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SARI TOIVONEN

Phenotypic Diversity and Genetic Epidemiology of Placental Abruption

Doctoral dissertation

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Department of Obstetrics and Gynaecology
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Chromosome and DNA laboratory of the Division of Diagnostic Services
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ABSTRACT

Placental abruption (PA, abruptio placentae) is defined as premature separation of the normally sited placenta. This obstetric emergency complicates 0.2–1.3 per cent of all deliveries and it accounts for up to one third of all cases of perinatal mortality. Moreover, PA brings about fetal hypoxia and prematurity and thus short- and long-term morbidity. Placental abruption also remains a significant cause of maternal morbidity via increased rates of Caesarean section, hypovolaemia and disseminated intravascular coagulopathy. Since the aetiology and pathogenesis of placental abruption are largely enigmatic, this adverse obstetric outcome is still unpredictable and thus unpreventable.

This study was designed to elucidate the pathophysiology, epidemiology, risk factors, outcomes, and genetic background of PA. The study population consisted of 170 women with PA and 22,905 healthy control women who gave birth at Kuopio University Hospital between 1989–2002. The overall incidence of the placental abruption was 0.74%. We found that cigarette smoking, pre-eclampsia, grand multiparity, velamentous umbilical cord insertion, prior fetal demise, advanced maternal age (> 35 years) and previous miscarriage were independent reproductive risk factors of PA. We also showed that the outcome of PA is still poor, as the perinatal mortality rate was 10% and 59.4% of the newborns were premature. The recurrence rate of PA was as high as 11.9%. The obstetric prognosis after PA was comparable to that of the general obstetric population if there was no recurrence of PA. We also assessed the risk of PA in first-degree relatives of index patients, and cases of placental abruption appeared to cluster only in families with recurrent placental abruption.

In studies of the polymorphism in two genes involved in placental haemodynamics and in detoxification processes, we found that the low activity haplotype of the microsomal epoxide hydrolase (EPHX) gene was protective against placental abruption. We found no association between Glu298Asp polymorphism in the gene for endothelial nitric oxide synthase (eNOS) and placental abruption in this Finnish population.

CONCLUSIONS: Despite investigation of risk factors, much remains unclear about the underlying disease mechanisms of PA and the condition is still unpredictable. The genetic component appears to be modest. Most cases of placental abruption seem to be sporadic, with no family history and no recurrence.

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To my children Oona, Elmo, Nelli, Luna and Leo

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Hamina, June 2009 *Sari Toivonen*

ABBREVIATIONS

ART	assisted reproduction technology/treatments
bp	base pair
cDNA	complementary DNA
cM	centiMorgan
CNS	central nervous system
CTG	cardiotocography
CVD	cardiovascular disease
DIC	disseminated intravascular coagulopathy
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
eNOS	endothelial nitric oxide synthase
EPHX	epoxide hydrolase
FVL	factor V Leiden mutation
H	Hegar gestational week
HELLP	haemolysis, elevated liver enzymes and low platelets
HLA	human leucocyte antigen
ICSI	intracytoplasmic sperm injection
IUGR	intrauterine growth restriction
IVF	*in vitro* fertilisation
LD	linkage disequilibrium
LOD	logarithm of the odds
MIAC	microbial invasion of the amniotic cavity
MoM	multiples of the median
MSAFP	maternal serum alpha-fetoprotein
NICU	neonatal intensive care unit
NK	natural killer leukocytes
NO	nitric oxide
NOS	nitric oxide synthase
OR	odds ratio
PA	placental abruption
PCR	polymerase chain reaction
PE	pre-eclampsia

PlGF	placental growth factor
PNM	perinatal mortality
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RR	relative risk
SD	standard deviation
SGA	small for gestational age
SIDS	sudden infant death syndrome
SNP	single nucleotide polymorphism
TNF-α	tumour necrosis factor-alpha
TYR	tyrosine
us	ultrasonography
VEGF	vascular endothelial growth factor

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I –V).

I Toivonen S, Heinonen S, Anttila M, Kosma V-M, Saarikoski S. Reproductive risk factors, Doppler findings and outcome of affected births in placental abruption – a population-based analysis. Am J Perinatology 2002;19:451-59.

II Toivonen S, Heinonen S, Anttila M, Kosma VM, Saarikoski S. Obstetric prognosis after placental abruption. Fetal Diagn Ther 2004;19:336-41.

III Toivonen S, Keski-Nisula L, Saarikoski S, Heinonen S. Risk of placental abruption in first-degree relatives of index patients. Clin Genet 2004;66:244-6.

IV Toivonen S, Romppanen E-L, Hiltunen M, Helisalmi S, Keski-Nisula L, Punnonen K, Heinonen S. Low activity haplotype of the microsomal epoxide hydrolase gene is protective against placental abruption. J Soc Gynecol Investig 2004;11:540-4.

V Toivonen S, Keski-Nisula L, Romppanen E-L, Helisalmi S, Punnonen K, Heinonen S. Endothelial nitric oxide synthase polymorphism is not associated with placental abruption in Finnish women. Fetal Diagn Ther 2005;20:508-11.

