Multiple Inherited Thrombophilic Gene Polymorphisms in Spontaneous Abortions in Turkish Population

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The aim of this study was to investigate the possible role of multiple inherited thrombophilic gene variations in women with unexplained spontaneous abortions. For this purpose, the Factor V Leiden (FVL) (rs6025), Prothrombin G20210A (rs1799963), MTHFR C677T (rs1801133), PAI-1 4G/5G (rs1799889), ACE I/D (rs1799752), eNOS E298D (rs1799893), and Apo E E2/E3/E4 (rs429358) polymorphisms were genotyped and correlated in spontaneously aborted fetal materials, their mothers and fertile women. Twenty three abortion materials, 22 women with ≥1 unexplained fetal loss, and 22 control subjects with at least two healthy term infants as a control group were studied. Target SNPs for each gene were analyzed by real time-PCR technique after genomic DNA isolation from maternal blood-EDTA, control group blood-EDTA and spontaneously aborted fetal tissues. Some cases had a single thrombophilic polymorphism, but the rest of the patients and fetal materials had combined thrombophilic polymorphisms. The PAI-1 4G/5G+4G/4G (P= 0.0017), 4G/4G (P= 0.0253), eNOS 894GT+894TT (P=0.0011) genotypes and T allele (P=0.0185), Apo E E2/E3/E4/E3/E2/E2/E4 (P<0.0001) genotypes, E2 (P<0.0001) and E4 (P<0.0001) alleles were higher in spontaneously aborted fetal materials when compared to their mothers and control group. The Factor V Leiden rs6025, Prothrombin G20210A, MTHFR C677T, ACE I/D genotypes were different for each group but not statistically significant due to relatively small size of the samples (P>0.05). Our results indicated that combined thrombophilic gene variations may be associated with increased risk for spontaneous abortions and results need to be confirmed by larger sample size.

Key words: Spontaneous abortion, thrombophilia, polymorphism, fetus

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Spontaneous abortion is defined as a clinically recognized pregnancy loss before the gestation week of twenty. The World Health Organization (WHO) defines it as expulsion or extraction of an embryo or fetus weighting 500 g or less (1). A normal pregnancy is dependent on sufficient placental circulation and fetal vasculature. Abnormalities of placental vasculature may cause several gestational complications like pregnancy loss, intrauterine fetal death, intrauterine growth restriction and preeclampsia (2, 3). The risk of pregnancy loss is enhanced by a variety of etiological factors, including chromosomal abnormalities, uterine abnormalities, endocrinological defects, infections and environmental factors. However, in up to 50% of cases the exact underlying pathophysiological mechanisms remain undetermined.

Recurrent pregnancy loss (RPL) is an important obstetric complication which is defined as two or more miscarriages of under 20 weeks of gestation (4). Thrombophilias are inherited or acquired conditions that predispose an individual to thromboembolism (5). Inherited thrombophilia has been known as a cause of RPL. Various studies in the recent years have examined the incidence of specific thrombophilic polymorphisms in women with RPL. Some studies have reported an association between thrombophilic gene mutations and RPL (6, 7), whereas others have shown the lack of any association (8, 9).

In this study, we aimed to compare the thrombophilic polymorphisms in Factor V Leiden (FVL) rs6025, Prothrombin G20210A (rs1799963), MTHFR C677T (rs1801133), PAI-1 4G/5G (rs1799889), ACE I/D (rs1799752), eNOS E298D (rs1799983), and Apo E E2/E3/E4 (rs429358) polymorphisms.

