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Genetic Mutations Pai-1 4G/4G and ACE D/D that Reduce Fibrinolysis are responsible for most Serious Pregnancy Complications and Abortions and are Best treated with S/C LMW Heparin and Aspirin

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Abstract

Background: Abortions, stillbirths, PET and other serious pregnancy complications cause more than 3-4 million pregnancy failures annually worldwide. Hypofibrinolytic and thrombophilia mutations are responsible for a large number of these events.

Methods: A study of 11 such genetic mutations (Table 5) in 26 Cypriot ladies after failed pregnancies from serious complications, stillbirths and abortions was carried out between 2006 and 2020. All 26 ladies had significant hypo fibrinolysis and thrombophilia mutations. Hypofibrinolytic mutation PAI-1 4G/4G, was the commonest (X^2 p=0.0052) with 11 ladies, 9 of them also ACE D/D positive. Factor V Leiden was present in 5 ladies (X^2 p=ns).

Clinical results: After the diagnosis of hypo fibrinolysis ± thrombophilia, 19 of these 26 ladies proceeded with 1-2 more pregnancies using LMW s/c heparin and oral aspirin, all with healthy babies. The remaining 7 ladies were too stressed for another pregnancy. 12 ladies were referred after PET, 2 with stillbirths and 9 after caesareans with birthweights at 700, 900, 950, 1050+1080, 1200, 1250, 2050, 2080 and 2200g. One baby developed mental retardation. The 12th lady with PET at 20 weeks, was immediately treated with LMW s/c heparin and had a healthy baby at 32 weeks. The remaining 14 ladies had other earlier pregnancy complications, 9 of them ending as abortions. Three ladies had their pregnancies saved with an instant mutational study and therapy with LMW heparin, leading to healthy babies.

Conclusion: Hypofrinolytic mutations PAI-1 4G/4G and ACE D/D are serious mutations leading to abortions and pregnancy complications and should always be taken seriously in pregnancy. Thrombophilia mutations Factor V Leiden, Factor VR2, prothrombin G20210A, and MTHFR also lead to complicated pregnancies but less frequently. LMW s/c heparin in pregnancies with such mutations saves lives. It would be ideal if these mutations were always studied prior to every first pregnancy.

Keywords: Mutations PAI-1 4G/4G, ACE D/d In Pregnancy

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Introduction

Deaths after: 1. Live births, 2. Stillbirths and 3. Abortions (miscarriages).

Live births: A successful pregnancy with the birth of a healthy child is the wish and desire of every new family throughout the world. In Cyprus we now have 9,500-10,000 live births annually and Table 1 with data from our Ministry of Health describes all live birth findings in the years 2016 and 2017. In 2016 there were 9670 live births with 26 deaths up to the end of their 1st year of life, (2.7/1000 live births). In 2017 there were 9442 live births with only 9 deaths, (0,9/1000 live births). These are excellent results.

Stillbirths: Contrary to these very small death numbers after live births, in Cyprus we observe a higher loss of stillbirths after the 22nd week of the pregnancy. In 2016 we had 52 stillbirths, while in 2017 we had 53 stillbirths, (Table 2). These stillbirths are more common during the first pregnancy, 46%-60%, (Table 2). In the whole world there may be ~2,6-3.0 million stillbirths /yr., from many different causes and it is estimated that 50-60% occur in the 10 most underdeveloped countries with deficient gynecological and obstetrical care [1]. These stillbirths cause great distress in all affected families. Careful research is urgently needed to understand the multiple causes of these unfortunate stillbirths and also to search for their effective prevention. Excellent clinical work and research during the last 27 years, 1994-2021, after the historic discovery of the most significant Thrombophilia Factor V Leiden, in Holland in 1994 by Prof Bettina [2,3] and most other similar mutations such as prothrombin G20210A, Factor VR2, PAI-1 4G/4G, and ACE D/D, has linked the presence of such thrombophilia and hypofibrinolytic genetic mutations to stillbirths and most serious pregnancy complications such as pre-eclampsia, (PET), the HELLP syndrome and placental abruption with serious placental bleeding. [4-10]. Thrombophilia mutation Factor V Leiden promotes the formation of placental arteriolar thrombi, while hypofibrinolytic mutations PAI-1 4G/4G and ACE D/D reduce their removal, both leading to serious uteroplacental ischemia that is very dangerous, particularly in the early days and weeks of each pregnancy [6-10]. A correct pre-pregnancy diagnosis of such mutations followed by LMW s/c heparin and oral jr aspirin therapy with the onset of the pregnancy may prevent thousands of serious pregnancy complications and stillbirths.