CONTENTS

1. INTRODUCTION

Placental abruption (PA) was first described in 1775 by E Rigby in "An Essay on the Uterine Haemmorrhage, which Precedes the Delivery of the Full Grown Foetus" *(: Illustrated with Cases)*. It is defined as the partial or complete premature separation of a normally sited placenta after twenty-two weeks of gestation, but prior to delivery of the infant. It is estimated to occur in 0.2 – 1.3% of all deliveries and it is one of the major causes of fetal and neonatal mortality, accounting for up to one third of cases of perinatal mortality (Kåregård and Gennser, 1986; Ylä-Outinen *et al.* 1987; Saftlas *et al.* 1991; Ananth and Wilcox, 2001; Petterson *et al.* 2002; Oyelese and Ananth, 2006; Black *et al.* 2008). Placental abruption is a major cause of third trimester bleeding and it is often an unpredictable obstetric emergency that may contribute to life-threatening hypovolaemic shock and disseminated intravascular coagulopathy (DIC), and further possible maternal or fetal death. Owing to its significant association with fetal hypoxia and prematurity, it may worsen neonatal outcome, increase the likelihood of admission to a neonatal intensive care unit (NICU) and lead to chronic neurodevelopmental complications and perinatal morbidity (Mikkola *et al.* 2006).

The aetiology of PA is multifactorial, and at present enigmatic, but defective early vascularisation during placentation (Dommisse and Tiltman, 1992), immunological dysfunction (Steinborn *et al.* 2004) and inflammatory processes (Ananth *et al.* 2006) may play significant roles in its development. Many maternal clinical factors during ongoing pregnancy such as pre-eclampsia (PE), hypertension, thrombophilia, polyhydramnion, intrauterine infection or preterm premature ruptured membranes are associated with an increased risk of PA. In addition, the risk seems to be increased in connection with higher maternal age and parity, multi-fetal pregnancies, specific fetal anomalies and maternal cigarette smoking. Similarly, cocaine abuse, trauma, leiomyomas and prior Caesarean section (Ananth *et al.* 1999a, 2001, 2005; Kramer *et al.* 1997; Yang *et al.* 2007) also appear to be associated with the development of PA.

Prior placental abruption remains the most significant risk factor as regards recurrent PA, suggesting a genetic background to this entity. However, so far only a few genetic studies have been carried out in association with PA and the results have been controversial (Zhang *et al.* 2007; Zdoukopoulos and Zintzaras 2008). The main purpose of the present work was to clarify clinical epidemiology and pathophysiology of placental abruption and to find susceptibility genes in the Finnish population. This new information may prove to be important in targeting therapeutic and/or preventive strategies in regard to placental abruption.

2. REVIEW OF THE LITERATURE

2.1. Normal and abnormal placentation

The placenta is the specific organ of metabolic interchange between the mother and fetus. It is mainly of embryonic origin, derived from the outermost embryonic membrane (chorion frondosum) and the maternal part is formed from the uterine mucosa (decidua basalis). The capillaries carrying blood of embryonic circulation end up in the chorionic villi which lie in the intervillous space and are exposed to the maternal blood circulation. Maternal and fetal blood does not mix directly, but the intervening tissue (the placental membrane) forms an intervascular barrier that is thin enough to permit the absorption of oxygen and nutritive materials. It also allows some harmful substances, such as drugs and viruses, to enter the fetal blood, but it also releases carbon dioxide and nitrogenous waste from the fetus. The placenta also has vital endocrine functions during pregnancy. At term, a normal placenta is disc-shaped, about 4 cm thick and 14 cm in diameter. The fetal surface of the placenta is smooth, being formed of the adherent amnion, with the umbilical cord normally attached near its centre. The maternal surface of a detached placenta looks rough and lobular because of the torn decidual tissue adhering to the chorion (Pugh, 2000).The weight of the placenta averages from $1/5^{th}$ to $1/7^{th}$ of the fetal weight, depending on gestational age (Heinonen *et al.* 2001a).

Placentation begins 6 days after fertilisation, when the blastocyst becomes implanted in the endometrium and is embedded in the endometrial stroma. The area around the embryoblast then differentiates into two layers: outer syncytiotrophoblasts and inner cytotrophoblasts. The placental trophoblast cells (cytotrophoblasts) invade the maternal decidua and adjacent spiral arteries (terminal branches of uterine arteries), thus establishing the uteroplacental circulation (Sadler, 2006). Cytotrophoblasts partly occlude (or"plug") the arterial lumina and thus limit the flow of maternal blood into the narrow intracellular space for the first nine weeks of gestation. A low oxygen environment, where the placenta and embryo develop, has been proposed to protect newly forming organs and differentiating cells against free oxygen radicals. Hypoxia seems to stimulate trophoblast differentiation, proliferation and angiogenesis (Mayhew, 2001; Burton and Jauniaux, 2004; Red-Horse *et al.* 2004). The spiral arteries recanalize and the placental oxygen concentration rises threefold during the 10^{th} and 12^{th} gestational weeks, increasing oxidative stress within the placenta when the "plugs" dissolve (Burton and Jauniaux, 2004). Normal placentation involves vascular remodelling from high-resistance uteroplacental spiral arteries into low-resistance blood

vessels to provide a high-flow circuit for perfusion of the intervillous space. Progressively invading trophoblast replaces part of the maternal endothelium and media in the arteries and part of the smooth muscle is replaced by fibrous tissue, and the walls of the spiral arteries loose their arterial structure and the luminal diameter enlargesThese changes associated with normal implantation are complete by the 23rd gestational week . These vascular changes extend from the intervillous space into the decidua and to the inner third of the myometrium. (Burton and Jauniaux, 2004). Fetal well-being is also critically dependent on regulated growth and activity of placental villous arborisations. Sprouting and maturation of the villi continue throughout gestation. Later in gestation trophoblast proliferation is mainly connected with renewal and repair processes, so that the border between mother and fetus will be thinner and more efficient in diffusive transport (Mayhew, 2001).

