Original Article

Genetic Architecture of Pregnancy Loss: Co-inheritance of Risk Factors in Bosnian Women

(co-inheritance / polymorphisms / SNPs / risk variants / pregnancy loss)

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Abstract. Pregnancy-related complications (PRC) represent a serious public health and healthcare challenge. In European countries, infertility among couples varies from 5 to 24 %. The cause of PRC may include autoimmune and metabolic factors, correctness of the karyotype and variants of selected genes. The impact magnitude of genetic variants in one of PRC, pregnancy loss (PL), is still unexplored. Therefore, in this study, raw data on 12 single-nucleotide polymorphisms (SNPs) that were published separately in 2017–2019 were re-examined. We analysed the co-inheritance of 12 SNPs: rs6025 *FV*, rs429358 and rs7412 *ApoE*, rs1799752 *ACE*, rs1799889 *PAI–1*, rs1799963 *PT*, rs1801133 *MTHFR*, rs9468 and rs1800547 *INV* 17q21.31, rs731236 and rs1544410 *VDR*, and rs10421768 *HAMP*. Each time, the same study group of 154 women with PL, mean age 33 (± 5.4) years, and 154 mothers without PL, mean age 31.4 (± 6.7) years, with at least one live-born child, a control group, was investigated. In Bosnian women, no relationship of the co-inheritance pattern of any of the studied variants with PL was confirmed: P was in the range 0.248–1.0. In conclusion, the role of co-inheritance of heterozygotes and homozygotes or homozygotes of selected genes in PL has not been fully confirmed.

Introduction

Infertility affects approximately 186 million people worldwide and 25 million in the EU. In European countries, it varies from 5 % to 24 % in reproductive-aged couples, and one of its causes is pregnancy loss (PL). However, even in developed countries, PLs are not systematically recorded, suggesting the numbers could be even larger. Moreover, approximately 50 % of PLs globally are diagnosed as idiopathic or remain unexplained (https://www.eshre.eu/-/media/sitecore-files/Publications/PolicyAuditonFertilityAnalysis9EUCountries-FINAL16032017.pdf [available: July 18, 2023]; Hong and Marren, 2018).

Various factors are listed as potential PL triggers: age, genetic abnormalities, hormonal regulation, endometrium and placental function, inflammatory response, and last but not least, changes in blood coagulation. Pregnancy is a physiological state, in which a state of continuous intravascular coagulation, mainly located in the placenta, occurs. On the one hand, local activation of the coagulation system in the placenta may contribute to the severe coagulation development. On the other hand, it protects against perinatal haemorrhage. It is suggested that pregnant women with hereditary thrombophilia (HT) may have a higher PL risk compared with pregnant women without HT (Liu et al., 2021; Wen et al., 2023). There are many genes enabling successful reproduction. Most single-nucleotide polymorphisms (SNPs) have a neutral function, but some may have regulatory or splice-site ones, can affect gene expression and lead to altered cell functions (Yan et al., 2021).

In 2017–2019, we published separately 12 single nucleotide polymorphisms (SNPs), which we had tried to link to PL. The variants we studied were linked to HT or

Received July 28, 2023. Accepted October 12, 2023.

This study was supported by the Pomeranian Medical University, Department of Studies in Antropogenetics and Biogerontology statutory funds, No. WNoZ-307/S/2023 (G.A.).

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Abbreviations: *ACE* − angiotensin I converting enzyme gene, *ApoE* − apolipoprotein E gene, *FV* − factor V gene, *HAMP* − hepcidin antimicrobial peptide gene, HT − hereditary thrombophilia, *INV* 17q21.31 − inversion at 17q21.31, *MTHFR* − methylenetetrahydrofolate reductase gene, *PAI1* − plasminogen activator inhibitor 1 gene, PL − pregnancy loss, PRC − pregnancy-related complications, *PT* − prothrombin gene, SNPs − single-nucleotide polymorphisms, *VDR* − vitamin D receptor gene.

previously were suggested as risk factors for PL or fertility, as follows: rs6025 *FV*, rs429358 and rs7412 *APOE*, rs1799752 *ACE*, rs1799889 *PAI-1*, rs1799963 *FII* (PT), rs1801133 *MTHFR*, rs9468 and rs1800547 *INV* 17q21.31, rs731236 and rs1544410 *VDR*, and rs10421768 *HAMP* (Mahmutbegovic et al., 2017a, b; Adler et al., 2018a, b, c, 2019).