Abortions (Miscarriages): A very large number of fetal deaths also occurs in the early days and weeks, before the 22nd week of a pregnancy and these are known as spontaneous abortions or miscarriages. Vaginal loss of blood with or without pain are the usual clinical symptoms and signs of pending abortions. Today, the exact number of all abortions worldwide still remains unknown because no accurate relevant data exist anywhere in the world. Chromosomal abnormalities, ectopic pregnancies, placental abnormalities with insufficiency and hypo fibrinolysis with

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thrombophilia are responsible for most abortions which cause very significant distress and unhappiness in all affected women all over the world [11].

Table 1: Birth findings in all live births in Cyprus in the years 2016 and 2017.

2016					
Duration of Pregnancy (Weeks)	**Total Births**	**Treated in an Intensive care pediatric ward**		**Treated in an Ordinary pediatric ward**	
		Survival	Deaths	Survival	Deaths
Less than 37 weeks	1073	443	12	614	4
Over 37 weeks	8526	203	6	8313	4
Not stated	71	8	0	63	
Total Live Births	**9670**	654	18	8990	8
2017					
Less than 37 weeks	1068	443	5	618	2
Over 37 weeks	8360	182	1	8176	1
Not stated	14	1	0	13	
Total Live Births	**9442**	626	6	8807	3

Table 2: Stillbirths (foetal deaths over 22 weeks) in Cyprus in the years 2016 and 2017.

	2016	**2017**
No previous pregnancy	31 **(60%)**	24 **(46%)**
One previous pregnancy	15 (29%)	18 (34%)
Two previous pregnancies	5 (10%)	7 (13%)
Three previous pregnancies	1 (2%)	4 (7%)
Total stillbirths	**52**	**53**

Pregnancy: The great significance of genetic Hypo fibrinolysis and Thrombophilia mutations in pregnancy

Thrombophilia mutations include

1. Lack of Antithrombin III, protein C and protein S,
2. Thrombophilia mutations, such as Factor V Leiden, Factor VR2, Prothrombin G20210A, β-fibrinogen-455 G>A and Factor XIII V34L.
3. MTHFR mutations such as MTHFR C677T and A1298C either in a homozygous or a double heterozygous state.

Hypo fibrinolysis mutations include

1. Plasminogen Activator Inhibitor-one, 4G/4G, (PAI-1 4G/4G),
2. Angiotensin Converting Enzyme D/D, (ACE D/D).

It has been recognized recently that the synthesis of plasminogen activator inhibitor-one (PAI-1) is increased by angiotensin II and therefore mutation ACE D/D that produces angiotensin II increases even more the hypofibrinolytic action of PAI-1 4G/4G. The co-presence of these two mutations stops almost completely the lysis and removal of placental fibrin and thrombi, which is of critical significance in the early placental stabilization phase, leading to the development of severe placental ischemia and insufficiency, [6-8,10,12]. The discovery of Antithrombin III deficiency occurred some 56 years ago, in 1965, by Norwegian Prof Olav Engberg, [13-15]. This heterozygous mutation is rare, present in only 0.2% of the general population and

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found in 0.5-7.5% of all venous thromboses. The discovery of protein C deficiency followed in 1981, at the Scripps Research Institute in San Diego, USA, [16-18]. The heterozygous deficiency of protein C is also a rare event, present in only 0.2% of the general population and in 2.5-6% of all venous thromboses. Lack of protein S was first described in 1984, in Oklahoma, USA, with a frequency of 1.3-5% in patients with venous thromboses [19]. The discoveries of these three genetic anticoagulant and factors were certainly important but have not influenced significantly daily clinical medicine. It was in 1994, only 27 years ago, when the most significant thrombophilia Factor V Leiden was discovered by Prof Bernita, [2,3] that clinical medicine changed significantly. Factor V Leiden is indeed the most common inherited cause of venous thromboses, found in 3-15% of most populations and in 30-60% of all people with venous thromboses, DVT and pulmonary embolism. The function of normal Factor V is to respond to activated protein C, (aPC) so as to regulate and control the production of activated thrombin from prothrombin with normal clot production as a result. Mutated Factor V Leiden however is resistant to aPC, leading to continuous overproduction of activated thrombin that leads to deposition of fibrin, coagulation and thromboses in peripheral veins and also in coronary, cerebral, renal and most other arteries and veins. Pregnancy however is a very special and unique situation. In pregnancy, especially in the very early stages, thrombophilia and increased hypo fibrinolysis from the presence of hypofibrinolytic mutations PAI-1 4G/4G and ACE D/D, lead to a significantly increased presence of placental thrombi, leading to severe placental ischemia and resulting to many abortions, stillbirths and many serious pregnancy complications. Therefore, the co-existence of mutations PAI-1 4G/4G and ACE D/D invariably results in very bad pregnancy outcomes due to placental ischemia, [6,7,9,10,12,20,21]. Correct diagnosis of the presence of these two mutations and treatment with LMW s/c heparin and oral jr aspirin in all pregnancies with these two mutations is life saving for both mothers and fetuses. The presence of mutated Factor V Leiden that results in an increased production of activated thrombin and deposition of fibrin may also lead to uteroplacental microthrombus's, contributing to abortions, stillbirths and major pregnancy complications but much less frequently than in pregnancies with the hypofibrinolytic mutations PAI-1 4G/4G and ACE D/D. The rare combination however of PAI-1 4G/4G, ACE D/D and Factor V Leiden is very serious indeed and invariably leads to a failed pregnancy. The presence of thrombotic MTHFR mutations such as MTHFR C677T and A1298C either in a homozygous or a double heterozygous state together with other thrombophilia mutations such as prothrombin G20210A, β-fibrinogen 455G>A and Factor XIII V34L are also pathogenic [22] and also lead to serious pregnancy complications.

