Research Article

Etiopathogenesis of Sheehan’s Syndrome: Roles of Coagulation Factors and TNF-Alpha

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Sheehan’s Syndrome (SS) is defined as pituitary hormone deficiency due to ischemic infarction of the pituitary gland as a result of massive postpartum uterine hemorrhage [1]. Although the onset of SS can involve acute severe panhypopituitarism in some patients, the majority of SS patients are diagnosed with a clinically subtle partial pituitary deficiency and therefore their diagnoses and treatments are delayed for many years [2]. The prevalence of SS is not clearly known, presumably due to the great number of nondiagnosed patients. It is a rarely encountered disorder in developed countries due to good obstetric care [3, 4]. However, its prevalence is estimated to be still high in developing countries in which many deliveries take place at home [5]. Zargar et al. estimated the prevalence of SS to be 3.1% among women in India, and about two-thirds of those women had given birth at home [6].

1. Introduction

Sheehan’s Syndrome (SS) is defined as pituitary hormone deficiency due to ischemic infarction of the pituitary gland as a result of massive postpartum uterine hemorrhage [1]. Although the onset of SS can involve acute severe panhypopituitarism in some patients, the majority of SS patients are diagnosed with a clinically subtle partial pituitary deficiency and therefore their diagnoses and treatments are delayed for many years [2]. The prevalence of SS is not clearly known, presumably due to the great number of nondiagnosed patients. It is a rarely encountered disorder in developed countries due to good obstetric care [3, 4]. However, its prevalence is estimated to be still high in developing countries in which many deliveries take place at home [5]. Zargar et al. estimated the prevalence of SS to be 3.1% among women in India, and about two-thirds of those women had given birth at home [6].

As the etiopathogenesis of SS is not clear, disorders of coagulation have been investigated in some studies. It has been reported that disseminated intravascular coagulation (DIC) can cause postpartum hypopituitarism [7, 8]. In addition, Cakir et al. found protein S deficiency in 2 out of 12 patients with SS [9]. Importantly, in another study, Gokalp et al. investigated inherited hypercoagulation as a risk factor of SS. They found that frequency of MTHFR (methylenetetrahydrofolate reductase) C677T and MTHFR A1298C polymorphisms was significantly higher among their 38 patients with SS compared to the healthy control group [10]. In addition, Factor II (G20210A), Factor V (G1691A), and PAI-1 4G/5G mutations were also more common among the SS patients, but no significant differences were observed.

Our aim in this study was to investigate gene polymorphisms of MTHFR C677T and A1298C, Factor II (G20210A), Factor V (G1691A), and PAI-1 (plasminogen activator inhibitor-1) 4G/5G that are associated with inherited...
hypercoagulation and TNF-α (tumor necrosis factor-alpha) 308 G > A that is associated with apoptosis of pituitary cells due to autoimmunity. Knowing the frequencies of these polymorphisms among SS patients will be helpful in understanding their roles in the etiopathogenesis of SS.

2. Patients and Methods

2.1. Study Design. Fifty-three patients who were previously diagnosed with SS and 43 healthy women were enrolled in this study which was conducted between 2011 and 2013. The blood samples of patients with SS who were followed up by the Endocrinology Department of Erciyes University Medical School were collected following 12 hours of fasting. The healthy female volunteers in the control group were chosen from hospital staff and their relatives. Written informed consent was obtained from all participants before registering them for the study.

In addition to the demographic information, the medical history and drug use of the participants were examined in detail. The most important inclusion criterion for the SS group was having the exact diagnosis of SS. Therefore, it was emphasized that all of the following criteria for diagnosis were met: (a) at least one pituitary hormone deficiency found in basal levels or via dynamic tests if required; (b) massive postpartum uterine hemorrhage history at last delivery; (c) agalactia and amenorrhea after the last delivery; (d) exclusion of all other causes of pituitary deficiency; (e) observation of partial or complete empty sella on magnetic resonance imaging (MRI). Exclusion criteria for SS patients were having comorbidity or being on other treatments than glucocorticoid and thyroid hormone replacement therapies which were adequately performed. In order to avoid the effects of replacement therapies of growth hormone (GH) and gonadal steroids on genotyping studies, such treatments were stopped 3 months before the blood collection for the genetic analyses. In addition, women in the control group did not have any history of disease and were not on any drug therapies.

