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Genetics of thromboembolic disorders in Sri Lanka

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Introduction

Thromboembolic disorders are reported to affect 1 in 1000 people in the general population. They are a group of complex disorders with a genetic component. When one draws pedigrees and mark on them individuals who are affected with any complex disorder three patterns emerge (Figure 1). The commonest one shows one family member affected with the disorder, everyone else is normal. This is called the sporadic occurrence of the disorder in the family. There is no sign of a significant genetic contribution to the development of the condition. The second pattern shows several members of the family, usually in the same generation, affected with a disorder in their early adult life. In this case the disorder is said to be familial – several first and second degree relatives developing the same disorder as they age due to shared environmental factor/s – junk food, bad habits, smoking, alcohol, sedentary lifestyle or even environmental pollution – acting on an underlying genetic predisposition to bring about the development of the disorder. But still there is no sign of a strong genetic contribution. The third pattern, the hereditary pattern, the one that shows a definite genetic contribution, shows many first degree relatives in several generations of the family affected with the disorder. The disorder can be seen to run in the family according to a known pattern of inheritance (e.g. autosomal dominant). When one probes into more details one finds that the disorder occurs at an earlier age than in the general population.

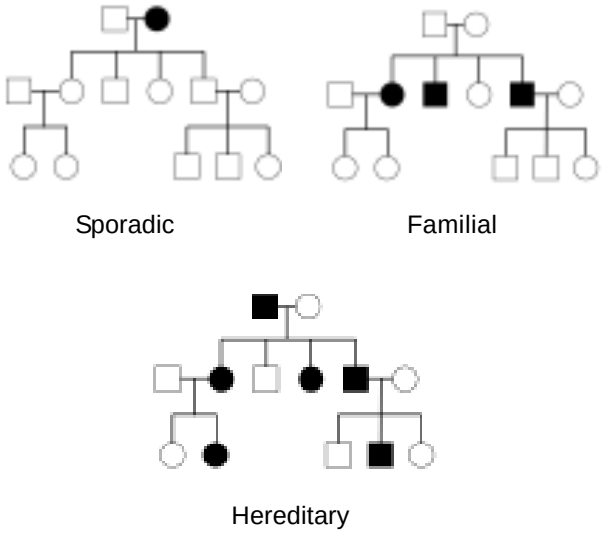

Figure 1. Pedigree Patterns of Complex Disorders.

These three patterns – sporadic, familial, and hereditary – are found when we examine the pedigrees of families affected with any thromboembolic disorder. The families that exhibit, the hereditary pattern, usually have rare genetic disorders, caused by high penetrant, low frequency, genetic variations, in one gene. In contrast the common complex disorders, usually found in families exhibiting the familial occurrence of the disorder, are caused by low penetrant, high frequency genetic variations in several genes that interact with each other and the environment. As a result the pattern of inheritance of such disorders is not clear. They however leave behind genetic traces, which could be unmasked through epidemiological studies, pedigree analysis, and genetic testing. Genetically predisposed thromboembolic disorders when caused by high penetrant variants can exhibit a hereditary pattern in pedigrees; they are however rare. In most cases the variants have low penetrance, and as such, in pedigrees show the familial pattern.

What underlies a thromboembolic disorder is a thrombophilia or a tendency for blood clotting. Genetic thrombophilia, which appear in pedigrees to have familial or hereditary patterns, was in the past believed to be caused by variants in different genes in different families. In other words, they were believed to be a collection of several individual monogenic disorders. These disorders seemed to run in families as autosomal dominant traits with reduced penetrance. Today, however, we know that genetic thrombophilia is an oligogenic disorder, meaning they are caused by variants in several genes rather than one. The penetrance of the disorder in variant carriers is reduced. It depends largely on the presence of other factors and the number of variants that the individual has. In these families, individuals with two variants will have thrombotic events more frequently and earlier in life than their relatives with a single variant. Over the years our understanding of the genetic basis of thromboembolic disorders has grown (Figure 2). In the mid 1960s when the first thrombophilic genetic defect was

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identified, only a few cases of thrombophilia could be explained by their presence. As time went by more and more variants were identified in different genes, and the percentage of cases explained by their presence continued to grow.