Total genomic DNA was isolated from either 10-15 mg of abortion material tissue by the QIAGEN QIAamp DNA Mini Kit or peripheral blood samples containing EDTA from the mothers and control cases by the QIAGEN QIAamp DNA Blood Mini Kit. The polymorphic alleles were amplified by real- time polymerase chain reaction (PCR) technique in Light Cycler 2.0 (Roche, Switzerland) using Light Mix kits (TIB MOBIOL, Germany). Briefly, Light Cycler, Roche Fast Start Master mix, master mix (water, PCR- grade, Mg\textsuperscript{2+}, stock solution, Primer mix and HybProbe mix) and DNA template were used for real-time
amplification. The multiple PCR consisted of a denaturation step of 10 min at 95 °C, followed by 45 cycles of 5 s at 95 °C, 10 s at 60 °C, and 15 s at 72 °C, and a melting step of 20 s at 95 °C, 20 s at 40 °C, a continuous mode at 85 °C, a cooling step of 30 s at 40 °C for all four regions. A software program (Light Cycler 2.0, Roche, Switzerland) was used for the detection of the mutated and normal genotype profiles of the target gene in the groups included in the study. The wild, heterozygous and homozygous profiles were analysed in channel 640 with melting curve analysis (Figure 1).

**Statistical analysis**

Statistical analysis has been done with medcalc statistical program. Chi-square test was used to analyze the differences between the abortion materials, mothers and fertile women as control group. Genotype percentages and allele frequencies were compared between aborted materials group-mothers group and aborted materials group-control group. Odds ratio (OR) and P-values were used to estimate the risk for the polymorphisms in the groups.

**Results**

Peripheral blood-EDTA samples from mothers and control cases (fertile women) and tissue samples from aborted materials were examined for genotyping in the current study. Mean age of the cases and the weeks of abortions are provided. The mean age of the mothers were 29.6 (21-40) years and the mean age of fertile women were 37.2 (30-46) years. The mean abortion week was 9.4 (5-18) weeks, twenty abortions were in first trimester, three abortions were in second trimester. There was no other diseases in the groups.

The fetus, mothers and control groups in this study were designed to determine the association between point mutations in the factor V Leiden (FVL), protrombin G20210A, methylenetetra-hydrofolate reductase (MTHFR) C677T, plasminogen activator inhibitor-1 (PAI-1) 4G/5G, Angiotensin-converting enzyme I/D (ACE I/D), endothelial nitric oxide synthase (eNOS) E298D, apolipoprotein E (Apo E) E2/E3/E4 and spontaneous abortions. By real time PCR technique, we evaluated these polymorphisms in the study groups (23 abortion materials, 22 mothers, 22 fertile women), and the results were compared between the groups. The estimate risk was examined by odds ratio.

10 aborted materials and 6 mother results for PAI 4G/5G, 3 mother results for eNOS E298D, 3 aborted materials and 2 mother results for Apo E polymorphisms, 2 mother results for ACE I/D polymorphism were missed because of unclear PCR results. Genotype frequencies and statistical results are presented in table 1. The statistical analysis was done in two different ways. All genotype and allele frequencies were compared between the groups. In statistical analysis, we used four different comparings:1. Percentages of wild genotype versus heterozygous mutant+homozygous mutant genotypes.2. Percentages of wild genotype +heterozygous mutant genotype versus homozygous mutant genotypes. 3. Percentages of wild
genotype versus homozygous mutant genotypes. 4. Allele frequencies.

For PAI-1 4G/5G polymorphism, 4G/5G+4G/4G genotypes percentages (P=0.0017) and 4G/4G genotype (p=0.0253) were higher in aborted materials comparing with their mothers (Table 1). 4G/4G genotype was also higher in aborted materials comparing with the control group (P=0.0028) (Table 2). For eNOS G894T polymorphism, GT+TT genotype percentages (P=0.0011)

### Tables 1. The genotypes and allelle frequency of target PAI-1 4G/5G, eNOS E298D gene polymorphisms in aborted materials and their mothers in the current limited results.

| Genes/SNPs      | Groups                  | Aborted Materials (n:13) | Mothers (n:16) | Statistical Analysis |
|-----------------|-------------------------|--------------------------|----------------|---------------------|
| PAI-1 4G/5G    |                         | n/%                      | n/%            |                     |
| 5G/5G          |                         | 4/30.8                   | 2/12.5         |                     |
| 5G/4G          |                         | 5/38.4                   | 9/56.2         |                     |
| 4G/4G          |                         | 4/30.8                   | 5/31.3         |                     |