2.2. Hormonal Analyses. Basal levels of hormones were analyzed to determine hypopituitarism in all patients. In addition, the insulin tolerance test or glucacon stimulation test was performed to identify growth hormone (GH) deficiency and secondary adrenal failure. Basal hormone levels including free T4 (normal: 0.88–1.72 ng/dL), thyroid stimulating hormone (TSH; normal: 0.57–5.6 mU/mL), adrenocorticotropic hormone (ACTH; normal: 0–46 pg/mL), cortisol (normal: 9–23 µg/dL), prolactin (PRL; normal for postmenopausal women: 2.4–29.8, and premenopausal women: 3.3–29.8 ng/mL), follicle stimulating hormone (FSH; normal for postmenopausal women: 23.9–119.1, and premenopausal women: 2.0–9.8 mU/mL), luteinizing hormone (LH; normal for postmenopausal women: 16.3–54.8, and premenopausal women: 0.7–17.3 mU/mL), estradiol (E2; normal for postmenopausal women: 14.4–44.5, and premenopausal women: 18.9–246.7 pg/mL), and insulin-like growth factor-1 (IGF-1; reference intervals varied by age) were measured in the hormone laboratories of Erciyes University Medical School.

Methods of assays and commercial kits were as follows: GH: immunoradiometric assay (IRMA), Immunotech SAS, France; IGF-1: IRMA, Immunotech SAS, France; ACTH: IRMA, Cisbio Bioassays, France; cortisol: radioimmunoassay (RIA), Immunotech s.r.o, Czech Republic; PRL, TSH, fT4, FSH, LH, estradiol: Immunoassay, Siemens Advia centaur XP-USA.

2.3. Genotyping Studies. 2 mL venous blood samples were collected in EDTA-containing tubes for DNA analyses. Total genomic DNA was extracted by standard methods and DNA samples were stored at –20°C until the analyses of polymorphisms. Genotyping studies were conducted in Erciyes University Genome and Stem Cell Center (GENKOK). A total of 20 µL PCR mixture with 5 µL sample DNA was analyzed by using a LightCycler FastStart DNA Master HybProbe to perform the genotypings of MTHFR C677T and A1298C, Factor II (prothrombin) G20210A, and Factor V G1691A according to manufacturer’s instructions (Roche Diagnostics, Germany). In addition, Roche LightCycler 480 Software was used for detecting different genotypes of these polymorphisms.

Genotypes of the TNF-α-308 G/A and PAI-1 4G/5G polymorphisms were detected by analyses of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) results. PAI-1 4G/5G polymorphisms were analyzed by forward primer 5’-CACAGAGAGGTCTG-GCCACGT-3’ and 5’-CAAAGAGACCTTTGTCCTC-3’ reverse primer. The genomic region of interest was amplified by PCR using 30 cycles with a denaturation temperature of 94°C for 3 min, 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec. The final extension step at 72°C was extended for 1 min. Amplified 98 base pair (bp) products were digested overnight with Bst I at 55°C and subjected to 4% agarose gel electrophoresis [11].

The PCR procedure was performed in a total volume of 50 µL containing 5 µL genomic DNA, 10x PCR buffer, dNTPs (2.5 mM), MgCl₂ (1.5 mM), Taq DNA polymerase (1 U/µL), 5’AGGCAATAGGTTTTGAGGGCCAT-3’ and 5’TCCTCCCTGCTCCGATTCCG-3’ forward primers, and 5’TCTCCCTGCTCCGATTC3’ reverse primers for TNF-α [12]. The cycling conditions consisted of denaturation at 95°C for 5 min, followed by 30 consecutive cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final elongation at 72°C for 5 min. The 107 bp PCR products were separated by electrophoresis on a 2% agarose gel and visualized with UV illumination and ethidium bromide staining. After amplification, the PCR products were digested overnight at 37°C with Nco I restriction enzyme and analyzed by 3% agarose gel electrophoresis.