Recently, several studies for PL and factors of HT, among other variants of *FV* (rs6025), *PAI-1* (rs1799889), *FII* (rs1799963) and *MTHFR* (rs1801133), have been published (Bigdeli et. al., 2018; Jusic et al., 2018; Peres et al., 2019). Unfortunately, these reports do not show co-inheritance of risk alleles or genotypes. Additionally, reports had differences and missing information that prevented their comparison in PL risk assessment.

In the past, several of the above-mentioned variants had been studied extensively, but only a few authors investigated the co-inheritance of hetero- and homo- or homozygotes (Brenner et al., 1999; Sarig et al., 2002; Sotiriadis et al., 2007; Mitic et al., 2010; Farahmand et al., 2016; Chatzidimitriou et al., 2017). Such results had not been previously analysed in the group of Bosnian women or published to date.

Therefore, the aim of this study was to reassess our raw data and evaluate the co-inheritance of hetero- and homo- or homozygotes in the above-mentioned polymorphisms in women with and without PL and to compare them to existing results for other populations.

Material and Methods

Material

Each of the variants was examined in a group of 154 women with PL, mean age 33 (± 5.4) years, and 154 mothers without PL, mean age 31.4 (± 6.7) years, with at least one live-born child. A detailed characteristic of the groups was published by Adler et al. (2018a).

Table 1 shows twelve variants of genes that were investigated in 2017–2019 and published separately.

Methods

Articles available in PubMed, Scopus, and Web of Science databases were analysed using time descriptors: 1999–2023 and key words: pregnancy loss, miscarriage, co-inheritance, combined polymorphisms. The co-inheritance had been defined as having one or two mutated alleles of *FV* (hetero- or homozygotes) and two polymorphic alleles of other genes (homozygotes). The smallest number of genotypes in a co-inheritance was two.

Co-carriers were divided into three groups: [1]heterozygotes of *FV* and homozygotes of other investigated genes, [2]homozygotes of investigated genes (without *FV* homozygotes), and [3]rare homozygotes of *FV* and homozygotes of other investigated genes.

Inclusion and exclusion criteria

Inclusion criteria: 1) women with one or more PLs were selected as the study group, and women without PLs and with at least one live-born child as the control group; 2) case-control studies on association between genes we have previously studied and PL; 3) publications in English, Bosnian, Serbian, Croatian or Russian.

Exclusion criteria: 1) reviews; 2) disproportion in the study and control group sizes; 3) unknown number of subjects or controls with co-inherited genotypes.

Statistical analysis

The statistical analysis was performed using the R CRAN computer software version 4.1.1 (2021-08-10) (R Core Team, 2021). Fisher's exact test was used for comparisons of haplotype distribution for each population, and for the same haplotypes in total, in woman with PL vs without PL. The null hypothesis H(0) assumed no significant differences in haplotype prevalence linked to the PL occurrence. $P < 0.05$ was considered the critical significance level required to reject H(0).

Table 1. Polymorphisms investigated by authors (2017–2019)

Gene	Reference sequence	Changes	Code	Biological effect	References
FV	rs6025	c.1691 G>A	GA/AA	thrombophilia	(Mahmutbegovic et al., 2017a)
PAI1	rs1799889	c. −675 4G/5G	4G4G	thrombophilia	(Mahmutbegovic et al., 2017b)
ACE	rs1799752	I/D	DD	thrombophilia	(Mahmutbegovic et al., 2017b)
PT	rs1799963	c.20210 G>A	AA	thrombophilia	(Mahmutbegovic et al., 2017a)
MTHFR	rs1801133	c.677 C>T	TT	thrombophilia	(Mahmutbegovic et al., 2017a)
ApoE	rs429358 and rs7412	c.388 T>C and c.526 C>T, resp.	E4E4	thrombophilia	(Adler et al., 2018a)
INV 17q21.31	rs9468 and rs1800547	T>C and A>G, resp.	H2H2	number of offspring	(Adler et al., 2019)
VDR	rs731236 and rs1544410	c.1056 T>C and c.1024 +283G>A, resp.	tt, bb	regulates embryo implantation	(Adler et al., 2018b)
HAMP	rs10421768	c. −582 A>G	GG	iron deficiency	(Adler et al., 2018c)

Results

In Bosnian women, in group[1] (heterozygotes of *FV* and homozygotes of other investigated genes), the most common patterns of co-inheritance were: GA-4G4G-DD, N = 2 and GA-4G4G, N = 2 in women with PL, and in women without PL, GA-DD, N = 2. Other patterns occurred individually in women with and without PL or in women with or without PL.