At term, uterine contractions normally cause separation of the placenta during the third part of labour in four consecutive phases: latent (placental site wall remains thin while placenta-free wall is thick), contraction (thickening of placenta-site wall), detachment (actual separation of the placenta from the adjacent uterine wall), and expulsion (sliding of the placenta out of the uterine cavity). This process begins mostly from the lower pole of the placenta and proceeds sequentially upwards in the uterine cavity (Herman *et al.* 1993; Herman *et al.* 2002).

2.1.1. Placental abruption

Placental abruption refers to premature separation of the normally implanted placenta from the underlying maternal uterine surface prior to the delivery of the infant (Page *et al.* 1954, Oyelese and Ananth, 2006). The separation occurs when uterine vessels bleed into the decidua basalis near its interface with the placental cytotrophoblastic shelf and anchoring villi. The resulting retroplacental haemorrhage usually tracks between the membranes and the uterus and then escapes through the cervix and is visible as vaginal bleeding (Figure 1).

Occasionally, a blood clot may be retained between the detached placenta and uterus, leading to a concealed haemorrhage (Figure 1). This usually occurs when the placental margins remain adherent, despite of the haemorrhage behind the centre of the placenta. This may form the classic Couvelaire uterus when bleeding penetrates into the myometrium towards the serosa. During a marginal placental separation the fetal membranes may also retain their attachment to the uterine wall and the blood may then pass through the membranes to the amniotic cavity. Sometimes the fetal head hinders the descent of the haematoma, thus preventing the blood from flowing through the cervix. Abruption may produce either retroplacental, preplacental or subchorionic haemorrhage

(Oyelese and Ananth, 2006). Concealed abruptions seem to have a poorer outcome compared with abruptions with antepartum haemorrhage, probably as a result of later detection; hence a larger placental separation area and more fetal distress (Chang *et al.* 2001).

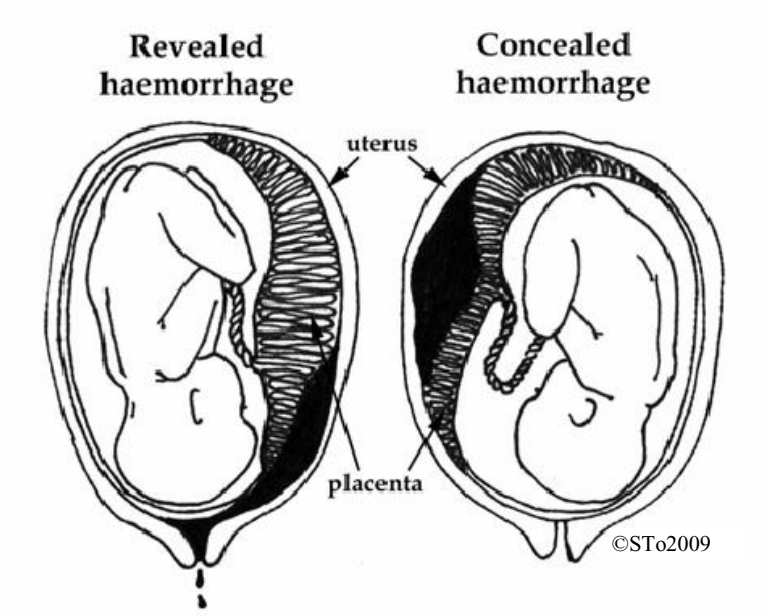

Figure 1. Revealed and concealed abruption.

Separation may be total or partial depending upon the degree of separation of the placental-decidual interface. In addition, the severity of PA depends on the site of separation, since PAs that are retroplacental or situated near the umbilical cord insertion seem to be more serious than those situated in marginal areas of the placenta (Nyberg *et al.* 1987a). Sometimes small, marginal and/or self-limited PAs may remain undetected and these are usually of no clinical significance. It is likely that the placental position (anterior or posterior wall) does not influence the severity of PA (Nagayama and Suzuki, 2006). The hypoxia producing mechanism of PA may be direct as a result of separation of the placenta from the maternal blood supply and thus from oxygen exchange, but also because of increased frequency and sometimes duration of contractions, prolonging the interruption of oxygen delivery to the intervillous space (Matsuda *et al.* 2005). In some studies placental abruption has been graded in severity classes according to the affected surface area in relation to the placental surface area, and some researchers have added signs of the fetal distress to the grading system (Kayani *et al.* 2003). The classical system of severity grading by Page, King and Merrill dates back to 1954, and is based on clinically recognizable maternal symptoms (Table 1).

Table 1. Severity grading of placental abruption.

Grade	**Clinically recognizable symptoms**
0	Clinically unrecognized before delivery (diagnosis based upon examination of the placenta)
1	Vaginal bleeding only, or mild uterine tetany, but no evidence of maternal shock
2	Uterine tetany, ordinarily with uterine tenderness, possibly vaginal bleeding, fetal distress (or death), but no evidence of maternal shock
3	Evidence of maternal shock or coagulation defect, uterine tetany, intrauterine death of fetus

2.1.2. Diagnosis of placental abruption

The diagnosis of PA is primarily based on findings in clinical examination, but it may be supported by sonographic, laboratory and histopathological findings (Table 2).

Table 2. Diagnosis of placental abruption.