2.4. Statistical Analyses. Statistical analyses were performed by using SPSS 15.0 (SPSS Inc., Chicago, IL). Demographic data were presented as mean ± SD. Binomial variables were analyzed using Pearson’s chi-square test or Fisher’s exact test. Moreover, the odds ratios (OR) were calculated with 95% confidence intervals (CI) using logistic regression analysis. Comparisons of genotype frequencies were made between
patients and controls, and $P$ values less than 0.05 were considered as statistically significant.

3. Results

Panhypopituitarism was detected in 38 (71.7%) patients with SS, while partial hypopituitarism was detected in 15 (28.3%) patients. Importantly, all patients had gonadotropin and GH deficiencies, but deficiencies of ACTH, TSH, and prolactin were not found in all of the patients (Table 1).

When comparing groups, it was revealed that there was no significant difference between the mean age of the 53 cases with SS and the 43 healthy controls, $63.2 \pm 12.5$ and $60.3 \pm 9.3$ years, respectively. In addition, no difference was detected in terms of mean body mass indexes (BMIs). The mean BMI was $28.9 \pm 2.8$ kg/m$^2$ in the SS group and $29.2 \pm 3.3$ kg/m$^2$ in healthy women. Moreover, the SS and healthy control groups did not significantly differ according to polymorphism rates of Factor II (G20210A), Factor V (G1691A), MTHFR (C677T and A1298C), PAI-1 (4G/5G), and TNF-α ($\sim 308$ G > A) genes, except for PAI-1 (4G/5G). The PAI-1 (4G/5G) mutation was detected in 26 (60.5%) of the 43 control cases and in 20 (37.7%) of the 53 SS cases. Frequencies and comparisons of polymorphisms are demonstrated in Table 2.

4. Discussion

The physiological enlargement of the pituitary gland during pregnancy plays a significant role in onset of SS, because severe bleeding does not lead to pituitary deficiency in women unless they are pregnant. Even though the pathogenesis of SS has not yet been fully clarified, the basis of its pathology has been identified as infarction and ischemic necrosis that develops due to the interruption of arterial blood flow in the anterior pituitary gland [13]. However, the cause of the interruption in the blood flow is not clear. Considered potential mechanisms are arterial thrombosis similar to that seen in stroke, development of arterial spasm as a result of severe hypotension that is due to massive uterine bleeding, or compression of pituitary vessels due to relatively small sella turcica volume associated with enlargement of the pituitary during pregnancy [14]. Furthermore, autoantibodies detected in many patients against the pituitary gland have been suggested as a contributing factor in the etiopathogenesis of SS [15].

In the etiopathogenesis of SS, our study findings did not yield a significant difference between the control and SS patient groups in terms of mutations of blood composition anomalies that lead to inherited hypercoagulation. In other words, in the SS group none of the genes had higher polymorphism rates than those in the control group. There are numerous other acquired (prolonged immobilization, pregnancy, oral contraceptive pills, advanced age, obesity, cigarette use, hypertension, etc.) or genetic (protein C or S deficiency, antithrombin-III deficiency, platelet GPllb/IIIa HPA-Ib mutation, elevated levels of Factors VII, VIII, IX, and XII, Von Willebrand disease, fibrinogen, etc.) factors that are known to cause hypercoagulation. An important limitation of our study is that not all of the parameters associated with hypercoagulation were investigated. Therefore, it is hard to conclude, based only on our study findings, that inherited hypercoagulation is not involved in SS.

Although, the mutation rates of MTHFR C677T and A1298C genes show social differences, homozygous mutations are estimated to be 10%, and heterozygous mutations are about 40% [16, 17]. Approximately, 15% of the population is reported to carry both heterozygous genetic mutations together, due to their close relations [17]. Although the rates of MTHFR gene polymorphisms were not higher in the SS group than in the control group in our study, more important issues are whether the SS patients had vitamin B6, vitamin B12, and/or folate deficiency and thus a disorder in MTHFR gene expression during their last pregnancies.