Results

Obstetric complications and mutation findings in all 26 studied pregnant ladies

Tables 3a+3b describe in detail all significant clinical and obstetric events and complications in all 26 studied pregnant ladies together with their genetic Thrombophilia and Hypofibrinolytic findings. Table 3a describes the 19 ladies that after their initial negative pregnancy experience and subsequent correct mutation studies, proceeded to one or two new successful pregnancies with LMW s/c heparin and oral jr aspirin therapy, all with excellent results, normal healthy babies and no complications. Table 3b describes the remaining 7 ladies that because of severe stress, did not wish to proceed with a new pregnancy. In the earlier years of our study, the commonest cause of referral was an episode of severe pre-eclamptic toxemia (PET), leading to a stillbirth or an early caesarean with a low birthweight premature baby. There were altogether 12 pregnant ladies with PET (Table 3a: patients 1-

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5,16,17 and Table 3b: 2-5,7). Two pregnancies (Table 3a, patients 2 and 4), unfortunately ended with dead embryos, stillbirths, at 27 and 24 weeks. Nine pregnancies ended with emergency caesareans and premature low birthweight babies (700, 900, 950, 1050+1080, 1200, 1250, 2050, 2080 and 2200g), that needed intensive nursing care for several weeks. Unfortunately, patient 2 in table 3b delivered a baby at 26-28 weeks with a weight of 950g that despite all efforts remains mentally retarded. These are very unhappy events for young women and their families during their first pregnancies and every effort should be made to prevent these complications. An unusual but excellent outcome occurred recently in 2019, in the 12th lady with PET at 20 weeks, (patient 16 in Table 3a), who was investigated promptly, while still pregnant. She had five mutations, ACE D/D, Het Factor VR2, Homo MTHFR 1298CC, Het β-fibrinogen-455G>A and Protein S deficiency. She was treated immediately with Tran date (labetalol) and LMW s/c heparin and jr aspirin. This treatment enabled the pregnancy to continue until the 32nd week, when a healthy baby was born with a weight of 2100g. This unique experience proves the great benefit of an early diagnosis of thrombophilia and reduced fibrinolysis so as to allow immediate LMW s/c heparin therapy with excellent results. Table 4 compares the birth weights in the 6 ladies with 2 stillbirths and 4 urgent caesareans after a serious PET, who after the correct diagnosis of reduced fibrinolysis ± thrombophilia was made, they proceeded to a second and third pregnancy with LMW s/c heparin and jr aspirin. The new birth weights were impressive, especially in the two ladies with stillbirths in their 1st pregnancy. These 2 ladies had 2 more pregnancies each, patient 2 at 3400g and 3000g and patient 4 at 2860g and 3030g. The weight changes in the remaining 4 were: (900/2860g, 2080/3000g, 700/2230g and 2050/2700g) All underlying mutations are described in detail in table 4. The remaining 14 ladies were referred to our center because of other early pregnancy problems. Nine patients, (Table 3a: 6-8,10,11-13,15,19), ended with one or more early abortions and were subsequently investigated for Thrombophilia / Reduced Fibrinolysis mutations. All had positive findings and correct interpretation of their genetic findings led to a new successful pregnancy with LMW s/c heparin and jr aspirin and healthy normal babies. Three other patients are very instructive because their initial pregnancies were saved. Patients 9 and 14 in Table 3a showed vaginal loss of blood at 12 and 13 weeks and immediate genetic studies while pregnant, showed reduced fibrinolysis and thrombophilia mutations. Patient 9 had the 3 mutations PAI-1 4G/4G, Factor VR2 and lack of Protein S. Patient 14 had 5 mutations, ACE D/D, Homo MTHFR 1298CC, het MTHFR C677T, het β-Fibrinogen-455G>A and het Factor XIII V34L. Instead of waiting for the pregnancies to abort, treatment with LMW s/c heparin and oral jr aspirin was started immediately, while pregnant. The vaginal loss of blood stopped and a normal pregnancy continued leading to two healthy, full term babies. The third patient, number 18 in Table 3a is similar but here the indication for urgent investigation and a correct diagnosis of thrombophilia followed by LMW s/c heparin with jr aspirin therapy, was the finding by ultrasonography of a severely reduced uteroplacental blood flow at 12 weeks. The immediate initiation of LMW s/c heparin led to a healthy baby at full term. The 13th lady, patient 6 in Table 3b is also very instructive. This lady had tried for 8 years to have a baby but without success, all efforts ending in abortions. When she finally came to our center, she turned out to have the worst possible genetic scenario with the presence of hypofibrinolytic PAI-1 4G/4G and ACE D/D mutations, Factor V Leiden and the Homozygous 1298CC mutation. This combination of very severe hypo fibrinolysis and severe thrombophilia mutations is unlikely to ever allow a pregnancy to succeed without the use of LMW s/c heparin and jr aspirin that she had never unfortunately tried. This lady is now aged 48 and a successful new pregnancy is very unlikely. It is very disappointing that a