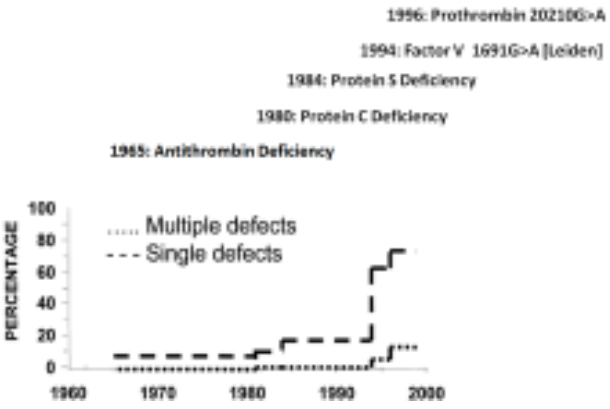

Figure 2. How the percentage of thromboembolic cases that could be explained by the underlying genetic aetiology increased as knowledge in the field grew over the years. The Y axis shows the percentage of thromboembolic cases that could be explained by the underlying genetic aetiology. The X axis shows the years. Modified and adopted from *Thromb Haemost* 2001; **86**: 92-103.

Today we know that these variants, while interacting with each other, interact with other factors such as abnormalities in blood flow (atrial fibrillation, left ventricular dysfunction, bed rest, paralysis, etc) and contact surface abnormalities (atherosclerosis, vascular injury/trauma, abnormal or mechanical heart valves, indwelling vascular catheters) to bring about the development of the thromboembolic disorder that brings the patient to hospital whether it is stroke, myocardial infarction, deep vein thrombosis, pulmonary embolism or any other disorder related to arterial or venous thromboembolism. Let me illustrate this using a family tested in our Unit.

An 8 year old boy was referred to us for genetic thrombophilia testing. He had developed signs and symptoms of a stroke after being in bed for five days because of fever. His MRI scan showed multiple cerebral infarcts. He underwent genetic thrombophilia testing and was found to have the *F5* 1691G>A (Leiden) variant. A variant that conferred on him an increased risk of thrombophilia, the effects of which manifested after being bed ridden for five days with fever, resulting in him developing a blood clot that blocked one of his cerebral arteries. When we see a patient like this we immediately think about the family – Who did he get this variant from – father or mother? Who else in this family is a carrier of the variant? What thromboembolic disorders, if any, have the others variant carriers in the family had? The pedigree of this family (Figure 3) shows the complex nature of the problem we are dealing with and the difficulties faced when interpreting genetic test results and giving advice to patients and their clinicians.

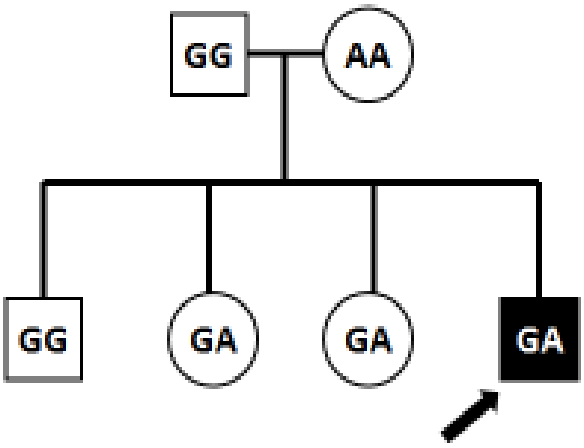

Figure 3. The pedigree of the 8 year old child with a stroke. *F5* 1691G>A genotype of every family member is shown in the figure. The 8 year old child, shown in the dark square, is the youngest in the family. He is the only one with a thromboembolic event, all others are normal, including his 40 year old mother who is homozygous for the variant.