Then, in group[2] (homozygotes other investigated genes without *FV* homozygotes), the most common patterns of co-inheritance were: 4G4G-DD, N = 11 vs N = 7, and tt-bb, N = 8 vs N = 10, in women with and without PL, respectively, while the co-inheritance of 4G4G-tt-bb occurred in six women, both with and without PL. The patterns of co-inheritance: DD-TT, N = 3 vs N = 1, and 4G4G-DD-tt-bb, none vs N = 3, occurred in women with and without PL, respectively. Other patterns occurred in a smaller number of women with and/or without PL.

The general prevalence of co-inheritance in Bosnian women with and without PL was comparable: 30.5 % (N = 47) and 29.9 % (N = 46), respectively. Also, concerning co-inheritance patterns, there was no statistically significant difference in prevalence between the groups.

Furthermore, in women with PL in the 1st trimester of pregnancy, N = 7 were co-carriers of heterozygotes of *FV* and homozygotes of other investigated genes ([1]group), and N = 33 were co-carriers of homozygotes of the investigated genes without *FV* homozygotes. Among women with PL in the 2nd trimester of pregnancy, N = 1 was co-carrier of heterozygote of *FV* and homozygote of other investigated genes, and N = 11 were co-carriers of homozygotes of the investigated genes without *FV* homozygotes ([2]group), (P = 0.66).

Table 2 shows co-inheritance patterns in Bosnian women, by gene order and codes as presented in Table 1.

Both worldwide and in Europe, we found very few studies on co-inheritance. Moreover, in the published reports, the authors examined a lower number of gene polymorphisms than presented in our study. Therefore, their compared patterns of co-inheritance are less various compared to ours.

In two previously presented reports, authors examined co-inheritance in Greek women with and without PL. In the group examined by Chatzidimitriou et al. (2017), women aged 35.3 (±5.1) and 35.1 (4.5) years, respectively, homozygotes of the investigated genes without *FV* homozygotes (group[2]), co-inheritance of DD-TT occurred in N = 5 women with PL, and in women without PL co-inheritance, it was not found. The examined difference was not statistically significant (P = 0.161).

In another group of Greek women aged 32.2 (±4.7) and 32.2 (±5.3) years, respectively, and belonging to the group[1] according to our breakdown, N = 1 woman was a co-carrier of GA-TT pattern, while among women without PL, the co-inheritance was not found (Sotiriadis et al., 2007). These differences were not statistically significant (P = 1.000).

In Serbian women of similar age, 30.1 y.o. (range 20–42 y.o.) and 34.2 (range 23–55 y.o.), respectively, belonging to the group[3] (with rare homozygotes of *FV* and homozygotes of other investigated genes), the authors identified co-inheritance of the AA-TT pattern in N = 3 women with PL, while in women without PL, co-inheritance was not found (Mitic et al., 2010). These differences were also not statistically significant (P = 0.251).

Two reports were also published from outside Europe. In the first one, in Iranian women with PL, aged 30.4 (±5.1) years, in N = 7, co-inheritance of the GA-TT pattern occurred (group[1]), but in women without PL aged 29.9 (±4.1) years, co-inheritance was not found. The authors found a statistically significant difference in the prevalence of co-inheritance between women with and without PL (P = 0.006) (Farahmand et al., 2016). The second report, Brenner et al. (1999), examined Israeli women with and without PL, aged 30.1 (±5) years and 31.0 (±6) years, respectively, and stated co-inheritance of the GA-TT pattern (group[1]) in N = 5 vs N = 1, respectively. The difference of co-inheritance between the studied groups was statistically significant (P = 0.012).

Table 3 shows co-inheritance patterns in women from other countries, by gene order and codes as presented in Table 1.

Discussion

Successful pregnancy outcome depends on many endo- and exogenous factors, among other genetic, epigenetic, and environmental (Arias-Sosa et al., 2018). It is suggested that PLs are frequently linked to abnormalities at the maternal-foetal interface, but the exact mechanisms remain unexplained. Disturbances of placental vasculature may result in a number of complications, including PL. This condition may be induced by pregnancy itself, in which an acquired hypercoagulable state is physiologically observed.

Connections between coagulopathies and diseases of placentation are compelling, because 50 up to 65 % women with unexplained PL had diagnosed thrombophilia, which suggests that it may be a significant risk factor (Brenner et al., 1999; Sarig et al., 2002). The thrombus formation in placenta vessels reduces placental blood flow and oxygen supply to the developing foetus, leads to the death of the trophoblast cells, and may lead to PL (Vaiman, 2015; Chatzidimitriou et al., 2017).