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genetic Thrombophilia / Fibrinolysis genetic test was never carried out during these 8 years. Clearly the money spent during these 8 years far exceeds the E200-250 needed for such a genetic test at the first pregnancy! The 14th lady, patient 1 in table 3b, is also very instructive. Her first and only pregnancy in the year 1990 at age 20 was complicated by a then unexplained serious iliofemoral vein thrombosis and pulmonary embolism that needed an early delivery and an IVC umbrella. The correct diagnosis was made 21 years later in 2011! after she was referred to our unit for SLE. She then received a Thrombophilia / Fibrinolysis test that revealed hypofibrinolytic mutations PAI-1 4G/4G, ACE D/D, Double heterozygous MTHFR C677T/A1298C, Het β-Fibrinogen-455G>A and SLE. This combination of serious mutations easily explained all her medical problems. She is currently very well at age 50 with long term oral jr aspirin. In contrast to the unhappiness after their failed first pregnancies, you cannot imagine the enormous happiness of these 19 ladies and their families in table 3a that had normal pregnancies after the correct diagnosis of severe hypo fibrinolysis and thrombophilia was made and the appropriate treatment with LMW s/c heparin and jr aspirin given during their new pregnancies. None of these repeat pregnancies in these 19 ladies had the slightest problem. These mothers asked repeatedly the question why the Thrombophilia / Fibrinolysis test had not been carried out earlier, before their disastrous first pregnancies.

Statistical analysis of all 11 relevant mutations in these 26 ladies

Very important Table 5, describes in detail the incidence and statistical significance of all 11 relevant genetic mutations in the 26 studied pregnant ladies, compared to a normal control Cypriot population. Hypofibrinolytic mutation, PAI-1 4G/4G turned out to be the commonest mutation present with 11 positive patients and the highest statistical significance, (x^2 p=0.0052). Nine of these 11 PAI-1 4G/4G positive ladies were also ACE D/D positive, making fibrinolysis even poorer. Mutation Factor VR2 was the 2nd commonest mutation (x^2 p=0.017). Only 5 of these 26 ladies had the Factor V Leiden. (x^2 p =ns). These findings in our study are very important, indicating that reduced fibrinolysis is much more common and much more important than primary thrombophilia regarding the development of early uteroplacental ischemia responsible for PET, stillbirths and most abortions (Table 5). The strong combination of PAI-1 4G/4G together with ACE D/D in the 26 ladies with serious pregnancy complications strengthens the conclusion that in pregnancy, hypo fibrinolysis is the commonest and most important factor leading to these complications. These two mutations, PAI-1 4G/4G and ACE D/D should always be taken seriously and never ignored, especially during pregnancies.

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Table 3A: 19 pregnant ladies with recurrent pregnancy losses and severe pregnancy complications. After appropriate blood tests with findings of severe reduced fibrinolysis and thrombophilia, they had one or more normal pregnancies with LMW s/c heparin and Jr aspirin and healthy babies.