#### Allele Frequency

| Allele Frequency | P value | Odds ratio       | CI (95 %) |
|------------------|---------|------------------|-----------|
| 5G               |         |                  |           |
| 4G               | <0.0017*| 3.2572           | 1.5580-6.8098 |

#### Allele Frequency

| Allele Frequency | G       | T       |
|------------------|---------|---------|
|                  | 39(0.848)| 7(0.152)|
|                  | 27(0.711)| 11(0.289)*|

*: Significant, *Pearson Chi-Square; AB: Aborted Materials; M:Mothers

### Tables 2. The genotypes and allelle frequency of target PAI-1 4G/5G, eNOS E298D gene polymorphisms in aborted materials and fertile couples (controls) in the presented results.

| Genes/ SNPs               | Groups                  | Aborted Materials (n:13) | Fertile Women (n:22) | Statistical Analysis |
|---------------------------|-------------------------|--------------------------|----------------------|---------------------|
| PAI-1 4G/5G              |                         | n/%                      | n/%                  |                     |
| 5G/5G                    |                         | 4/30.8                   | 9/41                 |                     |
| 5G/4G                    |                         | 5/38.4                   | 10/45.5              |                     |
| 4G/4G                    |                         | 4/30.8                   | 3/13.5               |                     |

#### Allele Frequency

| Allele Frequency | 5G       | 4G       |
|------------------|----------|----------|
|                  | 13(0.500)| 13(0.500)*|
|                  | 28(0.636)| 16(0.364)|

#### Allele Frequency

| Allele Frequency | G       | T       |
|------------------|---------|---------|
|                  | 39(0.848)| 35(0.795)|
|                  | 7(0.152)| 11(0.205)*|

*: Significant, *Pearson Chi-Square
Tables 3. Shows the genotypes and allele frequency of ApoE E2/E3/E4 polymorphisms in aborted materials and their mothers in the current limited results.

| Genes/SNPs   | Groups                | Aborted Materials (n:21) | Mothers (n:20) |
|--------------|-----------------------|--------------------------|----------------|
|              |                       | n/%                      | n/%            |
| ApoE E2/E3/E4|                       |                          |                |
| E3/E3        | 7/33.3                | 14/70                    |                |
| E3/E2        | 11/52.4               | 4/18                     |                |
| E3/E4        | 2/9.5                 | 1/5                      |                |
| E2/E4        | 1/4.8                 |                          |                |
| Allele Frequency |                   |                          |                |
| E3          | 27/0.643              | 34/0.850                 |                |
| E2          | 12/0.286*             | 5/0.125                  |                |
| E4          | 3/0.071*              | 1/0.025                  |                |

| Statistical Analysis | P value | Odds ratio | CI (95 %) |
|----------------------|---------|------------|-----------|
| E2                   | <0.0001 | 3.7188     | 2.3288-5.9384 |
| E4                   | <0.0001 | 5.8594     | 3.0172-11.3789 |

*: Significant, *Pearson Chi-Square

Tables 4. The genotypes and allele frequency of ApoE E2/E3/E4 polymorphisms in aborted materials and fertile couples in the presented results.

| Genes/SNPs   | Groups                | Aborted Materials (n:21) | Fertile Women (n:22) |
|--------------|-----------------------|--------------------------|-----------------------|
|              |                       | n/%                      | n/%                   |
| ApoE E2/E3/E4|                       |                          |                       |
| E3/E3        | 7/33.3                | 12/54.5                  |                       |
| E3/E2        | 11/52.4               | 4/18                     |                       |
| E3/E4        | 2/9.5                 | 3/23                     |                       |
| E2/E4        | 1/4.8                 | 1/4.5                    |                       |
| Allele Frequency |                   |                          |                       |
| E3          | 27/0.643              | 33/0.750                 |                       |
| E2          | 12/0.286*             | 5/0.114                  |                       |
| E4          | 3/0.071*              | 3/0.136                  |                       |

| Statistical Analysis | P value | Odds ratio | CI (95 %) |
|----------------------|---------|------------|-----------|
| E2                   | <0.0001 | 5.8594     | 3.0172-11.3789 |
| E4                   | <0.0001 | 5.8594     | 3.0172-11.3789 |