The heterozygous mutations in Factor II (prothrombin) G20210A are encountered at a 2-3% rate among Caucasians [18]. Plasma Factor II levels (prothrombin) increase as a result of mutations. In that case, risk of venous thrombosis is higher than arterial thrombosis risk. Venous thrombosis risk among individuals carrying this mutation is increased by 2-3 times. One study reported that coronary artery risk is increased by 1.31 times in individuals with prothrombin G20210A mutation [19].

While varying across different populations, the incidence rate of Factor V Leiden heterozygous mutation is approximately 5%, but its homozygous mutation is rare [20]. As a result of the mutation in the Factor V gene (G1691A), activated protein-C cannot inhibit Factor V molecules. This, in turn, results in a deterioration of the bleeding and coagulation balance in favor of coagulation. This mutation is reported to increase thrombosis risk fivefold when heterozygous, and by 10–80-fold when homozygous [21, 22]. Factor V Leiden has also been shown to increase complications such as miscarriage, preeclampsia, and abruptio placentae by a minimum of 2-3 times [20].

Plasminogen activator inhibitor-1 (PAI-1) inhibits tissue plasminogen activator (tPA) and urokinase which are proteins leading to plasminogen activation and fibrinolysis. The result is inhibition of fibrinolysis. The estimated frequencies of PAI-1 4G/4G, 4G/5G and 5G/5G are, respectively, about 35%, 50%, and...
Table 2: Distribution of thrombophilic and cytokine genes among Sheehan’s Syndrome cases and controls.

|                       | Sheehan's Syndrome patients (n: 53) | Controls (n: 43) | OR (95% CI)    | P value<sup>‡</sup> |
|-----------------------|-------------------------------------|------------------|----------------|--------------------|
|                       | n (%)                               | n (%)            |                |                    |
| **MTHFR C677T**       |                                     |                  |                |                    |
| Genotype              |                                     |                  |                |                    |
| CC (normal)           | 31  58.5                            | 25  58.1         | 1 (Ref)        | —                  |
| CT (heterozy.)        | 19  35.8                            | 13  30.2         | 1.179 (0.489–2.843) | 0.714 |
| TT (homozy.)          | 3  5.7                              | 5  11.6          | 0.484 (0.105–2.224) | 0.351 |
| P value<sup>†</sup>   | 0.538                               |                  |                |                    |
| **MTHFR A1298C**      |                                     |                  |                |                    |
| Genotype              |                                     |                  |                |                    |
| AA (normal)           | 17  32.1                            | 15  34.9         | 1 (Ref)        | —                  |
| AC (heterozy.)        | 28  52.8                            | 19  44.2         | 1.3 (0.525–3.219) | 0.570 |
| CC (homozy.)          | 8  15.1                             | 9  20.9          | 0.784 (0.241–2.549) | 0.686 |
| P value<sup>†</sup>   | 0.646                               |                  |                |                    |
| **Factor II G20210A** |                                     |                  |                |                    |
| Genotype              |                                     |                  |                |                    |
| GG (normal)           | 50  94.3                            | 42  97.7         | 1 (Ref)        | —                  |
| GA (heterozy.)        | 3  5.7                              | 1  2.3           | 2.520 (0.253–25.136) | 0.431 |
| AA (homozy.)          | —                                   | —                | —              | —                  |
| P value<sup>†</sup>   | 0.416                               |                  |                |                    |
| **Factor V G1691A**   |                                     |                  |                |                    |
| Genotype              |                                     |                  |                |                    |
| GG (normal)           | 47  88.7                            | 37  86           | 1 (Ref)        | —                  |
| GA (heterozy.)        | 5  9.4                              | 4  9.3           | 0.984 (0.247–3.925) | 0.982 |
| AA (homozy.)          | 1  1.9                              | 2  4.7           | 0.394 (0.034–4.511) | 0.454 |
| P value<sup>†</sup>   | 0.741                               |                  |                |                    |
| **PAI-1 4G/5G**       |                                     |                  |                |                    |
| Genotype              |                                     |                  |                |                    |
| 5G/5G (normal)        | 21  39.6                            | 7  16.3          | 1 (Ref)        | —                  |
| 4G/5G (heterozy.)     | 20  37.7                            | 26  60.5         | 0.256 (0.091–0.722) | 0.01  |
| 4G/4G (homozy.)       | 12  22.6                            | 10  23.3         | 0.4 (0.121–1.326) | 0.134 |
| P value<sup>†</sup>   | 0.03                                |                  |                |                    |
| **TNF-α –308 G>A**    |                                     |                  |                |                    |
| Genotype              |                                     |                  |                |                    |
| GG (normal)           | 43  81.1                            | 34  79.1         | 1 (Ref)        | —                  |
| GA (heterozy.)        | 9  17.1                             | 9  20.9          | 0.791 (0.283–2.210) | 0.654 |
| AA (homozy.)          | 1  1.9                              | —                | —              | —                  |
| P value<sup>†</sup>   | 0.6                                 |                  |                |                    |