N0	AGE	CLINICAL EVENTS	REDUCED FIBRINOLYSIS/THROMBOPHILIA
1	33	1st pregnancy 2005. Severe PET at 31 weeks. Emergency Caesarean. Birth weight 900g. Blood tests showed severe thrombophilia and reduced fibrinolysis. A combination of very serious mutations. 2ηd pregnancy 2008 with aspirin and s/c heparin. Normal pregnancy with no complications. Birth weight 2860g.	1.Homozygous PAI-1 4G/4G 2.ACE D/D 3.Heterozygous Factor V Leiden 4.Homozygous MTHFR 1298CC
2	30	1st pregnancy 2007. Severe PET with death of the embryo at 27 weeks. Blood tests showed positive cardiolipins with APS, SLE and thrombophilia. 2ηd pregnancy 2008 with aspirin and s/c heparin. Normal pregnancy. Birth weight 3400g. 3rd pregnancy 2012 with aspirin and s/c heparin. No complications. Normal pregnancy. Birth weight 3000g. Long term Jr aspirin.	1.APS with positive anticardiolipin. 2.SLE 3.Double heterozygous for MTHFR C677T/A1298C
3	28	1st pregnancy 2008. PET with Caesarean at 36 weeks. Birth weight 2080g. Blood tests showed thrombophilia and fibrinolysis 2ηd pregnancy 2012 with aspirin and s/c heparin and no complications. Birth weight 3000g.	1.ACE D/D 2.Heterozygous F VR2 3. Double heterozygous for MTHFR C677T/A1298C
4	29	1st pregnancy 2011. Severe PET with death of the embryo at 23-24 weeks. Blood tests showed severe reduced fibrinolysis and thrombophilia. 2ηd pregnancy 2012 with Jr aspirin and s/c heparin. No complications. Normal Caesarean at 38 weeks. Birth weight 2860g. 3rd pregnancy 2015 with Jr aspirin and s/c heparin. Birth weight 3030g.	1. Homozygous PAI-1 4G/4G 2.ACE D/D 3 Double heterozygous MTHFR C677T/A1298C 4. Heterozygous β-fibrinogen-455G>A

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 IJGMGT: April-2021: Page No: 01-17

5	31	1st pregnancy 2011. Severe PET at 27 weeks. Caesarean. Birth weight 700g. Blood tests showed severe reduced fibrinolysis and thrombophilia. 2ηd pregnancy 2016 with Jr aspirin and s/c heparin. No complications. Birth weight 2230g.	1. Homozygous PAI-1 4G/4G 2.ACE D/D 3.Heterozygous MTHFR C677T 4.β-fibrinogen-455G>A
6	28	Two unsuccessful pregnancies with early abortions ~ 2011. Blood tests showed severe thrombophilia 3rd pregnancy 2019 with Jr aspirin and s/c heparin and no complications. Delivery at 39 weeks. Birth weight 3290g.	1.Homozygous Prothrombin Factor G20210A 2.Double Heterozygous MTHFR C677T/A1298C
7	31	1st pregnancy 2012. Mild PET at 38 weeks. Birth weight 3400g 2ηd pregnancy 2013 Abortion at 10 weeks. Blood tests showed severe reduced fibrinolysis and thrombophilia 3rd pregnancy 2016 with Jr aspirin and s/c heparin. Normal pregnancy and delivery with a caesarean at 38 weeks. Birth weight 3500g.	1. Homozygous PAI-1 4G/4G 2.ACE D/D 3. Heterozygous Prothrombin G20210A 4. Heterozygous Factor XIII V34L 5. Homozygous MTHFR 1298CC
8	33	1st normal pregnancy 2012. 2015+2016. Two recurrent abortions at 6+8 weeks. Blood tests 2016 showed thrombophilia. 2ηd pregnancy 2017 with Jr aspirin and s/c heparin. Normal pregnancy with delivery at 38 weeks. Birth weight 3200g	1.ACE D/D 2.Heterozygous F VR2 3.Heterozygous Factor XIII V34L 4.Heterozygous MTHFR C677T 5.Heterozygous β-Fibrinogen-455G>A
9	27	1st normal pregnancy 2013 with birth weight 2590g. 2nd pregnancy 2017 with vaginal loss of blood at 12 weeks. Urgent blood tests showed reduced fibrinolysis with thrombophilia. Treated urgently with s/c heparin and Jr aspirin. Pregnancy continued normally with normal delivery at 38 weeks. Birth weight 2840g.	1. Homozygous PAI-1 4G/4G 2. Heterozygous Factor VR2 3. lack of protein S

