

# The Saudi Thrombosis and Familial Thrombophilia Registry

## *Design, rationale, and preliminary results*

Jalal N. Saour, MBChB, FRCPI, Mohammed M. Shoukri, MS, PhD, Layla A. Mammo, BSc Pharmacy, PhD.

### ABSTRACT

**الأهداف:** وصف السجل وعرض البيانات الأولية لعوامل تغير البروثرومين في الطفرات الجينية لدى السكان السعوديين.

**الطريقة:** تم جمع عينات دم من أشخاص سعوديين أصحاء وافقوا على الاشتراك بهذه الدراسة ومن مرضى التجلط الدموي (VT) من مختلف أطراف المملكة خلال الفترة من نوفمبر 2001م إلى يوليو 2007م. وتم استخلاص الحامض النووي DNA وخصونه تحت درجة  $-70^{\circ}\text{C}$  لدراسة وجود الطفرات لجينية في 5 عوامل معروفة مسببة لتجلط الدم. وسوف تشمل الدراسة المرضى المؤكدة إصابتهم بالتجلط الدموي فقط (VT). سيتم جمع المعلومات من خلال مقابلة شخصية ومن ثم ادخالها ببرنامج خاص بالحاسب الآلي. تم تصميم نماذج خاصة للموافقة على الاشتراك بالدراسة و جمع المعلومات وبرنامج للحاسب الآلي خاص بسجل تجلط الدم السعودي.

**النتائج:** نعلن هنا فقط نتائج فحص حامض نووي مستخلص من 902 من السعوديون الأصحاء الذين تم فحصهم للأحماض النووية التالية: عامل ليدن الخامس (FVL) و بروثرومين  $20210\text{G}>\text{A}$ ، و مختزلة الميثيلين تتراهيدروفولات  $\text{MTHFR} - 677\text{C}>\text{T}$ ، و مشبط منشط مولد البلازمين متعدد الأشكال النوع الأول  $4\text{G}/5\text{G}$ ، وعامل  $\text{FVHR2}$  الفردي. وجدنا أن نسبة وجود طفرة FVL بين الأفراد الأصحاء كانت 1.3%،  $0.7\%$   $\text{PT} 20210\text{G}>\text{A}$  و  $\text{MTHFR}$   $2.45\%$   $677\text{C}>\text{T}$ ، و  $10.1\%$   $4\text{G}/4\text{G}$  PAI، وعامل  $\text{FVHR2}$   $26.1\%$ .

**خاتمة:** تشير النتائج الأولية إلى انخفاض نسبة العوامل الخمسة المسببة للتجلط في السعوديين الأصحاء بالمقارنة مع أصحاء مجتمعات أخرى باستثناء عامل  $\text{FVHR2}$ .

**Objectives:** To describe the Registry and report preliminary data for the prevalence of 5 prothrombotic gene mutations in the normal Saudi population.

**Methods:** Blood from consenting healthy Saudi individuals and patients with venous thrombosis (VT)

from different regions of the Kingdom was collected from November 2001 until July 2007. The extracted DNA of each sample was kept at  $-70^{\circ}\text{C}$  until tested for 5 known prothrombotic factors using established methods. Only patients with confirmed VT were included. Data generated through direct interview were entered into the Saudi Thrombosis and Familial Thrombophilia (S-TAFT) Register. The consent and demographic data collection forms and the S-TAFT Register were developed using the SQL web based software.

**Results:** Nine hundred and two DNA samples of consenting healthy Saudi individuals were tested for factor V Leiden (FVL), prothrombin (PT)  $20210\text{G}>\text{A}$ , 5-10 methylenetetrahydrofolate reductase (MTHFR)  $677\text{C}>\text{T}$ , the  $4\text{G}/5\text{G}$  polymorphism of Plasminogen activator inhibitor type 1 (PAI-1  $4\text{G}/5\text{G}$ ), and factor V HR2 (FVHR2) haplotype. The incidence of FVL among healthy subjects was 1.3%, PT  $20210\text{G}>\text{A}$  0.7%, homozygous MTHFR  $677\text{C}>\text{T}$  2.45%, PAI  $4\text{G}/4\text{G}$  10.1%, and FVHR2 26.1%.

**Conclusion:** Our preliminary data from healthy Saudi individuals suggest that the incidence of the 5 prothrombotic risk factors is lower than in most other populations, except for FVHR2.