Genetic thrombophilic variants in the Sri Lankan population

My interest in genetics of thrombophilia began in 2001 when I embarked on my PhD research on genetics of pre-eclampsia. Genes, that had variants that increase the risk of thrombophila were hot candidate genes for pre-eclampsia at that time, because pre-eclampsia is a disorder where thromboembolism is a feature.[1]

There are three well known thrombophilic genetic variants that have grabbed the attention of the scientific and medical communities - *F5* 1691A (F5 Leiden), *F2* (Prothrombin) 20210A and *MTHFR* 677T. The consequence of carrying these variants, which underlie the predisposition to thrombophilia are well known. *F5* 1691G>A (Leiden) – activated Protein C Resistance; *F2* 20210G>A – elevated Prothrombin Levels; *MTHFR* 677C>T – hyperhomocystinaemia. These variants were the subject of our investigations.

Our first research project which was conducted in several stages over many years culminating in a publication in 2009[2] was aimed at examining whether these variants as well as other lesser known variants

in these genes were present in our population. To answer that question we established a population based DNA resource consisting of samples from the major ethnic groups in the Sri Lankan population – Sinhalese, Sri Lankan Tamils and Moors. These samples were genotyped using PCR/RFLP assays. We established that these variants were prevalent in our population as follows: *F5* 1671A - 2.3%, *F2* 20210A - 0%; *MTHFR* 677T – 10.3%. There were Sri Lankans harboring both the *F5* 1691A and the *MTHFR* 677T variants. The *F2* (prothrombin) 20210A variant was not detected among healthy volunteers because it was either absent or present in a very low frequency. In addition to these findings, the genotyping of more than one variant in the *F5* gene enabled us to identify the presence of a subset of *F5* Leiden variant carriers who were at double the risk of venous thromboembolism because they harbored the *F5* 4070G variant on the same gene which conferred the additional risk. Therefore, we established the prevalence of these thrombophilc genetic variants in healthy Sri Lankans, and demonstrated that there are individuals who harbor more than one deleterious variant.

Genetic thrombophilic variants in pre-eclampsia and recurrent pregnancy loss

Our research group has a special interest in the Genetics of Reproductive Disorders. Therefore we focused our attention on two projects that were aimed at answering the question whether these thrombophilic genetic variants increased the risk of pre-eclampsia and recurrent pregnancy loss, two disorders, that have a thromboembolic component. To conduct these studies, we recruited two groups of cases and controls. The case control genetic marker disease association studies into pre-eclampsia required the recruitment of strictly defined pre-eclampsia cases (180 primiparous Sinhalese women with pre-eclampsia defined by pregnancy complicated by blood pressure>140/90 mm Hg and proteinuria >+2) and normotensive pregnant controls (180 primiparous Sinhalese women who had uncomplicated pregnancies) matched for age and body mass index and not having any known risk factors for pre-eclampsia. The recruitment of subjects which was crucial for these studies was the subject of two of our papers.[3,4] The case control genetic marker disease association studies into recurrent pregnancy loss also required the recruitment of strictly defined cases (200 Sinhalese women with three of more recurrent pregnancy loss and no living children who had normal karyotypes) and controls (200 Sinhalese women with two or more living children and no history of pregnancy loss). The results of this work showed that the genotype frequencies at each variant site in the *MTHFR* 677C>T; 1298A>C; F5 1691G>A and 4070A>G genes and the haplotypes defined by them were not significantly associated with either preeclampsia or recurrent pregnancy loss.[5] These results are in agreement with similar studies from other countries. We did not test the *F2* variant in these studies as it was found to be non polymorphic in the population.

These negative results however, should not dissuade one from investigating for a genetic cause for thrombophilia when it comes to dealing with individual patients. The reason being that genetic thrombophilia is an oligogenic disorder so it could simply be that we were not looking at enough genetic variants in our case control studies to find an association. In fact there are several recent studies that report that the risk in fact increases depending on the number of variants found in all the thrombophilic genes, which is consistent with our current understanding of genetic thrombophilia.