Authors, year, country	***n* cases/ (*n* controls)/ deliveries**	**Clinical diagnosis**	**Us**	**Lab**	**Histo pathology**	**Study design**
Eriksen *et al.* 1991, Denmark	87/ 5,727	+	-	-	-	Re
Rasmussen *et al.* 1996a, Norway*	9,592/ 1,446,154	+	na	na	na	Re
Kramer *et al.* 1997, Canada	415/ 36,875	+	na	na	+	Re
Kyrklund-Blomberg *et al.* 2001, Sweden	4,003/ 795,459	+	na	na	na	Re
Salihu *et al.* 2005, USA	single 93,968/ 15,051,872 twins 5,051/ 413,619 triplets 353/ 22,585	+	+	na	na	Re
Lindqvist and Happach, 2006, Sweden	161/ (2,371)/24,207	+	-	-	-	Re
Tikkanen *et al.* 2006a, Finland	198/(396)/ 46,742	+	+	-	+/-	Pr
Ananth *et al.* 2006b, USA	179,204/ 30,378,902	+	**+/-**	na	na	Re

Us, ultrasonographic detection; Lab, laboratory findings; na, not applicable; Pr and Re, prospective and retrospective study; *gestational age >16weeks.

2.1.2.1. Clinical examination

Clinical findings may vary greatly but at least two of the following classical clinical criteria are usually required for diagnosis: 1) vaginal bleeding after 22 completed gestational weeks, 2) uterine tenderness or abdominal pain, 3) tetanic uterine contractions or increased baseline uterine tone which is usually monitored externally, 4) adherent retroplacental blood clot and/or depression or disruption of underlying placental tissue after vaginal birth or in Caesarean section, or signs of Couvelaire uterus found in Caesarean section. Moreover, fetal distress determined as non-reassuring fetal heart rate (FHR) patterns (bradycardia or recurrent late decelerations or decreased beat-to-beat variability or sinusoidal heart rate pattern) in cardiotocographic monitoring, decreased fetal movements, fetal acidosis or fetal death and bloody amniotic fluid, maternal hypotonia, shock or disseminated intravascular coagulopathy (DIC) may suggest a diagnosis of PA. However, even these first four hallmarks of PA are not always present. For example, Tikkanen and associates found that neither bleeding and nor pain was present in 19% of cases of placental abruptions (Tikkanen *et al.* 2006b). Some small and marginal placental abruptions may remain undetected, but they are usually of no clinical significance *per se*.

2.1.2.2. Ultrasonography

Acute PA is difficult to differentiate from thickened placenta in ultrasonographic examination, since acute bleeding is hyperechoic to isoechoic compared with the placenta. Resolving haematomas are easier to detect because they become hypoechoic within one week and sonolucent within two weeks after bleeding (Jaffe *et al.* 1981; Nyberg *et al.* 1987b; Merz, 1991). There is some evidence that the use of colour Doppler ultrasonography might help in differentation of isoechoic placental tissues with blood flow, and haematomas (Kikutani *et al.* 2003). Sonography is not considered sensitive enough (24%) for the detection of PA, but positive findings were associated with more severe cases of PA in a study by Glantz and Purnell (2002). With liberal use of ultrasonography, retroplacental haematoma has been detected in 15% of PA cases (Tikkanen *et al.* 2006b). So far there is no evidence available that 3D or 4D techniques give any extra advantage in revealing acute placental abruptions.

2.1.2.3. Laboratory diagnosis

Laboratory tests have not proven to be clinically useful in the diagnosis PA, although they have been evaluated in many clinical settings. The Kleihauer-Betke test for detecting fetal haemoglobin does not indicate PA in pregnant trauma patients (Dhanraj *et al.* 2004) and should not be used in the diagnosis of PA (Emery *et al.* 1995), since negative test result does not rule out PA. It is also of limited value in predicting the outcome of pregnant patients at risk of fetomaternal haemorrhage, but is of clinical importance when considering the need of Rh-isoimmunisation prevention (Dupre *et al.* 1993).

Assay of plasma fibrin D-dimer, which is a degradation product of cross-linked fibrin normally circulating at less than 0.5 mg/L, has been suggested to be a good laboratory test in the diagnosis of thrombosis and of DIC, and it has also been evaluated in association with PA. Neiger and associates found that assay of plasma fibrin D-dimer was not useful in the diagnosis of partial PA (1997), but Nolan and associates (1993) concluded that evaluation of D-dimer latex agglutination slide tests would be rapid and useful as regards improving the early diagnosis of placental abruption.

Williams *et al.* (1993) found that maternal serum CA-125 levels appear not to be useful in the diagnosis of abruptio placentae. Thrombomodulin (a vascular endothelial cell receptor for thrombin) has been suggested as an alternative analyte in the diagnosis of acute placental abruption in the setting of trauma or unexplained vaginal bleeding (Magriples *et al.* 1999). A plasma fibrinogen level below 15 mg/L and thrombocytopenia are highly suggestive of coagulopathy and are possible markers of severe PA (Matsuda *et al.* 2005). An elevated white blood cell count at admission (>20 000/mm^3) in pregnant trauma patients may also be an indicator of ongoing PA (Shah *et al.* 2002).

2.1.2.4. Histopathological diagnosis

Acute PA may not generally leave any gross evidence in the placental tissue such as adherent blood clots or indentation of the placental parenchyma. However, a depression in the maternal surface of the placenta and adherent strands of fibrin may be visible at the site of haematoma formation. There may be histological evidence showing intense congestion and dilatation of the fetal vessels in the affected area. Chorionic villous haemorrhage or defective physiological changes of the spiral arteries of the intramyometrial segments may also be present (Dommisse and Tiltman, 1992; Keeling, 2002). Prolonged placental abruption often leads to retroplacental haematoma and

its histological appearance is age-related. Pathologists assess the histological evidence of PA according to the presence of a retroplacental clot and villous compression or villous infarcts with decidual destruction. Moreover, subchorial thrombosis of maternal origin may be detected in the placenta (Keeling, 2002). Placental abruption diagnosed solely on the basis of histopathological examination has been reported to be more common than clinically diagnosed cases and found in up to 3.8% of consecutively examined placentas, although most of the findings were of no clinical value (Faye-Petersen *et al.* 2006).