*: Significant, *Pearson Chi-Square

and T allele frequency (P= 0.0185) were higher in aborted materials comparing with their mothers (Table 1). For Apo E2/E3/E4 polymorphism, the sum of the polymorphisms except E3/E3 (P<0.0001, P=0.0019), E2 allele frequency (P<0.0001, P= 0.0041) and E4 allele frequency (P<0.0001 for both) were higher in aborted materials comparing with their mothers and with control group, respectively (Tables 3-4). FVL, Prothrombin G20210A, MTHFR C677T, ACE I/D polymorphisms were not significant when comparing aborted materials with both their mothers and control group (P> 0.005) (Table 5).

Three abortion materials had both MTHFR C677T and eNOS E298D mutations, five abortion materials had both MTHFR C677T and ACE I/D mutations, eight abortion materials had both PAI-1 4G/5G and ACE I/D mutations, three abortion materials had both eNOS E298D and Apo E mutations, eight abortion materials had both ACE I/D and eNOS E298D mutations.

Discussion

Thrombophilia is one of the coagulation disorders which predisposes individuals to develop thrombosis (10). It includes acquired thrombophilia such as antiphospholipid antibody syndrome, activated protein C resistance, deficiencies in antithrombin III (ATIII), proteins C, S, and heritable causes such as factor V Leiden, Prothrombin (Factor II) G20210A, MTHFR C677T, PAI-1 4G/5G, ACE I/D, eNOS E298D, Apo E
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Table 5. The statistically significance of the genotypes and allele frequency of target genes for FVL, prothrombin G20210A, MTHFR C677T, ACE I/D SNPs in aborted materials, their mothers and fertile couples in the presented results.

| Genes/SNPs | Aborted Materials (n:23) | Mothers (n:22) | Fertile Controls (n:22) | P value | OR  | CI(95%) |
|------------|--------------------------|----------------|-------------------------|---------|-----|---------|
| FVL        |                          |                |                         |         |     |         |
| GG         | 21/95                    | 21/95.5        | 22/100                  |         |     |         |
| GA         | 2/5                      | 1/4.5          | -                       |         |     |         |
| AA         | -                        | -              | -                       |         |     |         |
| Allele     | Frequency                |                |                         |         |     |         |
| G          | 44/0.960                 | 43/0.980       | 44/1.000                | 0.0989  | 11.5759 | 0.6315 - 212.2057 |
| A          | 2/0.040*                 | 1/0.040*       | 0/0.000                 |         |     |         |
| Prothrombin|                          |                |                         |         |     |         |
| G20210A    | 23/100                   | 22/100         | 22/100                  |         |     |         |
| GG         | -                        | -              | -                       |         |     |         |
| GA         | -                        | -              | -                       |         |     |         |
| AA         | -                        | -              | -                       |         |     |         |
| Allele     | Frequency                |                |                         |         |     |         |
| G          | 46/1.000                 | 44/1.000       | 44/1.000                | 1.0000  | 1.0000 | 0.0196 - 50.8937 |
| A          | 0/0.000                  | 0/0.000        | 0/0.000                 |         |     |         |
| MTHFR      |                          |                |                         |         |     |         |
| C677T      | 11/48                    | 10/45.5        | 11/50                   |         |     |         |
| CC         | 11/48                    | 11/50          | 8/36.4                  |         |     |         |
| CT         | 1/4                      | 1/4.5          | 3/13.6                  |         |     |         |
| TT         | 33/0.720                 | 31/0.700       | 30/0.680                |         |     |         |
| Allele     | Frequency                |                |                         |         |     |         |
| G          | 13/0.280                 | 13/0.300       | 14/0.320                | 0.7773  | 1.0833 | 0.6222 - 1.8863 |
| C          | 30/0.650*                | 25/0.620*      | 25/0.570                | 0.8634  | 1.0611 | 0.5403 - 2.0836 |
| T          | 5/21.8                   | 4/20           | 5/22.8                  |         |     |         |
| I          | 6/26                     | 7/35           | 9/40.9                  |         |     |         |
| D          | 12/52.2                  | 9/45           | 8/36.3                  |         |     |         |
| Allele     | Frequency                |                |                         |         |     |         |
| I          | 16/0.350                 | 15/0.380       | 19/0.430                |         |     |         |
| D          | 30/0.650*                | 25/0.620*      | 25/0.570                | 0.8634  | 1.0611 | 0.5403 - 2.0836 |