Notes: heterozy.: heterozygous; homozy.: homozygous.
<sup>†</sup>Significance of χ² values which were obtained from a Chi-Square test.
<sup>‡</sup>Significance of odds ratios which were obtained from a logistic regression model.

Interestingly, our results showed that the 4G/5G polymorphism rate was 60.5% among healthy women, while it was 37.7% among SS patients. This finding may suggest that the 4G/5G polymorphism does not always lead to thrombophilia, unless accompanied by some acquired factors. TNF-α (tumor necrosis factor-alpha) is a cytokine that plays an important role in the regulations of immune functions, cell proliferation and differentiation, apoptosis due
to autoimmunity, coagulation, adipocyte, lipid and glucose metabolism. The 308 G/A polymorphism of TNF-α which leads to elevated TNF-α levels is known to have an important impact on the development of autoimmune diseases, metabolic syndromes, cancers, and psychiatric disorders [30, 31]. Additionally, an association between high TNF-α levels and hypercoagulation has also been detected [32].

Autoimmunity is a potential risk factor in the development of SS and 35% of patients have been shown to have anti-pituitary autoantibodies [33]. These antibodies are presumably generated in reaction to the necrotic pituitary gland tissue found after ischemic infarction and seem to be responsible for the chronic but progressive course of SS [15]. On another note, increased estrogen levels during pregnancy make adenohypophysial cells, particularly the lactotroph cells, more vulnerable to apoptosis due to autoimmunity as a result of elevated expression of the TNF-α gene in rats [34]. This condition may also be associated with the vulnerability of an enlarged pituitary to ischemic necrosis at the end of pregnancy. Estrogen levels gradually increase during pregnancy and peak close to completion of the full term. In this regard, we hypothesized that autoimmunity caused by TNF-α 308 G/A mutation can be a contributing factor in the etiopathogenesis of SS. However, no increase in the rate of TNF-α 308 G/A polymorphism was observed among the SS patients when compared to the control group.

5. Conclusion

As demonstrated in our study, the disorders suggested for SS etiopathogenesis and the associations among them are highly complicated. Thrombophilia and autoimmunity in SS are also multifaceted and complicated mechanisms. However, the results of our study showed no increase in Factor II, Factor V, MTHFR, PAI-1, and TNF-α gene polymorphism rates among SS patients compared to the control group. Hence, genetic factors other than these gene polymorphisms should be researched in the etiopathogenesis of SS. Clarifying SS etiopathogenesis via further studies will be particularly beneficial in identifying women susceptible to SS prior to disease development.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Halit Diri and Elif Funda Sener contributed equally to this work and should be considered co-first authors.