Co-inheritance research of potential genetic risk factors (including thrombophilic) and their relationship with PL is a rarity. Our research is the first on such a scale in Bosnia, but it showed that co-inheritance in Bosnian women with history of PL was not significantly different when compared to women without PL. These results confirm some of the previous research conducted in European populations (Sotiriadis et al., 2007; Mitic et al., 2010; Chatzidimitriou et al., 2017).

Table 2. Distribution of co-inheritance in Bosnian women with and without PL

Population, N	Co-inheritance	With PL	Without PL	P value	OR	95% CI	References
		N = 154	N = 154				
Bosnians, 308	**Co-inheritance[1]**						This study
	GA-DD-tt-bb	1	0	1	∞	(0.026–∞)	
	GA-TT-tt-bb	1	0	1	∞	(0.026–∞)	
	GA-4G4G-DD	2	1	1	2.01	(0.10–119.45)	
	GA-4G4G-TT	0	1	1	0	(0–39)	
	GA-DD-GG	0	1	1	0	(0–39)	
	GA-tt-bb	1	1	1	1	(0.01–78.96)	
	GA-4G4G	2	0	0.498	∞	(0.19–∞)	
	GA-DD	0	2	0.498	0	(0–5.32)	
	GA-tt	1	0	1	∞	(0.03–∞)	
	GA-H2H2	0	1	1	0	(0–39)	
	GA-bb	0	1	1	0	(0–39)	
	GA-TT	0	1	1	0	(0–39)	
	Co-inheritance[2]						
	DD-TT-tt-bb	1	1	1	1	(0.013–78.960)	
	DD-tt-bb-GG	1	0	1	∞	(0.03–∞)	
	TT-E4E4-tt-bb	1	0	1	∞	(0.03–∞)	
	4G4G-DD-tt-bb	0	3	0.248	0	(0–2.41)	
	4G4G-TT-tt-bb	0	1	1	0	(0–39)	
	4G4G-H2H2-tt-bb	0	1	1	0	(0–39)	
	4G4G-DD-TT	1	0	1	∞	(0.03–∞)	
	4G4G-tt-bb	6	6	1	1	(0.26–3.83)	
	DD-TT-tt-bb	0	1	1	0	(0–39)	
	4G4G-DD-GG	0	1	1	0	(0–39)	
	DD-tt-bb	2	1	1	2.01	(0.10–119.45)	
	4G4G-DD	11	7	0.333	1.73	(0.59–5.44)	
	4G4G-TT	2	1	1	2.01	(0.10–119.45)	
	4G4G-E4E4	1	0	1	∞	(0.03–∞)	
	tt-bb-GG	1	0	1	∞	(0.03–∞)	
	DD-TT	3	1	0.623	3.03	(0.24–160.47)	
	DD-GG	1	2	1	0.500	(0.01–9.66)	
	tt-bb	8	10	0.809	0.79	(0.26–2.29)	
	4G4G-H2H2	0	1	1	0	(0–39)	
	4G4G-bb	0	1	1	0	(0–39)	
	4G4G-GG	0	1	1	0	(0–39)	

[1]heterozygotes of *FV* and homozygotes of other investigated genes; [2]homozygotes of investigated genes without *FV* homozygotes. OR – odds ratio; 95% CI – 95% confidence interval. All P values were calculated using the Fisher's test.

Two independent studies have been conducted in Greek women with and without PL. Relatively recently, Chatzidimitriou et al. (2012) tested 12 thrombophilic polymorphisms, including the following six: rs6025 *FV*, rs429358 and rs7412 *APOE*, rs1799752 *ACE*, rs1799889 *PAI-1*, rs1799963 *FII*, and rs1801133 *MTHFR*, identical to these investigated by us. Unfortunately, both groups of women with and without PL were very small, N = 48 and 27, respectively. For this reason, the authors did not find either E4E4 or TT homozygotes of *APOE* and *MTHFR*, respectively. Additionally, the yield of co-inheritance was too small for statistical analysis. One result that could be compared with the one obtained in our research was the DD-TT co-inheritance; in Greeks, it occurred in N = 5 women with PL. In our study, it was less frequent, 3 vs 1 in women with and without PL.