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 IJGMGT: April-2021: Page No: 01-17

10	32	1st pregnancy 2017. Vaginal blood loss + abortion at 8 weeks. Blood tests showed severe reduced fibrinolysis and thrombophilia. 2nd pregnancy 2018/2019 with s/c heparin and aspirin. Normal delivery at 40 weeks, July 2019. Birth weight 3200g.	1.Homozygous PAI-1 4G/4G 2. Heterozygous Factor VR2 3.Heterozygous β-Fibrinogen-455G>A 4.Heterozygous MTHFR A1298C
11	33	Failed artificial insemination 2017. Blood tests showed severe reduced fibrinolysis and thrombophilia. 2st pregnancy 2017/2018 with Jr aspirin and s/c heparin. Normal pregnancy with normal delivery at 38 weeks. Birth weight 3320g.	1.Homozygous PAI-1 4G/4G 2.ACE D/D 3.Heterozygous MTHFR 677TT 4.Homozygous Factor XIII V34L
12	29	1st pregnancy 12/2017 with abortion at 8 weeks. Blood tests showed severe thrombophilia due to several (5) mutations. 2nd pregnancy with Jr aspirin and s/c heparin. Normal delivery January 2019 with birth weight 3320g.	1.ACE D/D 2.Heterozygous Factor VR2 3.Heterozygous β-Fibrinogen-455G>A 4.Heterozygous Factor XIII V34L 5.Heterozygous MTHFR C677T
13	37	Two failed early pregnancies 2015, 2016. Blood tests 2017 showed severe thrombophilia. 3rd pregnancy with Jr aspirin and s/c heparin led to a normal pregnancy with delivery at 39 weeks, July 2018. Birth weight 3900g.	1.Double heterozygous MTHFR C677T/A1298C 2.Heterozygous β-Fibrinogen-455G>A
14	29	1st pregnancy 2018 with vaginal loss of blood at 13 weeks. Urgent blood tests showed thrombophilia. Immediate therapy with Jr aspirin and s/c heparin led to stabilization with a normal pregnancy and delivery at 40 weeks. Birth weight 2740g	1.ACE D/D 2.Homozygous MTHFR 1298CC 3.Heterozygous MTHFR C677T 4.Heterozygous β-Fibrinogen-455G>A 5. 5.Heterozygous Factor XIII V34L
15	33	1st pregnancy 2016 with abortion at 8 weeks. Blood tests showed SLE and mild thrombophilia.	1.Positive Lupus anticoagulant 2. Heterozygous MTHFR C677T 3. Heterozygous β-Fibrinogen-455G>A

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 IJGMGT: April-2021: Page No: 01-17

		2nd pregnancy 2019 with Jr aspirin and s/c heparin. Normal pregnancy. Caesarean at 37 weeks. Birth weight 3070g.	
16	44	1st pregnancy 2000. Twins with early caesarean due to PET. 2ηd pregnancy 2019 with PET at 20 weeks. Urgent blood tests showed 5 mutations with thrombophilia and hypo fibrinolysis. Treated immediately with Trindade, Jr aspirin and LMW s/c heparin. Improved up to 32 weeks and delivery with Caesarean. Birth weight 2100g	1.ACE D/D 2.Heterozygous Factor VR2 3. Homozygous MTHFR 1298CC 4. Heterozygous β-Fibrinogen-455G>A 5. Protein S deficiency
17	33	1st pregnancy 2016 with severe PET and Caesarean at 35 weeks. Birth weight 2050g. Blood tests showed severe thrombophilia. 2ηd pregnancy 2019 with Jr aspirin and S/C heparin. Delivery at 38 weeks. Birth weight 2700g.	1. Heterozygous Factor V Leiden 2. Homozygous MTHFR 677TT
18	30	1st pregnancy in 2018/2019. U.S. at 12 weeks showed reduced uteroplacental arterial flow. Urgent blood tests showed severe thrombophilia. Immediate treatment with S/C heparin and Jr aspirin stabilized the pregnancy. Delivery at 40 weeks with Caesarean 8/2019. Birth weight 3310g.	1. Heterozygous Factor VR2 2. Double Heterozygous MTHFR C677T/ A1298C
19	39	1st pregnancy 2017 Abortion at 12 weeks 2nd pregnancy 2018. Abortion at 12 weeks. Blood tests showed severe reduced fibrinolysis and thrombophilia. 3rd pregnancy 2019 with Jr aspirin and S/C heparin. Normal pregnancy and delivery at 38 weeks. Birth weight 2550g.	1.Homozygous PAI-1 4G/4G 2.ACE D/D 3.Double Heterozygous C677T/A1298C 4. Heterozygous β-Fibrinogen-455G>A

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 IJGMGT: April-2021: Page No: 01-17

Table 3B: 7 pregnant ladies with severe complications during their first pregnancy. Very distressed. No desire for a repeat pregnancy.

