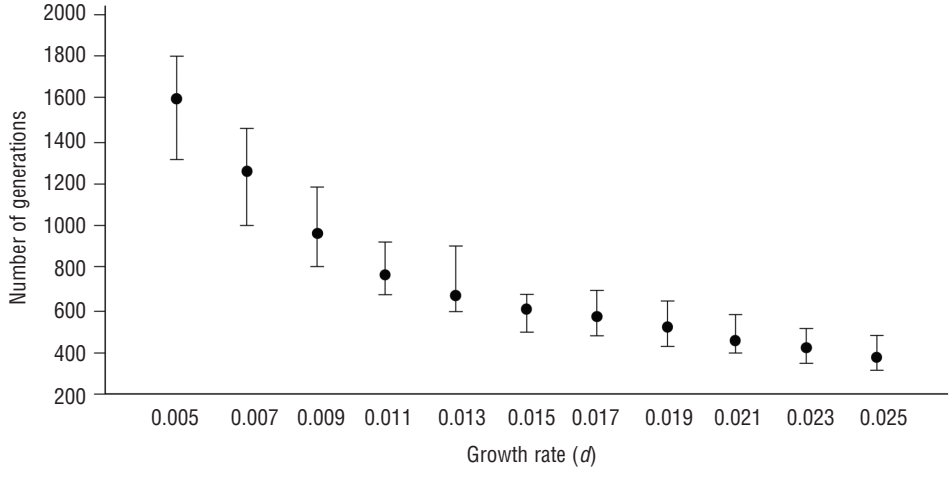

Figure 3. Estimated allele age of the c.2561G>A mutation calculated with the DMLE+ software based on the oscillation in growth rates, considering 0.025 as the growth rate in year 1 AD.

carriers' survival or jeopardize their health, even in the homozygous state. It is not, therefore, subject to negative selection phenomena, although it is hard to imagine that it might confer a selective advantage, given that hemorrhage, for example, after childbirth or trauma, was an important cause of morbidity/mortality in the past.

The CA**A**CGC haplotype shared by our patients does not seem to be peculiar to Italian type 2N patients because it was also found in the CEPH (*Centre d'Etude du Polymorphisme Humain*) family pedigrees obtained from multigenerational Caucasian families living in Utah and coming from Western and Northern Europe. One individual was found to carry both the R854Q mutation (identified in the study as single nucleotide polymorphism rs41276738) and the CA(G/A)CGC haplotype seen in our patients with type 2N VWD. Although we cannot be sure that this haplotype is linked to the allele carrying the R854Q mutation, this finding gives the impression that this might be so, also in the light of the low incidence of the CA_CGC haplotype in the general population.

Finally, with regards to the time that the R854Q mutation first occurred in the Italian population, we surmise that this happened later than in other European countries, because the allele frequency we calculated for R854Q in the Italian general population (0.6%) is lower than that in the French or Dutch populations in Europe, or in Caucasian Americans (1-2%).

In conclusion, our findings suggest that Italian cases of type 2N VWD associated with the R854Q mutation descend from a common ancestor who lived more than 10,000 years ago. It would be interesting to see whether the other R854Q-associated type 2N VWD patients in Europe descend from the same ancestor.

Funding

This study was supported by funds from the MIUR (ex 60%, 2009).