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*From the Department of Medicine (Saour), Department of Biological Computing and Scientific Calculations (Shoukri), Coagulation Research Unit, and Biological and Medical Research (Mammo), King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia.*

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*Address correspondence and reprint request to: Dr. Jalal N. Saour, Consultant Internist and Director of Thromboembolic Service, Department of Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia. Tel. +966 (1) 4424238. Fax. +966 (1) 4424238. E-mail: jalal.saour1945@gmail.com*

The incidence and prevalence of venous thrombosis (VT) and venous thromboembolism (VTE) and their trend in Saudi Arabia is not known. However, there is a good reason to suspect that they will increase as the population, ages, patients undergo more major surgery, survive myocardial infarction, cerebrovascular accident and chemotherapy for malignancies. As in other countries, the increase may be mitigated if preventive measures are used for patients both in hospitals and in communities.<sup>1,2</sup> Familial thrombophilia (FT) defined as a genetically determined lifelong tendency to VT is now increasingly recognized as a major cause of VT<sup>3,4</sup> particularly the recurrent and familial type.<sup>5</sup> Identifiable causes are now increasing at a fast rate so that a new factor is being identified every few years.<sup>4,5</sup> This happened with the background of an explosion in basic research using DNA-Bank from patients with idiopathic and familial VT, such as The Leiden Thrombophilia Study.<sup>6</sup> Twenty years ago, we were able to identify causes of thrombophilia in only 5% of patients with VT. This has now increased to approximately 20% for idiopathic VT and increase up to 50% for recurrent or familial VT.<sup>3,7</sup> Several studies have associated factor V Leiden (FVL),<sup>7-9</sup> prothrombin (PT) 20210 G>A gene mutation<sup>7,10</sup> homozygosity to 5,10 methylenetetrahydrofolate reductase (MTHFR) 677 C>T<sup>11</sup> as independent risk factors for VT. Other studies have described the possible association of VT with a specific factor V gene polymorphism due to A>G at position 4070 (FV HR2)<sup>12,13</sup> and the insertion/deletion of 4G/5G at position 675 of the plasminogen activator inhibitor type 1 (PAI-I) gene.<sup>14</sup>

Registries provide information on disease incidence, prevalence, trends and data on which health planners can make short and long term decisions. Disease Registries also help in assessing the outcome of treatment for different diseases. To our knowledge The Saudi Thrombosis and Familial Thrombophilia (S-TAFT) Registry is the only registry for thrombosis and FT not only in the Kingdom of Saudi Arabia but also in the Gulf and Middle East.

We hereby describe the Registry and report preliminary data for the prevalence of 5 prothrombotic gene mutations in the normal Saudi population. The objectives of the S-TAFT Registry is to be the tool to collect data on the occurrence of VT, VTE, and

FT in different regions of the Kingdom ultimately leading to estimating the incidence, prevalence, and trends nationwide; to identify patients with FT and asymptomatic carriers; to estimate the incidence of 5 reported prothrombotic genetic risk factors namely FVL, FVHR2, PT 20210 G>A, MTHFR 677C>T, and PAI-1 4G/5G insertion deletion polymorphism in a control population and later compare them to patients with VT; to counsel patients and asymptomatic carriers regarding genetic risk, risk of future VTE, and advise regarding prophylaxis; to enhance national awareness of VT, VTE, and FT; to collect data to facilitate clinical and basic research into VT, VTE, and FT in the Kingdom and to provide necessary data to the Ministry of Health to allocate future resources.

**Methods.** The S-TAFT Registry has been approved by the King Abdulaziz Institute for Science and Technology (KACST) and the Research Advisory Council (RAC) at King Faisal Specialist Hospital and Research Center (KFSHRC), Riyadh, Kingdom of Saudi Arabia, which includes the approval of the Clinical Research Committee, Bio-Ethics Committee and the Basic Research Committee. Blood collection from different regions of the Kingdom started between November 2001 until July 2007. All "forms" used in this study were designed and approved by the RAC. A special designed "consent form" was also used. The aim and benefits of the study were explained to all patients during the routine appointment at the Anticoagulation Clinics by the attending physician(s). We informed the patient that the enrollment was voluntary and that he/she can withdraw at any time, and withdrawal from the study will not affect the patient's right for medical management. The patient was informed that the results of the test may or may not change his/her current management, but may help in preventing recurrence or change future management and that they will remain confidential. Written informed consent was obtained from all participants before the study period. Access to the S-TAFT Register is highly restricted. Thus, special access codes were given to the selected staff upon the approval of the Registry Committee. All personnel involved in data collection, entry, and analysis signed the confidentiality agreement. Only non-identifiable patient's data was made available to non-authorized personnel and presented in the periodical and annual reports. The S-TAFT Register is housed at the Registry Core Facility, Biostatistics, Epidemiology and Scientific Computing Department, Research Center, KFSHRC, Riyadh and is accessible by collaborating centers and hospitals across the Kingdom via the Internet using their assigned security passwords. As almost all patients with VT were managed in the hospitals, the data

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generated reflects the problem within the communities these hospitals serve. The patient population consists of individuals with VT. The clinical diagnosis of thromboembolic disease must always be confirmed by appropriate laboratory or radiological tests. The FT population includes the index patient that tested positive for any of the prothrombotic risk factors, their parents, siblings, and offspring above the age of 14 years. Patients with recurrence were identified in the S-TAFT Register as a distinctive subgroup. Patients with FT and their asymptomatic family members were identified as another distinctive subgroup.