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References

[1] H. L. Sheehan, “Postpartum necrosis of the anterior pituitary,” Journal of Pathology & Bacteriology, vol. 45, no. 1, pp. 189–214, 1937.
[2] F. Keleştimur, “Sheehan’s syndrome,” Pituitary, vol. 6, no. 4, pp. 181–188, 2003.
[3] E. C. Feinberg, M. E. Molitch, L. K. Endres, and A. M. Peaceman, “The incidence of Sheehan’s syndrome after obstetric hemorrhage,” Fertility and Sterility, vol. 84, no. 4, pp. 975–979, 2005.
[4] M. Regal, C. Páramo, J. M. Sierra, and R. V. García-Mayor, “Prevalence and incidence of hypopituitarism in an adult Caucasian population in northwestern Spain,” Clinical Endocrinology, vol. 55, no. 6, pp. 735–740, 2001.
[5] O. O. Famuyiwa, A. F. Bella, and A. O. Akanji, “Sheehan’s syndrome in a developing country, Nigeria: a rare disease or problem of diagnosis?” East African Medical Journal, vol. 69, no. 1, pp. 40–43, 1992.
[6] A. H. Zargar, B. Singh, B. A. Laway, S. R. Masoodi, A. I. Wani, and M. I. Bashir, “Epidemiologic aspects of postpartum pituitary hypofunction (Sheehan’s syndrome),” Fertility and Sterility, vol. 84, no. 2, pp. 523–528, 2005.
[7] T. Yamauchi, N. Yoshio, T. Mizuguchi, E. Negoro, N. Kamitani, and T. Ueda, “Acute fatty liver of pregnancy complicated with anterior pituitary insufficiency,” Internal Medicine, vol. 40, no. 12, pp. 1227–1231, 2001.
[8] J. J. Piech, P. Thiebaut, and J. P. Haberer, “Twin pregnancy with acute hepatic steatosis followed by hypopituitarism and diabetes insipidus,” Presse Medicale, vol. 14, no. 24, pp. 1421–1423, 1985.
[9] I. Cakir, F. Tanriverdi, Z. Karaça et al., “Evaluation of coagulation and fibrinolytic parameters in adult onset GH deficiency and the effects of GH replacement therapy: a placebo controlled study,” Growth Hormone and IGF Research, vol. 22, no. 1, pp. 17–21, 2012.
[10] D. Gokalp, A. Tuzcu, M. Bahceci et al., “Analysis of thrombophilic genetic mutations in patients with Sheehan’s syndrome: is thrombophilia responsible for the pathogenesis of Sheehan’s syndrome?” Pituitary, vol. 14, no. 2, pp. 168–173, 2011.
[11] E. F. Emirougullari, C. Saatci, A. Unal, A. Sahin, and Y. Ozkul, “Prothrombin, factor-V leiden and plasminogen activator inhibitor type 1 gene polymorphisms in hemodialysis patients with/without arteriovenous fistula thrombosis,” The Journal of Nephro-Urology, vol. 2, no. 2, pp. 314–319, 2010.
[12] M. Dundar, A. Kiraz, B. Balta et al., “The role of TNF-alpha and PAI-1 gene polymorphisms in familial Mediterranean fever,” Modern Rheumatology, vol. 23, no. 1, pp. 140–145, 2013.
[13] K. Kovacs, “Sheehan syndrome,” The Lancet, vol. 361, no. 9536, pp. 520–522, 2003.
[14] A. H. Tessnow and J. D. Wilson, “The changing face of Sheehan’s syndrome,” American Journal of the Medical Sciences, vol. 340, no. 5, pp. 402–406, 2010.
[15] R. Goswami, N. Kochupillai, P. A. Crock, A. Jaleel, and N. Gupta, “Pituitary autoimmunity in patients with Sheehan’s syndrome,” Journal of Clinical Endocrinology and Metabolism, vol. 87, no. 9, pp. 4137–4141, 2002.
[16] B. Zappacosta, L. Romano, S. Persichilli et al., “Genotype prevalence and allele frequencies of 5,10- methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms in Italian newborns,” Laboratory Medicine, vol. 40, no. 12, pp. 732–736, 2009.
[17] I. Weisberg, P. Tran, B. Christensen, S. Sibani, and R. Rozen, "A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity," Molecular Genetics and Metabolism, vol. 64, no. 3, pp. 169–172, 1998.