*: Significant; 95% CI for FVL; MTHFR C677T; and ACE I/D

E2/E3/E4. These thrombophilic factors may have an important role in influencing the pregnancy outcome. This is the first study to report the relationship between thrombophilic factors polymorphisms and pregnancy loss in spontaneously aborted fetal materials and their mothers.

The placenta has double blood supply, one maternal and one fetal. The maternal blood, originating from uterine spiral arteries, circulates in the intervillus space, comes into contact with the syncytiotrophoblast and drains back through decidual veins. The fetal blood supply within the placenta starts from the umbilical arteries. Blood circulates in the fetal villi and drains back via the umbilical vein. A hypercoagulable state within the fetal circulation could lead to fetal stem vessel thrombosis, placental infarction in the distribution

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of fetal vessels and a miscarriage. In a study, the presence of FVL mutation in fetuses has been reported to be associated with histologically proven placental infarction (11). In our study, FVL genotype was not significant in fetal carriage comparing with their mothers for spontaneous abortions. In the literature, many studies have been reported about the relationship of FVL and other trombophilic factors about usually recurrent pregnancy loss and women genotypes. Sottilotta et al. reported that FVL and the FII G20210A mutations were related with unexplained stillbirth, but not with recurrent pregnancy loss (12). In another study, FVL and MTHFR polymorphisms were found as not associated with recurrent pregnancy loss or pre-eclampsia (13).

Generally, studies belong to different countries and races, so that polymorphism results may differ. For example, a study from China reported that there was a significant association between MTHFR C677T and unexplained recurrent pregnancy loss (14). A study planned with couples, reported that MTHFR C677T polymorphism is a risk factor for RPL (15). Zetterberg et.al reported that combined MTHFR C677T and A1298C mutations were higher in spontaneously aborted embryos comparing with adult controls (16). We did not detect any association between MTHFR C677T polymorphism and spontaneous abortion in this study.

Differently, in a study fetal karyotypes were analyzed following miscarriages and 41.4% had chromosomal abnormalities. After analyzing their parents’ karyotypes they reported that there is a relationship between chromosome abnormalities and spontaneous abortion (17). In contrast usual studies, Udry et.al. investigated the relationship between paternal FVL and prothrombin G20210A genotypes and recurrent pregnancy loss. It was reported that paternal FVL mutation was associated with RPL (18). These results partly remind our study with including fetal and paternal genetic results to their studies. It is clear that it is important to include not only maternal results to pregnancy loss studies but also paternal and fetal results. A fetus takes the genes both maternal and paternal, so that a pregnancy loss can not be only related with women chromosome abnormalities or genotypes.

With an overview to this study, FVL, Prothrombin G20210A, MTHFR C677T, ACE I/D polymorphisms were not different in spontaneously aborted fetal materials and both mothers and control group. Only PAI-1 4G/5G, eNOS E298D and Apo E E2/E3/E4 polymorphisms had higher prevalence in fetal materials comparing with their mothers and fertile women. Studies about miscarriages usually researched recurrent pregnancy losses and women genotypes for thrombophilic factors. Subrt et. al. reported that PAI-1 (-675) 4G/4G homozygous genotype was associated with increased risk of RPL independently from the antiphospholipid antibodies (19). In a different study, it was reported that ACE I/D, PAI-1 4G/5G and NOS3 4a/4b were not associated with first trimester recurrent miscarriage (20). Agarwal et.al. reported that there was no association between Apo E gene polymorphisms and RPL (21). In our results Apo E2 and E4 alleles and genotypes with these alleles were significant comparing with E3 allele and E3/E3 genotype between fetus and mother groups. Fetal Apo E genotype is found as associated with spontaneous abortion.