Inclusion criteria were healthy Saudi nationals including blood bank donors, hospital staff, and volunteers with no medical illness from the control group. Consenting Saudi adults (>14 years), of both gender, with confirmed VTE disease formed the patient group. Asymptomatic parents, full siblings, and offspring above the age of 14 years of index patients who tested positive for FT will be tested for that particular risk factor. Exclusion criteria were Saudi individuals (control group) with any medical illness, with clinical diagnosis of VT, and VTE without collaborating laboratory or radiology evidence. Half siblings of the index patients will not be tested. The managing physician(s) will counsel the patients and their relatives who tested positive for the relevant genetic mutation. Physicians will delineate the risk of asymptomatic carriers, and the future offspring have for developing VTE, and will advise regarding prophylaxis at times of increased risk. In general, prophylaxis is not presently recommended for asymptomatic carriers. Such patients may need aggressive management during difficult crisis such as complicated VT, major surgery, pregnancy and delivery, or when they have a critical medical illness. Patient Educational Materials were developed at KFSHRC and copies were supplied to collaborating hospitals. Members of the thromboembolic services at the KFSHRC regularly visit the collaborating center(s) to deliver the lectures on management and prevention of VT. Hospital wide preventive strategies for VT were developed at the KFSHRC and updated recently. This in the future will be shared with collaborating institutes, if they are interested. The results of the normal control population for the 5 genetic risk factors tested were compared with the worldwide normal populations studies, and later to those of patients with confirmed VT.

Data collection was prospective and via direct interview. Data were documented on special Demographic Data Collection Form (\*Appendix 1 & 2) prior to loading onto the S-TAFT Registry Data Base Software referred to as "The Register." Information

entered will include name, medical record number, age, gender, place of birth, current address, consanguinity, family history of VT, diagnosis, risk factors for VT, and when appropriate whether or not he was on preventive therapy prior to VT. Results of tests carried out for FT will also be entered as they become available. Data on outcome including complications during therapy and recurrence of VT, are continuously updated.

**Data analysis and statistical methods.** Annual report on demographic data were provided. Data generated from genetic testing were analyzed to evaluate the probability values, confidence intervals, and odd ratio using the standard statistical methods. The Chi square were used to evaluate the allele frequency in normal and patient population. The Univariate analysis was used to screen each factor separately, and the multivariate logistic regression to investigate the joint effect of different risk factors. Moreover, to identify the proportional contributions of genetic factors to the variability of the disease, mixed-logistic regression model will be used. Variance components estimation were achieved via the Restricted Maximum Likelihood (REML) method of estimation.

**Results.** The DNA was extracted using venous blood collected from healthy consenting individuals and patients with confirmed VT, then stored at -70°C until tested. The healthy control population consisted of normal volunteers staff of the KFSHRC and normal blood donors from the blood bank at KFSHRC. The latter group was mainly relatives of patients undergoing surgery. As KFSHRC is a tertiary care facility, these patients (and their blood donor family members) came from all over the Kingdom, as does the Saudi staff of the KFSHRC. Using established or slightly modified methods, aliquots of 902 DNA samples from consenting control group were tested for FVL, FVHR2, PT 20210 G>A, MTHFR 677C>T, and PAI-1 4G/5G polymorphism. Three factors were tested in 902 healthy controls (793 men and 109 women, aged 29±8 years). Eight (1.2%) were heterozygous to FVL and one (0.1%) was homozygous. Six (0.7%) were heterozygous to PT 20210 G>A and none were homozygous. Two hundred and twenty (24.39) were heterozygous to MTHFR 677 C>T and 22 (2.43%) were homozygous. Two factors were tested in 651 healthy controls. One hundred and forty (21.5%) were heterozygous to FVHR2 and 15 (2.3%) were homozygous. A total of 155 (23.9%) tested positive for FVHR2. Two hundred and fifty-two (38.7%) carried the PAI-1 5G/5G polymorphism, 333 (51.1%) carried the 4G/5G polymorphism and 66 (10.1%) carried the high-risk 4G/4G polymorphism. Table 1 compares the 5 prothrombotic genetic mutations

\*The full text including Appendix is available in PDF format on Saudi Medical Journal website ([www.smj.org.sa](http://www.smj.org.sa))

**Table 1** - Comparison of the incidence of 5 prothrombotic gene mutations in healthy Saudi controls with Worldwide distribution.