Table 3. Distribution of co-inheritance in women with and without PL from European and non-European countries

Population, N	Co-inheritance	With PL	Without PL	P value	OR	95% CI	References
Greeks, 75	**Co-inheritance**[2]	N = 48	N = 27	0.161	∞	(0.53–∞)	(Chatzidimitriou et al., 2017)
	DD-TT	5	0				
Greeks, 201	**Co-inheritance**[1]	N = 99	N = 102	1	∞	(0.03–∞)	(Sotiriadis et al., 2007)
	GA-TT	1	0				
Iranians, 680	**Co-inheritance**[1]	N = 330	N = 350	**0.006**	∞	(1.55–∞)	(Farahmand et al., 2015)
	GA-TT	7	0				
Israelis, 182	**Co-inheritance**[1]	N = 76	N = 106	**0.012**	∞	(1.32–∞)	(Brenner et al., 1999)
	GA-TT	5	0				
Serbs, 275	**Co-inheritance**[3]	N = 147	N = 128	0.251	∞	(0.36–∞)	(Mitic et al., 2010)
	AA-TT	3	0				

[1]heterozygotes of *FV* and homozygotes of other investigated genes; [2]homozygotes of investigated genes without *FV* homozygotes; [3]rare *FV* homozygotes and homozygotes of other investigated genes. OR – odds ratio; 95% CI – 95% confidence interval. All P values were calculated using the Fisher's test. Statistically significant P values (P < 0.05) are bolded.

However, similarly to our study, it was not statistically different in the group of Greek women with and without PL (P = 0.161).

In earlier studies, Storiadis et al. (2006) tested five thrombophilic polymorphisms in Greek women with and without PL, including three identical to these investigated by us, rs6025 *FV*, rs1799963 *FII*, and rs1801133 *MTHFR*. Although the research was conducted in larger groups than the previous one (order as above: N = 99 and 102, respectively), unfortunately, the authors found only one co-inheritance in women with PL, GA-TT (P = 1.0). In our study, there was one woman without PL with co-inheritance of GA-TT.

On the other hand, Mitic et al. (2010) provided data on Serbian women, investigating three polymorphisms, rs6025 *FV*, rs1799963 *FII*, and rs1801133 *MTHFR*, that were also investigated in Bosnian women. They reported the co-inheritance in a small percentage of women, 2.7 %, and in three women with PL, they found co-inheritance AA-TT, including rare *FV* homozygotes. However, in women without PL this co-inheritance was not found. The difference between the groups was statistically insignificant (P = 0.251). This was the only study to find AA homozygotes of *FV*.

Both Farahmand et al. (2016) and Brenner et al. (1999) investigated polymorphisms of three genes *FV*, *FII*, and *MTHFR* in Iranian and Israeli women, respectively. In Iranian women, both GA-TT and GA-AA-TT co-inheritance differed statistically significantly between the groups (P = 0.006). Furthermore, a similar result was obtained for GA-TT co-inheritance in Israeli women (P = 0.012).

In Bosnian women, the most common patterns of co-inheritance were: 4G4G-DD, N = 11 and 7, and tt-bb, N = 8 and 10, in women with and without PL, respectively, while the co-inheritance of 4G4G-tt-bb occurred in six women in both groups.

Unfortunately, the comparison of our results with those obtained by other authors is problematic. These authors did not investigate similar polymorphisms of genes, or the group was too small, and the results prevented them from finding homozygotes and examining co-inheritances. Additionally, not all co-inheritance patterns were shown. All these factors interfered with comparison of the results. It is noteworthy that among the published results, the most common genotypes were GA and TT.

Starting from 2006, the authors wonder which polymorphisms may be significant risk factors for PLs (Goodman et al., 2006; Padda et al., 2021). When planning subsequent studies, the authors should consider gathering sufficiently large groups and in order to account for the interactions, examine as many polymorphisms previously indicated as risk factors as possible. Furthermore, studies in different populations should be conducted, as it is difficult to conclude whether a specific pattern of co-inheritance may be a risk modifier in different populations and ethnic groups. The prevalence of variants of the studied genes in the general population is also significant.

It must be emphasized that a key factor for the occurrence of co-inheritance of variants is their occurrence frequency in the general population. In European populations, the highest summarized frequency of alleles rs6025A *FV*, rs1799963A *FII*, and rs1801133T *MTHFR* was observed as follows: over 40 % in Italy, Greece, and Bosnia and Herzegovina, and the lowest, below 30 % in the Czech Republic and Sweden (Adler et al., 2015). For this reason, we researched co-inheritance in the Bosnian population.

Conclusion

The involvement of co-inheritance has not been fully confirmed.

Conflict of interest

There is no conflict of interest.

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