2.1.2.5. Differential diagnostics

Bleeding from a low-set placenta is also an obstetric emergency that may end with hypovolaemic shock and stillbirth. Usually it is less painful than PA and it is possible to predict in most cases by ultrasonography during the second trimester. Second and third trimester pain and bleeding may also be caused by labour and on rare occasions by uterine rupture, which is usually characterized by the absence of uterine contractions and hypertonus. Vasa praevia and cervico-vaginal neoplasms may also produce painless vaginal bleeding. In addition, there are several other disorders producing pain without bleeding to be considered in differential diagnostics, such as diseases in the adnexal area, the gastro-intestinal tract, or urinary tract problems (Cunningham *et al*.2001; Konje and Taylor, 2006).

2.1.3. Incidence

Placental abruptions complicate 0.2–1.3% of all deliveries, i.e. 1 in 75 to 225 births (Huisjes *et al.* 1979; Kåregård and Gennser, 1986; Ylä-Outinen *et al.* 1987; Saftlas *et al.* 1991; Rasmussen *et al.* 1996a; Barron and Hill, 1998; Tikkanen *et al.* 2006a). Abruption that leads to a stillbirth occurs in 0.12% of deliveries, i.e. one in 830 births (Ananth and Wilcox, 2001) and every 5th stillbirth is caused by PA (Petterson et al. 2002). The incidence of PA seems to have an increasing trend (Ananth *et al.* 2005a), which may partly be explained by better detection by means of ultrasonography and other methods, but the true increase in the number of PA cases is also possibly due to the increased Caesarean section rates (Hemminki and Meriläinen, 1996; Getahun *et al.* 2006; Yang *et al.* 2007) and the increasing number of illicit drug abusers (of cocaine and crack) (Little *et al.* 1999). Advanced maternal age, assisted reproduction treatment (ART) and increased smoking among fertile-aged women may also contribute to this trend. The incidence of PA is highest at 24–

26 weeks of gestation, being up to 8.6% and then dropping precipitously with advancing gestation to 0.3% untill term (Rasmussen *et al.* 1996a; Sheiner *et al.* 2002 and 2003; Oyelese and Ananth 2006).

Table 3. Incidence of placental abruption.

Study, year, country	**Cases/ deliveries**	**Incidence %**	**Criteria includes also**
Kåregård and Gennser 1986, Sweden*	3,959/ 894,619	0.44	103 twin pairs, congenital anomalies
Eriksen *et al.* 1991, Denmark	87/ 5,727	0.5	anomalous fetuses (8.0%/3.2%)
Rasmussen *et al.* 1996a, Norway	9,592/ 1,446,154	0.66	traumas
Cnattigius *et al.* 1997, Sweden	5,712/ 1,075,711	0.53	
Kramer *et al.* 1997, Canada#	415/ 36,875	1.1	multiple births (2.1%)
Kyrklund-Blomberg *et al.* 2001, Sweden	4,003/ 795,459	0.51	anomalous fetuses (0.51%/0.50%) (congenital heart anomalies: 1.75/ 0.49)
Salihu *et al.* 2005, USA	93,968/ 15,051,872 twins 5,051/ 413,619 triplets 353/ 22,585	0.62 twins 1.22 triplets 1.56	twins and triplets
Lindqvist and Happach, 2006, Sweden	161 / 24,207	0.67	
Tikkanen *et al.* 2006a,b; Finland	198 / 46,742	0.42	twins placenta previa cases
Ananth *et al.* 2006b, USA	179,204/ 30,378,902	0.6	

na, not applicable; *28 completed gestational weeks; # 20 completed gestational weeks

Usually pregnancies with congenital fetal anomalies are excluded from such studies because of the increased risks of miscarriages, stillbirths and adverse pregnancy outcomes in these pregnancies (Linhart *et al.* 2000).

2.1.4. Pathophysiology

The primary aetiology of PA is unknown and its pathogenesis is still poorly understood. Usually abruption begins when uterine spiral arteries bleed into the decidua basalis near its interface with the placental cytotrophoblastic shelf and anchoring villi. These ruptured vessels may be either congenitally defective as a result of impaired placentation or damaged later during pregnancy, as in the case of intrauterine hypoxia or uteroplacental underperfusion (Kramer *et al.* 1997; Ananth *et al.*

2006a). Early placental damage is evidenced by second trimester feto-maternal alpha-feto-protein (AFP) leakage (Ryynänen, 1981; Van Rijn *et al.* 1999; Tikkanen *et al.* 2007) as well as low first trimester pregnancy-associated plasma protein A (PAPP-A) levels and elevated concentrations of β-human chorion gonadotropin (hCG) (Chandra *et al.* 2003, Dugoff *et al.* 2004).

The ensuing haemorrhage causes a split in the decidua and subsequent haematoma formation. The haematoma expands and causes further uterine vascular disruption and the resulting obliteration of the intervillous space leads to further ischaemia and destruction of the adjacent placental tissue. The immediate triggering event leading to abruption is thought to be either acute vasospasm or thrombosis in possibly abnormally remodelled spiral arteries. These changes cause ischaemic necrosis and then haemorrhage, possibly subsequent to reperfusion (Redline, 2006; Oyelese and Ananth, 2006). In rare occasions, such as sometimes in traumas, the haemorrhage originates from the feto-placental vessels (Pearlman, 1990). On addition, fetuses may also be involved in the pathophysiology of PA, as shown by the fact that the numbers of fetal anomalies is doubled in cases of PA (Raymond and Mills, 1993).