AGE	CLINICAL EVENTS	REDUCED FIBRINOLYSIS/ THROMBOPHILIA
20	1st pregnancy 1990. Two weeks post-delivery, left ilia femoral vein thrombosis with pulmonary embolism. IVC umbrella. 1993. Diagnosed with SLE. 2011 Blood tests showed severe reduced fibrinolysis and thrombophilia. 1993-2020. Now aged 50. Treaded for SLE. Long term Jr aspirin 75mg daily. Very well.	1. Homozygous PAI-1 4G/4G 2.ACE D/D 3.Double Heterozygous MTHFR C677T/A1298C 4.Heterozygous β-Fibrinogen-455G>A 5.SLE
27	1st pregnancy 2007. Severe PET 26-28 weeks. Caesarean. Birth weight 950g child. Mentally retarded. Blood tests showed severe thrombophilia. Long term treatment with Jr aspirin and Folic acid	1.Heterozygous Factor V Leiden 2.Homozygous MTHFR 1298CC 3.Heterozygous β-Fibrinogen-455G>A
28	1st pregnancy 2007. Severe PET with 4g proteinuria. Caesarean at 36 weeks. Birth weight 2200g. Blood tests showed thrombophilia. Long term therapy with folic acid.	1.ACE D/D 2.Double heterozygous MTHFR C677T/A1298C
27	1st pregnancy 2009. Twins. PET at 32 weeks. Caesarean with birth weights 1080g and 1050g. Blood tests showed severe thrombophilia and reduced fibrinolysis. Long term treatment with Folic acid and Jr aspirin.	1.ACE D/D 2.Heterozygous Factor V Leiden 3.Homozygous MTHFR 677TT
29	1st pregnancy 2014. PET with placental abruption and hemorrhage at 31 weeks. Birth weight 1250g. Blood tests showed severe thrombophilia.	1.Heterozygous prothrombin G20210A 2. Heterozygous Factor VR2

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 IJGMGT: April-2021: Page No: 01-17

		3.Double heterozygous MTHFR C677T/A1298C
38	2010-2019. Multiple pregnancy attempts, all ending will abortions. Blood tests 2015 showed severely reduced fibrinolysis and severe thrombophilia. The worst possible combination of mutations against a successful pregnancy. Unlikely to have a baby with these mutations. No pregnancy with s/c heparin and Jr aspirin ever attempted!!	1.Homozygous PAI-1 4G/4G 2.ACE D/D 3. Heterozygous Factor V Leiden 4.Homozygous MTHFR 1298CC
32	1st pregnancy 2017. Severe PET at 29 weeks. Caesarean with birth weight 1200g. Blood tests showed severely reduced fibrinolysis and severe thrombophilia. Long term Folic acid and Jr aspirin.	1.Homozygous PAI-1 4G/4G 2.ACE D/D 3. Homozygous MTHFR 677TT 4.Antithrombin III deficiency

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 **IJGMGT: April-2021: Page No: 01-17**

Table 4: Improved birth weights in 6 ladies with previous PET, after a pregnancy with LMW s/c heparin and Jr aspirin.

Table 3a patient	**Birth weight after PET**	**Birth weight at 2nd ± 3rd pregnancy LMW s/c heparin and Jr aspirin**	**Fibrinolysis/thrombophilia mutations**
1	900g	2860 g	1.Homozygous PAI-1 4G/4G 2.ACE D/D 3.Heterozygous Factor V Leiden 4.Homozygous MTHFR 1298CC
2	Stillbirth	3400+3000g	1.APS with positive anticardiolipin. 2.SLE 3.Double heterozygous for MTHFR C677T/A1298C
3	2080g	3000g	1.ACE D/D 2.Heterozygous VR2 3. Double heterozygous for MTHFR C677T/A1298C
4	Stillbirth	2860+3030g	1. Homozygous PAI-1 4G/4G 2.ACE D/D 3 Double heterozygous MTHFR C677T/A1298C 4. Heterozygous β-fibrinogen-455G>A
5	700g	2230g	1. Homozygous PAI-1 4G/4G 2.ACE D/D 3.Heterozygous MTHFR C677T 4.β-fibrinogen-455G>A
17	2050g	2700g	1.Homozygous Prothrombin Factor G20210A 2.Double Heterozygous MTHFR C677T/1298C

DOI: https://doi.org/10.36811/ijgmgt.2021.110006 IJGMGT: April-2021: Page No: 01-17

Table 5: The incidence and significance of all 11 relevant mutations in the 26 studied pregnant ladies.