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Fay PJ, Cumans JV, Walker FJ. von Willebrand factor mediates protection of FVIII from activated protein C-catalyzed inactivation. J Biol Chem. 1991;266(4): 2172-7.
2. Nishino M, Girma J-P, Rothschild C, Fressinaud E, Meyer D. New variant of von Willebrand disease with defective binding to factor VIII. Blood. 1989;74(5):1591-9.
3. Mazurier C, Dieval J, Jorieux S, Delobel J, Goudemand M. A new von Willebrand factor (vWF) defect in a patient with FVIII deficiency but with normal levels and multimeric patterns of both plasma and platelet vWF. Characterization of abnormal vWF/FVIII interaction. Blood. 1990;75(1): 20-6.
4. Casonato A, Pontara E, Zerbinati P, Zucchetto A, Girolami A. The evaluation of factor VIII binding activity of VWF by means of an ELISA method: significance and practical implications. Am J Clin Path. 1998;109(3):347-52.
5. Mazurier C, Meyer D, Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand disease - results of an international survey. Thromb Haemost. 1996;76(2):720-24.
6. Foster PA, Fulcher CA, Marti T, Titani K, Zimmerman TS. A major factor VIII binding domain resides within the amino terminal 272 amino acid residues of von Willebrand factor. J Biol Chem. 1987;262 (18):8443-6.
7. VWFdb. International Society of Thrombosis and Haemostasis Scientific and Standardization Committee VWF information homepage. http://www.vwf.group.shef.ac.uk/, accessed January 2010.
8. Meyer D, Fressinaud E, Gaucher C, Lavergne JM, Hilbert L, Ribba AS, et al. Gene defects in 150 unrelated French cases with type 2 von Willebrand disease: from the patient to the gene. INSERM Network on Molecular Abnormalities in von Willebrand Disease. Thromb Haemost. 1997;78(1):451-6.
9. Kroner PA, Friedman KD, Fash S, Scott JP, Montgomery RR. Abnormal factor VIII is linked with the substitution of glutamine for arginine in von Willebrand factor in a variant form of von Willebrand disease. J Biol Chem. 1991;266(29):19146-9.
10. Mazurier C, Goudemand J, Hilbert L, Caron C, Fressinaud E, Meyer D. Type 2N von Willebrand disease: clinical manifestations, pathophysiology, laboratory diagnosis and molecular biology. Best Pract Res Clin Haematol. 2001;14(2):337-47.
11. Schneppenheim R, Budde U, Krey S, Drewke E, Bergmann F, Lechler E, et al. Results of a screening for von Willebrand disease type 2N in patients with suspected haemophilia A or von Willebrand disease type 1. Thromb Haemost. 1996;76(4):598-602.
12. Casonato A, Gaucher C, Pontara E, Zucchetto A, Zerbinati P, Mazurier C, et al. Type 2N von Willebrand disease due to Arg91Gln substitution and a cysteine deletion in exon 18 of the von Willebrand factor gene. Br J Haematol. 1998;103(1):39-41.
13. Casonato A, Pontara E, Sartorello F, Bertomoro A, Girolami A. Interaction of factor VIII and von Willebrand factor investigated in a large cohort of von Willebrand disease patients: evalution of type 2N prevalence (abstract). J Thromb Haemost. 2003;1:0093.
14. Woods AI, Meschengieser SS, Blanco AN, Salviù MJ, Farias CE, Kempfer AC, et al. Clinical features and laboratory patterns in a cohort of consecutive Argentinian patients with von Willebrand disease. Haematologica. 2001;86(4):420-7.
15. Eikenboom JCJ, Reitsma PH, Peerlinck KMJ, Briet E. Recessive inheritance of von Willebrand's disease type I. Lancet. 1993; 341(8851):982-6.
16. Bowen DJ, Standen GR, Mazurier C, Gaucher C, Cumming A, Keeney S, et al. Type 2N von Willebrand disease: rapid genetic diagnosis of G2811A (R854Q), C2696T (R816W), T2701A (H817Q) and G2823 (C858F) -- detection of a novel candidate type 2N mutation: C2810T (R854W). Thromb Haemost. 1998;80(1): 32-6.
17. Casonato A, Pontara E, Sartorello F, Cattini MG, Perutelli P, Bertomoro A, et al. Identifying carriers of type 2N von Willebrand disease: procedures and significance. Clin Appl Thromb Hemost. 2007;13 (2):194-200.
18. Daidone V, Gallinaro L, Grazia Cattini M, Pontara E, Bertomoro A, Pagnan A, et al. An apparent silent nucleotide substitution (c.7056C>T) in the von Willebrand factor gene is responsible for type 1 von Willebrand disease. Haematologica. 2011; 96(6):881-7.
19. Reeve JP and Rannala B. DMLE+: Bayesian linkage disequilibrium gene mapping. Bioinformatics. 2002;18(6):894-5.
20. Luria SE, Delbrück M. Mutations of bacteria from virus sensitivity to virus resistance. Genetics. 1943;28(6):491-511.
21. Borroni B, Bonvicini C, Galimberti D, Tremolizzo L, Papetti A, Archetti S, et al. Founder effect and estimation of the age of the Progranulin Thr272fs mutation in 14 Italian pedigrees with frontotemporal lobar degeneration. Neurobiol Aging 2011; 32(3):555.e1-8.
22. Slatkin M, Rannala B. Estimating the age of alleles by use of intraallelic variability. Am J Hum Genet. 1997;60(2):447-58.
23. Pritchard JK, Seielstad MT, Perez-Leuzaun A, Feldmen MW. Population growth of human Y chromosomes: a study of Y chromosome microsatellites. Mol Biol Evol. 1999;16(12):1791-8.
24. Tuley EA, Gaucher C, Jorieux S, Worrall NK, Sadler JE, Mazurier C. Expression of von Willebrand factor Normandy. An autosomal mutation that mimics hemophilia A. Proc Natl Acad Sci USA. 1991;88(14):6377-81.
25. Jorieux S, Gaucher C, Goudmand J, Mazurier C. A novel mutation in the D3 domain of von Willebrand factor markedly decreases its ability to bind factor VIII and affects its multimerization. Blood. 1998:92 (12):4663-70.
26. Casonato A, Sartorello F, Cattini MG, Pontara E, Soldera C, Bertomoro A, et al. An Arg763Cys mutation in the consensus sequence of the von Willebrand factor propeptide cleavage site is responsible for a new von Willebrand disease variant. Blood. 2003;101(1):151-6.
27. Bellissimo DB, Christopherson PA, Flood VH, Gill JC, Friedman KD, Haberichter SL, et al. Von Willebrand factor mutations and new sequence variations identified in healthy controls are more frequent in the African-American population. Blood. 2012; 119(9):2135-40.