[18] F. R. Rosendaal, C. J. M. Doggen, A. Zivelin et al., "Geographic distribution of the 20210 G to A prothrombin variant," Thrombosis and Haemostasis, vol. 79, no. 4, pp. 706–708, 1998.

[19] Z. Ye, E. H. C. Liu, J. P. T. Higgins et al., "Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66 155 cases and 91 307 controls," The Lancet, vol. 367, no. 9511, pp. 651–658, 2006.

[20] J. L. Kujovich, "Factor v Leiden thrombophilia," Genetics in Medicine, vol. 13, no. 1, pp. 1–16, 2011.

[21] S. Middeldorp, J. R. Meinardi, M. M. W. Koopman et al., "A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism," Annals of Internal Medicine, vol. 135, no. 5, pp. 322–327, 2001.

[22] F. R. Rosendaal, T. Koster, J. P. Vandenbroucke, and P. H. Reitsma, "High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance)," Blood, vol. 85, no. 6, pp. 1504–1508, 1995.

[23] Y. Matsubara, M. Murata, I. Isshiki et al., "Genotype frequency of plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism in healthy Japanese males and its relation to PAI-1 levels," International Journal of Hematology, vol. 69, no. 1, pp. 43–47, 1999.

[24] J. Kvasniˇcka, J. Hájková, P. Bobˇc´ıkov´a et al., "Prevalence of thrombophilic mutations of FV Leiden, prothrombin G20210A and PAI-1 4G/5G and their combinations in a group of 1,450 healthy middle-aged individuals in the Prague and Central Bohemian regions (results of FRET real-time PCR assay)," Casopis Lekaru Ceskych, vol. 151, no. 2, pp. 76–82, 2012.

[25] M.-L. P. J. van Goor, E. G. García, F. L ee bee k, G. -J. Browne rs, P. Koudstaal, and D. Dippel, "The plasminogen activator inhibitor (PAI-1) 4G/5G promoter polymorphism and PAI-1 levels in ischemic stroke. A case-control study," Thrombosis and Haemostasis, vol. 93, no. 1, pp. 92–96, 2005.

[26] L. L. Gong, J. H. Peng, F. F. Han et al., "Association of tissue plasminogen activator and plasminogen activator inhibitor polymorphism with myocardial infarction: a meta-analysis," Thrombosis Research, vol. 130, no. 3, pp. e43–e51, 2012.

[27] A. Dossenbach-Glaninger, M. Van Trotsenburg, M. Dossenbach et al., "Plasminogen activator inhibitor 1 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss," Clinical Chemistry, vol. 49, no. 7, pp. 1081–1086, 2003.

[28] W. Koch, M. Schrempf, A. Erl et al., "4G/5G polymorphism and haplotypes of SERPINE1 in atherosclerotic diseases of coronary arteries," Thrombosis and Haemostasis, vol. 103, no. 6, pp. 1170–1180, 2010.

[29] K. Jood, P. Ladenwall, A. Tjärnlund-Wolf et al., "Fibrinolytic gene polymorphism and ischemic stroke," Stroke, vol. 36, no. 10, pp. 2077–2081, 2005.

[30] H. T. Idriss and J. H. Naismith, “TNF alpha and the TNF receptor superfamily: structure-function relationship(s),” Microscopy Research and Technique, vol. 50, no. 3, pp. 184–195, 2000.

[31] W. P. Cawthorn and J. K. Sethi, "TNF-𝛼 and adipocyte biology," FEBS Letters, vol. 582, no. 1, pp. 117–131, 2008.

[32] M. G. Macey, S. I. Wolf, C. P. D. Wheeler-Jones, and C. Lawson, “Expression of blood coagulation factors on monocytes after exposure to TNF-treated endothelium in a novel whole blood model of arterial flow," Journal of Immunological Methods, vol. 350, no. 1-2, pp. 133–141, 2009.