The number of genetic variants that are found to confer increased risk of thrombophilia is ever growing. Genetic technology has just reached a stage where we can genotype all these variants at once in a cost effective manner. Hence, it would be possible to re-genotype these samples for all known variants, and at that point, we may have different results to report.

Genetic thrombophilia testing as a diagnostic service

The commencement and successful completion of these research projects gave us the opportunity to establish genetic testing for thrombophilic genetic variants in our laboratory as a diagnostic service. There are many guidelines on when to test for thrombophilic genetic mutations. The list of indications for testing derived from these guidelines which we use to guide clinicians when they seek our advice on who should be tested is as follows:

- Idiopathic thrombosis and/or age <50 years with thrombosis
- History of recurrent thrombosis
- Unusual site of thrombosis (mesenteric, splenic, portal, hepatic, cerebral)
- First-degree relative(s) with thrombosis, particularly at age 50 years
- Thrombotic event during pregnancy
- Thrombotic event while taking oral contraceptives

The variants that we test when we perform the genetic thrombophilia test include *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T. From January 2006 to February 2013, over a period of 7 years, we tested 902 persons for these variants. They included 869 patients as well as 33 asymptomatic family members of *F5* Leiden or *F2* 20210A variant carriers. Over the years we have kept the referring clinicians as well as

the scientific community informed of the results of genetic testing through a series of scientific communications at medical and genetic conferences.[6-16] Analysis of our database gives fascinating insights into the genetic aetiology of thromboembolic disorders in the Sri Lankan population. An overview of that data is presented here.

The demographic details of patients we tested are in table 1. It is clearly from this table that those who were tested were a young population of patients (mean age 30 years; range 1 to 82) who experienced serious conditions which are summarised in table 2. There was a wide array of referrals from both medical and surgical specialties for conditions associated with both arterial as well as venous thromboembolism. Table 3 shows the variants that were detected. All variants including the *F2* 20210A, which was not found in healthy volunteers, were found in these patients, thus justifying its inclusion in the genetic thrombophilia testing panel. In addition, there was even an asymptomatic homozygote for the *F5* Leiden variant identified through family screening – the mother of the 8 year old boy with a stroke in the family illustrated above.

The high prevalence of the *MTHFR* 677T variant in this population, at almost double the frequency seen in the general population, is noteworthy for the following reasons. There is a well documented correlation between the *MTHFR* 677 genotype and homocysteine levels. *MTHFR* 677 TT homozygotes are prone to hyperhomocysteinaemia when their folate levels are low. Given our food habits, which include cooking green leafy vegetables to the point where all folate is depleted before consumption, folate supplementation may be required to maintain normal homocysteine levels among Sri Lankans, which when high contributes to thrombophilia. In this respect it is interesting to note the work of Prof. Shanthi Mendis and her colleagues in Peradeniya in the mid 1990s. They reported in several studies that even normal healthy Sri Lankans had higher levels of homocysteine than their counterparts in the west.[17,18]

In the family illustrated above, the mother was a homozytoge for the *F5* Leiden variation. At 40 years of age she was normal without a thromboembolic event. She has however a 15 fold increased risk of venous thromboembolisim compared to a woman who does not have the variant if she were to be given hormone replacement therapy around the menopause. Additionally, two more siblings though genetically predisposed have remained symptom free. You may wonder how they have been spared. The answer is not straightforward. Perhaps they do not have yet another, but unknown, genetic variant present in the boy who

Table 1. Demographic characteristics of patients who underwent genetic thrombophilia testing

Characteristic	*n (%)*
Age	
upto 20 years	173 (19.2%)
21 to 40 years	536 (59.4%)
41 to 50 years	155 (17.2%)
Over 50 years	38 (4.2%)
Sex	
Male	376 (41.7%)
Females	526 (58.3%)
Race	
Sinhalese	768 (85%)
Tamils	53 (5.9%)
Moors	47 (5.2%)
Burger	1 (0.1%)
Not Recorded	33 (3.7%)