2.1.4.1. Vascular disease

Abnormal endovascular trophoblast invasion and thus impaired spiral artery remodelling may be connected to early oxidative stress (Schwarzler *et al.* 1999) and abnormalities in circulating angiogenic factors (such as deficiency of placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) and excess of anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng)) that have been associated with the development of uteroplacental diseases, such as pre-eclampsia, intrauterine growth restriction (IUGR) and placental abruption (Signore *et al.* 2006). However, prediction of PA has not been possible in second trimester by using assay of pro-and antiangiogenic factors, such as PlGF, sFlt-1 and sEng (Signore *et al.* 2006; Tikkanen *et al.* 2007).

In the deficient implantation process the vessels remain superficial and maintain their muscular wall structure and so remain sensitive to vasomotor stimuli (Redman and Sargent, 2000 and 2005; Khong, 2004). This may in turn predispose these high resistance spiral arteries to endothelial injuries leading to ruptures that manifest as placental abruptions (Dommisse and Tiltman, 1992; Signore *et al.* 2006). Dommisse and Tiltman (1992) demonstrated inadequate trophoblast invasion in placental bed biopsies after abruption. Further, Khong (2004) and Oyelese and Ananth (2006) have suggested that impaired vascular development leads to utero-placental insufficiency, which could be a common aetiological determinant of PE, IUGR and PA. Evidence thus far suggests that

low placental blood flow and inflammatory activation of the endothelium may be prerequisites as regards these abnormalities exerting their deleterious effects (Redline, 2006).

Microthrombal obliteration of the spiral arteries due to thrombophilias, causing villous infarcts, may also play a role in placental vascular lesions on the maternal side. When the ensuing ischaemic destruction of the endothelium is followed by reperfusion, the adjacent decidua ruptures and haemorrhage manifests as clinical pre-delivery detachment of the placenta (Redline, 2006).

2.1.4.2. Immunological rejection

An excessive anti-fetal immune response is suspected to play a significant role in the pathogenesis of PA (Steinborn *et al.* 2004). In normal pregnancy the humoral immune response is up-regulated and the cell-mediated immune response is down-regulated, which protects the pregnancy, where the fetus represents a semi-allograft. Fetal villous cytotrophoblasts and syncytiotrophoblasts normally express human leukocyte antigens (HLA) -G and -E that can block the cytotoxicity of maternal natural killer (NK) cells and thus facilitate the invasion of trophoblast cells into the maternal tissues during early gestation. The levels of soluble HLA-G have been shown to be strongly decreased in the pregnancies with PA (Steinborn *et al.* 2003). In women with PA the balance is changed more towards increased cell-mediated immunity with activated T helper-1 (TH1) lymphocytes and NK cells, leading to defective trophoblast invasion in spiral arteries as well as remarkably decreased soluble HLA-DR levels. This is considered to mirror the exaggerated fetal allograft rejection (Steinborn et al. 2003).

In addition, the increased maternal antibody production against paternal antigens leads to the elevated interleukin-6 (IL-6) release by fetal monocytes, which may be assessed by the presence of anti-HLA-antibodies in the maternal circulation. Significantly increased amounts of anti-HLA antibodies have been detected in the circulation of women with placental abruption. This indicates the existence of an increased humoral immune response of the mother against the semi-allogenic fetus and this is suggested to have a decisive role in the pathogenesis of placental abruption (Steinborn *et al.* 2004).

Maternal immunological responses against male-specific minor histocompatibility (HY-) antigens may play a role in recurrent placental abruption (Nielsen *et al.* 2007). Ghidini and Salafia (2005) found more chronic inflammation lesions at the implantation site in the placentas of extremely premature male infants compared with female infants, and the authors considered this male fetal gender association suggestive of a maternal immune response against the invading interstitial trophoblast.

2.1.4.3. Inflammatory processes

Chorioamnionitis is significantly associated with PA in preterm (OR 3.6, 95% CI 1.7–10.5) and term pregnancies (OR 2.8, 95% CI 1.3–6.1) (Nath *et al.* 2007). Both infections and other inflammatory processes leading to tissue damage are mediated by cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) (Peltier 2003). These cytokines upregulate the production of the matrix metalloproteinases (MMPs), which may cause destruction of the extracellular matrices of trophoblastic tissue and also destruction of the cell-cell interactions securing the placenta, thus resulting in premature detachment of the placenta. On the other hand, thrombin-enhanced interleukin-8 (IL-8) may explain decidual neutrophil infiltration, which in turn may activate MMPs to contribute to the extracellular matrix degradation involved in fetal membrane rupture and thus abruption-associated premature rupture of the fetal membranes (PROM) (Rosen *et al.* 2002; Lockwood *et al.* 2005; Nath *et al.* 2007).

Nakatsuka and associates (1999) suggested that nitric oxide (NO) and its metabolites generated by apoptosis and hypoxia may serve as mediators in a causal pathway leading to cell death and subsequent placental abruption in chorioamnionitis. Kiss *et al.* (1998) speculated that acute hypoxia or inflammation would impair endothelial nitric oxide synthase (eNOS) or NO production by the trophoblast. This may contribute further to platelet adhesion, aggregation and thrombus formation as well as altered placental vascular resistance, finally resulting in PA. Both ascending and haematogenic infections have been associated with the origin of microbial invasion of the amniotic cavity (MIAC) (Han *et al*. 2006). However, Aaltonen and associates (2005) showed that proinflammatory cytokines do not cross normal term placenta and they concluded that the inflammatory response in amniotic fluid is of fetal origin.