Type of mutation	Saudi control (n=902) %	Worldwide distribution %
Factor V Leiden	1.3	3 - 9.8
Prothrombin 20210G>A	0.66	0.85 - 2.1
MTHFR 677 C>T (homozygous)	2.43	6 - 32
Plasminogen activator inhibitor type 1 4G/4G	10.19	41 - 63
Factor V HR2	23.8	0 - 31
MTHFR - 5-10 methylenetetrahydrofolate reductase		

tested in the normal Saudi population to worldwide distribution. The genotype frequency was in Hardy-Weinberg equilibrium for PT 20210 G>A, FVHR2 and MTHFR 677 C>T and was not for FVL and PAI-1 4G/4G polymorphism.

**Discussion.** Venous thrombosis and VTE are an important cause of mortality and morbidity. Both are related to venous stasis, abnormalities in blood vessels, and a hypercoagulable state.<sup>3,15,16</sup> Hypercoagulable states can be acquired, namely, age, immobility, paralysis, pregnancy, surgery, obesity, congestive heart failure, myocardial infarction, cerebrovascular accident (CVA), fracture of the hip and lower extremities, indwelling catheter in femoral veins, nephrotic syndrome, inflammatory bowel disease, estrogen use, varicose veins, and previous VTE,<sup>3,17</sup> or congenital, namely, antithrombin III deficiency, protein C deficiency, protein S deficiency, factor V Leiden (FVL), prothrombin (PT) 20210 G>A, homozygosity to 5-10 methylenetetrahydrofolate reductase (MTHFR) 677C>T, dysfibrinogenemia, disorders of plasminogen and plasminogen activators, antiphospholipid syndrome, and hyperhomocysteinemia.<sup>4,17</sup> Patients with FT are often normal and asymptomatic. Those who are heterozygous for one abnormality only will develop symptomatic disease (VT) when exposed to an additional acquired risk factor such as surgery, pregnancy, prolonged bed rest, oral contraception.<sup>18,19</sup> Individuals who have more than one abnormality, or those who are homozygous to one prothrombotic gene mutation are at a much higher risk of VT. They usually present with idiopathic or familial VT, often at a young age. The usual clinical presentation of individuals with FT is that of a young asymptomatic individual who develops DVT with or without an additional acquired risk factor(s). Thrombosis at unusual sites example: visceral or cerebral VT and recurrent abortion and miscarriages are less common presentations.<sup>20-22</sup> We

recognize the existence of population variance of the genetic risk factors we studied and the conflicting reports of their association with VT. Because of the scanty data from our part of the world, we studied several known polymorphisms such as FVL, prothrombin 20210G>A,<sup>23</sup> 5-10 methylenetetrahydrofolate reductase 677 C>T, the -675 insertion/deletion polymorphism of plasminogen activator inhibitor type-1 and FVHR2 in a healthy control population<sup>15,23,24</sup> and in patients with confirmed VT/VTE.<sup>25</sup>

Our data from the normal Saudi population show that the risk factors we tested (except for FVHR2) are lower than in most other populations. This was an unexpected finding in view of the fact that intermarriages are relatively common in the Kingdom, and our impression that VTE is relatively common. It is possible that a prothrombotic genetic mutation unique to our part of the world and yet to be identified may exist, and hence all DNA will be stored for future testing for new and novel genetic risk factors in the Saudi community. It is also possible that our impression of how common VTE is incorrect. These are 2 issues (of many) that a National Registry will answer. Data analysis in patients with VT continues and will be reported later. Of note is the fact that our data compares favorably to those from other countries in both number of individuals studied and the genetic risk factors. Although the normal population studied originated from different regions and tribes in the Kingdom, there was an inevitable over-representation of individuals from the Riyadh and central area where KFSHRC is based. Our current data cannot therefore be truly "National". This issue will however be resolved once the Registry becomes truly national when our colleagues from other institutes join us. Through this article we invite our colleagues from the health institutes through out the Kingdom to join us in this very worthwhile effort. The collaborating center will have to sign a collaboration agreement as required by the Research Advisory Council (RAC). King Faisal Specialist Hospital and Research Center will provide the necessary consent forms, demographic data collection forms, access to the web registry and training. The collaborating hospitals needs to secure and identify one person for training on the software and related registry policies. This will be carried out at the Registries Core Facility at the Research Center, KFSHRC. Trained user(s) will require computer(s) with Internet connection, Microsoft Office (Word, Excel, Power Point) and SPSS (for statistical analysis if needed) installed on the computer.

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