No	MUTATION	No IN THE 26 PREGNANT LADIES AND %	% OF MUTATION IN 100 HEALTHY PEOPLE	X^2 TEST P VALUE =
1	Factor V Leiden	5……19%	7%	n.s
2	F VR2	8…….31%	10%	P=-0.017
3	β-fibrinogen – 455G>A	12……46%	31%	n.s
4	Factor XIII V34L	5…….19%	22%	n.s
5	Prothrombin (G20210A)	3…….12%	10%	n.s
6	MTHFR 677 (Het)	16……62%	45%	n.s
7	MTHFR 677 (Hom)	3…….12%	13%	n.s
8	MTHFR 1298 (Het)	11……..42%	52%	n.s
9	MTHFR 1298 (Hom)	6…….23%	15%	n.s
10	ACE D/D	16……62%	44%	n.s
11	PAI-1 4G/4G	11……42%	15%	P=0.0052

Discussion

For many years, nephrologists and gynecologists managed pregnant ladies with medical and obstetric complications only after these had developed. Effective preventive therapy did not exist until after the discovery of Factor V Leiden, prothrombin G20210A, PAI-1 4G/4G, ACE D/D and most other relevant mutations in the years 1990-2000, with the realization that uteroplacental thrombotic angiopathy from these inherited mutations could be responsible for most cases of PET and the 3-4 million of stillbirths and abortions annually worldwide [23]. In Cyprus, our Karaiskakeion Institute introduced an extensive diagnostic Fibrinolysis/Thrombophilia screen around 2003-2004 [24] and from then on, we tried to relate pregnancy complications to these genetic mutation results, following all relevant new published information [25,26]. At the beginning, we carried out these mutation analyses several weeks to a few months or one to two years after the termination of a complicated pregnancy. In several countries the official recommendation is unfortunately to delay these mutation tests until the occurrence of 2-3 failed pregnancies, which is very sad. More recently we try to carry out these mutational investigations immediately after the development of a relevant pregnancy complication, even during the pregnancy and if appropriately positive, treatment with LMW s/c heparin is started immediately, so far with excellent results. The greatest difficulty today is the correct interpretation of all mutational findings in different clinical settings and specifically in pregnancy. Until recently, a serious mistake made in pregnancy was the belief that the absence of Factor V Leiden in pregnancy was interpreted as absolute lack of thrombotic predisposition. This is not correct, for in pregnancy as proven by our study and by many other published studies, PAI-1 4G/4G especially when accompanied by ACE D/D almost always leads to severe uteroplacental ischemia that leads to abortions, stillbirths, PET

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and other serious pregnancy complications [6-10,12,20,25,26]. What is very important to recognize is that in early pregnancy, the maintenance of normal uteroplacental circulation with normal oxygenation depends mostly on the effective clearance of fibrin. Therefore, reduced fibrinolysis from mutation PAI-1 4G/4G, especially in the presence of mutation ACE D/D, turns out to be the most important factor in the causation of abortions, stillbirths, PET and obstetric complications. Our results confirm the great pathogenetic significance of PAI-1 4G/4G and ACE D/D and in pregnancy these two mutations are much more common and far more important than Factor V Leiden.

Conclusion

The excellent results during the last several years in all ladies with severe hypo fibrinolysis ± thrombophilia that tried a new pregnancy with prophylactic LMW s/c heparin and jr aspirin have helped to establish worldwide the importance of this treatment. There is really no excuse to allow a lady to have a disastrous pregnancy when a simple blood test can help her have a safe and healthy pregnancy with a healthy baby. Hypofrinolytic mutation PAI-1 4G/4G and ACE D/D are important mutations leading to abortions, stillbirths and PET and should always be taken seriously. Factor V Leiden, Factor VR2, prothrombin G20210A, β-fibrinogen 455G>A and MTHFR mutations may also lead to problematic pregnancies but much less frequently. For all these reasons it would appear inexcusable not to carry out such a genetic mutation blood test before a pregnancy and ideally these mutations should always be studied prior to every first pregnancy. LMW s/c heparin in pregnancies with such mutations saves lives.

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