In conclusion, spontaneous abortion risk is related with PAI-1 4G/5G, eNOS E298D and Apo E E2/E3/E4 polymorphisms for fetal genotypes. If fetal genotypes are effective comparing with mother genotypes, this means father genotypes are also effective for pregnancy losses. Our results indicated that combined thrombophilic gene mutations may be associated with increased risk for spontaneous abortions. There is a need for larger studies to explore the effect of thrombophilic factors in family members for pregnancy losses.

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Conflict of Interests

The authors declared no conflict of interests.

References

1. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:839-54.
2. Khankin EV, Royle C, Karumanchi SA. Placental vasculature in health and disease. Semin Thromb Hemost 2010;36:309-20.
3. Reynolds LP, Borowicz PP, Caton JS, et al. Developmental programming: the concept, large animal models, and the key role of uteroplacental vascular development. J Anim Sci 2010;88:E61-72.
4. Dong Y, Li LL, Wang RX, et al. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of chromosome abnormalities or polymorphisms. Genet Mol Res 2014;13:2849-56.
5. Rabinovich A, Cohen JM, Prandoni P, et al. Association between thrombophilia and the post-thrombotic syndrome: a systematic review and meta-analysis. J Thromb Haemost 2014;12:14-23.
6. Sarig G, Younis JS, Hoffman R, et al. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertil Steril 2002;77:342-7.
7. Brenner B, Sarig G, Weiner Z, et al. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost 1999;82:6-9.
8. Pickering W, Marriott K, Regan L. G20210A prothrombin gene mutation: prevalence in a recurrent miscarriage population. Clin Appl Thromb Hemost 2001;7:25-8.
9. Carp H, Salomon O, Seidman D, et al. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. Hum Reprod 2002;17:1633-7.
10. Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: a systematic review. British journal of haematology 2006;132:171-96.
11. Dizon-Townson DS, Meline L, Nelson LM, et al. Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction. Am J Obstet Gynecol 1997;177:402-5.
12. Sottolitta G, Oriana V, Latella C, et al. Genetic prothrombotic risk factors in women with unexplained pregnancy loss. Thromb Res 2006;117:681-4.
13. Disanayake VH, Sirisena ND, Weerasekera LY, et al. Candidate gene study of genetic thrombophilic polymorphisms in pre-eclampsia and recurrent pregnancy loss in Sinhalese women. J Obstet Gynaecol Res 2012;38:1168-76.
14. Cao Y, Xu J, Zhang Z, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. Gene 2013;514:105-11.
15. Govindaiah V, Naushad SM, Prabhakara K, et al. Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss. Clin Biochem 2009;42:380-6.
16. Zetterberg H, Regland B, Palmer M, et al. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. Eur J Hum Genet 2002;10:113-8.
17. Zhang S, Gao L, Liu Y, et al. Reproductive outcome and fetal karyotype of couples with recurrent miscarriages. Clin Exp Obstet Gynecol 2014;41:249-53.
18. Udry S, Aranda FM, Latino JO, et al. Paternal factor V Leiden and recurrent pregnancy loss: a new concept behind fetal genetics? J Thromb Haemost 2014;12:666-9.
19. Subrt I, Ulcova-Gallova Z, Cerna M, et al. Recurrent pregnancy loss, plasminogen activator inhibitor-1 (-675) 4G/5G polymorphism and antiphospholipid antibodies in Czech women. Am J Reprod Immunol 2013;70:54-8.
20. Al Sallout RJ, Sharif FA. Polymorphisms in NOS3, ACE and PAI-1 genes and risk of spontaneous recurrent miscarriage in the Gaza Strip. Med Princ Pract 2010;19:99-104.
21. Agarwal M, Parveen F, Faridi RM, et al. Recurrent pregnancy loss and apolipoprotein E gene polymorphisms: a case-control study from north India. Am J Reprod Immunol 2010;64:172-8.