Harris *et al.* (1985) examined prematurely delivered placentas without overt PA and found that more than half of them showed significant antepartum peripheral haemorrhage, possibly partly of venous origin, and they speculated that associated decidual necrosis might activate a prostaglandin cascade that could lead to premature labour. Thus the cause-and-effect relationships of inflammatory lesions of placenta and PA still remain unclear.

2.1.4.4. Trauma and other mechanical causes of PA

In some high energy **trauma** cases, such as motor vehicle accidents, falls or assoults a single precipitating event is likely to begin the shearing of the placenta from the uterine wall. Some sort of trauma affects about 6–7% of all pregnancies (Connolly *et al.* 1997) and women hospitalized as a result of trauma have 9.22 higher (95% CI 7.79–10.91) PA rates than controls varying between 1,6% and 8%. The prevalence of PA has been as high as 24% among women who delivered when hospitalized as a result of trauma (El Kady *et al.* 2004; Shiff *et al.* 2002).

Placental abruption in **multiple pregnancies** is considered to have a different pathological mechanism compared with abruption in singleton pregnancies, since the intrapartal risks are mainly related to rapid volume changes of the uterus after the birth of the first infant (Ananth *et al.* 2001; Oyelese and Ananth 2006).

The vast majority of patients with PA have **multifactorial** risks and the PA is considered to be an outcome of a long chain of events in a **chronic pathological process** at the fetal-placental interface, with vaginal bleeding early in pregnancy, placental lesions and possibly small-for-gestational age (SGA) infants (Salafia *et al.* 1995; Ananth *et al.* 2006a). Altogether, the findings indicate immunological causes and thrombophilias and thus a particular genetic background of PA.

2.1.5. Clinical risk factors of PA

Clinical risk factors can roughly be divided into sociodemographic, behavioural, obstetric and fetal groups. Most of studies have not been focused on all known risk factors and therefore comparisons are difficult. Large epidemiological studies based on national statistics data or birth registers have not had all details recorded and thus are not available for comparison. Data on domestic violence, for example, is not included in any of these studies. Table 4 shows clinical risk factors in these studies. Tha recurrence rates of PA and thrombophilias are discussed later (section 2.1.8 and Table 10).

Table 4. Clinical risk factors of placental abruption.

Study	Kramer *et al.* 1997, Canada **	Kyrklund-Blomberg *et al.* 2001, Sweden	Linqvist and Happach 2006, Sweden	Tikkanen *et al.* 2006a,b; Finland
n PA /(matched pairs)/ *N* total (incidence of PA)	415/ 36,875 (1.1%)	4,003/ 795,459 (0.5%)	175/ 24,207 (0.7%)	198/ (396)/ 46,742 (0.42%)
Age ≤19 *vs.* 20-35y	0.89 (0.48-1.66)	aOR 0.8 (0.6-1.0)	na	na
Age >35 *vs*. 20-35y	1.50 (1.14-2.01)	aOR 1.3 (1.1-1.4)	2.6 (1.6-4.1)	1.7 (1.1-2.6)
Primipara	ns	aOR 1.1 (1.0-1.2)	ns	
Parity ≥3	ns	aOR 1.3 (1.1-1.5)	ns	2.6 (1.4-4.7)
Parity ≥4	ns	aOR1.6 (1.4-2.0)	na	na
Single *vs*. married	1.50 (1.13-1.98)	aOR 1.2 (1.01-1.3)	na	1.4 (1.0-2.0)
Education <12 y [10-11 y] {≥9 y}	ns	 [aOR 1.0 (0.9-1.1)] {aOR 1.2 (1.1-1.3)}	na	na
Lower occupational level	na	na	na	1.5 (1.0-2.1)
Smoking [1-9 cig/d] {≥10 cig/d}	 [1.04 (0.65-1.65)] {1.51 (1.07-2.11)}	 [aOR 1.9 (1.8-2.1)] {aOR 2.2 (2.0-2.5)}	2.3 (1.5-3.4)	2.1 (1.4-3.3)
Alcohol abuse	ns	na	na	11.0%/(4.6%) 2.6 (1.3-5.0)
Uterine malformations	na	na	na	9.4 (2.0-44)
Previous Caesarean section	na	na	na	1.9 (1.2-3.0)
Previous spontaneous abortion	na	na	1.4 (0.9-2.2)1st trim 3.2 (1.4-7.1)2nd trim	1.6 (1.1-2.4)
Previous stilbirth	na	na	13.1 (5.1-34.0)	ns
Repeated fetal loss	na	na	3.4 (1.3-8.9)	na
Family history of venous thromboembolism	na	na	3.4 (2.1-5.6)	na
History of PA	na	na	25.8 (9.8-68.3)	7.0 (1.9-26)
ART [infertility 1-2 y] {infertility ≥3 y}	na	 [aOR 1.2 (1.0-1.4)] {1.5 (1.2-1.8)}	na	subfertility ns
Male fetal sex	1.32 (1.07-1.62)	ns	1.3 (0.9-1.9)	
Multifetal gestation	ns	na	2.9 (1.1-7.7)	4.5%/(2.0%) ns
PPROM	2.44 (1.60-3.37)	5.9 (5.0-6.9)	na	ns
Pre-eclampsia [mild] {severe}	2.32 (1.58-3.42)	 [2.2 (1.9-2.7)] {5.6 (4.7-6.6)}	3.4 (1.4-8.2)	2.7 (1.3-5.4)
Essential hypertension	1.59 (1.02-2.50)	1.9 (1.2-3.2)	na	ns
PIH	1.59 (1.02-2.50)	1.5 (1.1-2.0)	na	ns
Chorioamnionitis	2.61 (1.65-4.12)	na	na	3.3 (1.1-10.2)
DM1	na	2.7 (1.9-3.7)	na	ns
GDM	na	0.8 (0.5-1.2)	na	ns
IUGR/ SGA [mild] {severe}	 [1.28 (0.89-1.82)] {3.99 (2.75-5.77)}	na	3.2 (1.8-5.5)	ns

ORs/aORs (adjusted odds ratios) are shown with 95% confidence intervals; *singleton pregnancies only; na, not applicable; ns, not statistically significant (p>0.05); **completed 20 gestational weeks; ART, assisted reproductive treatments; PPROM, preterm premature rupture of membranes; PIH, pregnancy-induced hypertension; DM1, Type 1 diabetes mellitus; GDM, gestational diabetes mellitus; IUGR, intrauterine growth restriction; SGA, small for gestational age.