Table 2. Indications for genetic thrombophilia testing

Indication for testing	*Total tested*	
	No	*%*
Cerebrovascular Accident	334	37%
Deep Vein Thrombosis	161	18%
Pregnancy Complications	150	17%
Thrombosis in Abdominal Veins	60	7%
Myocardial Infarction	41	5%
Cerebral Venous Sinus Thrombosis	32	4%
Pulmonary Embolism	31	3%
Arterial thrombosis at Other Sites	18	2%
Deep Vein Thrombosis and Pulmonary Embolism	16	2%
Thrombosis in Eye Veins	7	1%
Thrombosis in Neck Veins	7	1%
Thrombosis of Upper Limb Veins	3	0%
Venous Thrombosis at other Sites	3	0%
Other	6	1%
Family Screening	33	4%
Total	902	100

Table 3. Indications for genetic thrombophilia testing

Indication for testing		Mutation/Genotype							
		F5			*F2*		*MTHFR*		
		1691 G>A			*20210G>A*		*677C>T*		
		GG	*GA*	*AA*	*GG*	*GA*	*CC*	*CT*	*TT*
Cerebrovascular Accident		322	10	0	328	3	274	55	4
Deep Vein Thrombosis		154	6	0	158	2	127	31	2
Pregnancy Complications		149	1	0	149	1	122	27	1
Thrombosis in Abdominal Veins		60	0	0	58	2	45	15	0
Myocardial Infarction		39	2	0	41	0	29	11	1
Cerebral Venous Sinus Thrombosis		29	3	0	32	0	26	6	0
Pulmonary Embolism		31	0	0	31	0	22	9	0
Arterial thrombosis at Other Sites		18	0	0	18	0	13	4	1
Deep Vein Thrombosis and Pulmonary Embolism		16	0	0	15	0	11	3	1
Thrombosis in Eye Veins		6	1	0	7	0	5	2	0
Thrombosis in Neck Veins		6	1	0	7	0	6	1	0
Thrombosis of Upper Limb Veins		6	0	0	6	0	4	2	0
Venous Thrombosis at other Sites		3	0	0	3	0	2	1	0
Other		3	0	0	3	0	2	1	0
Family Screening		25	7	1	27	5	23	3	0
Total	n	867	31	1	883	13	711	171	10
	%		3.4	0.1		1.5		19.2	1.1

developed the stroke, which he had inherited from his father, which interacts with the *F5* Leiden variant inherited from the mother to bring about the event. Perhaps the others were never exposed to an environmental trigger like the 8 year old boy who was bed ridden for five days before developing the stroke. On the other hand the mother had gone through four pregnancies without any complications. These illustrate the complex issues we are faced with when counselling these patients. Therefore pre- and post test counselling, after appropriate consent is very important when offering genetic testing. The knowledge of this genotype data is useful to help the family avoid the kind of situation faced by the little boy with a stroke at the tender age of 8 years, as well as to help them with appropriate health education, such as hormonal contraception and hormone replacement for the mother.

Summary

In summary, genetic thrombophilia is an oligogenic disorder, one or more thrombophilic genetic variants interacting with each other and/or the environment, as illustrated in the family above, rather than a single genetic variant acting on its own that precipitates thromboembolic events. With the sequencing of a Sri Lankan Genome, as well as several other Sri Lankan exomes by our group, it has become possible for us to understand the variant profile of Sri Lankans and to simulate such interaction at genome level in our population using computer models. I hope that that such inquiry would lead to unique understanding of the pathophysiology underlying thromboembolic disorders in our population in the years to come. In conclusion, I have provided a glimpse of the central role genetics would play in health care, such as genetic testing that is more affordable and in understanding the genetic basis of disease where genetic testing will not be a curiosity but a necessity.

Acknowledgements

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