2.1.5.1. Sosiodemografic risk factors

Older mothers have higher risks of perinatal morbidity and mortality. Women over 40 years of **age** have been shown to have a higher tendency to experience PA (OR 1.8, 95% CI 0.99–3.34) compared with younger women of 20–24 (Joseph *et al.* 2005), which is in concert with the results of Kyrklund-Blomberg *et al.* (2001) (aOR 1.3, 95% CI 1.1–1.4) and Linqvist and Happach (2006) (OR 2.6, 95% CI 1.6–4.1). However, Ananth and associates (1996) have stated that the frequency of PA at advanced ages seems to be more dependent on parity than the age of the mother *per se*. Again, very young women seem to have an increased incidence of PA in some studies (Kåregård and Gennser, 1986; Ananth *et al.* 2005).

There seems to be **race** disparity as regards the risk of PA, since in studies carried out in the USA black women have been found to have a higher incidence of PA than women of other races (Ananth *et al.* 2005). Rathore and McMahon (2001) also assessed racial variation in the incidence of PA in a large epidemiological study (n=807 759) and showed that black women had the highest rate of PA (black 0.97% *vs*. white 0.68% *vs*. other race 0.56%, p= 0.001). On the other hand, in the same study they found that compared with white women, black women had an OR of 0.76 (95% CI 0.70–0.82) for PA after controlling for factors such as maternal age, tobacco and cocaine use and perinatal care level.

Marriage seems to be slightly protective against PA in some studies, since being unmarried has been a risk factor of PA (OR 1.4–1.50) compared with being married (Kramer *et al.* 1997; Tikkanen *et al.* 2006a).

A high maternal **body mass index** (BMI) does not alone seem to be of statistical significance as regards PA (Kramer *et al.* 1997; Tikkanen *et al.* 2006a; Salihu *et al.* 2009). However, in an Asian population a low pre-pregnancy BMI seemed to have a slight impact on PA (aOR 1.3, 95% CI 1.0–1.6) compared with women with normal BMI (Hung *et al.* 2007).

2.1.5.2. Behavioural (i.e. reversible) risk factors

Cigarette smoking has been strongly associated with PA in several studies, with ORs of 1.4 to 2.5 compared with non-smokers (Raymond and Mills, 1993; Cnattingius, 1997; Ananth *et al.* 1999b; Tikkanen *et al.* 2006a; Høgberg *et al.* 2007). There also seems to be a dose-dependent risk of PA up to a threshold of ten cigarettes a day (Ananth *et al.* 1999b; Kyrklund-Blomberg *et al.* 2001). Accordingly, Cnattingius (1997) found smokers to have a higher risk of PA (OR 2.0, 95% CI

1.9–2.1) when smoking 1– 9 cigarettes a day compared with non-smokers, and when smoking more than 10 cigarettes a day the OR was assessed to be as high as 2.5 (95% CI 2.3–2.7). Interestingly, Tikkanen and associates (2006a) found that paternal smoking correlated even more strongly with the increased risk of PA than maternal smoking alone, and when both parents smoked there was further multiplication of the risk. Studies involving the use of biochemical markers such as urinary cotinine have demonstrated that women consistently underreport their smoking behaviour (Bardy *et al.*1993). Of Finnish women, 14.5% have smoked during the pregnancy and 10.4% continued smoking after the first trimester in 2007 (Stakes 2008).

The following mechanisms have been suggested for the increased PA risk in cigarette smokers: nicotine has vasoconstrictive effects in the placenta and in the fetal circulation, and increased carboxyhaemoglobin concentrations hinder oxygenation, causing ischaemia and infarcts that may lead to capillary fragility and further haemorrhage (Kaminsky *et al.* 2007). Hyperhomocysteinaemia seems to result in endothelial cell injury (Ray and Laskin, 1999; Eskes 2001) and this, combined with the vasoconstrictive effects of nicotine, may partly explain why smokers have an increased risk of PAs. Moreover, according to Raatikainen and associates (2007a, 2007b), smoking, and especially continuation of smoking during pregnancy, tends to accumulate with other behavioural pregnancy risk factors, such as continuation of alcohol consumption and under-attendance at antenatal care centres, which in turn seems to contribute to an increased risk of PA. The (calculated) effect of discontinuation of smoking might reduce placental abruptions by as much as 7–25% (Ananth *et al.* 1999b; Høgberg *et al.* 2007).

Several researchers have found that **alcohol** consumption increases the risk of PA (Kaminski *et al.* 1976; Halmesmäki 1988) with an OR of up to 2.6 (95% CI 1.3–5.0) compared with women with no alcohol abuse (Tikkanen *et al.* 2006a). This is not consistent with the results of some former studies (Raymond and Mills, 1993; Kramer and Usher, 1997), but the amount and mode of alcohol use may have resulted in the discrepancies between different studies. The real amount of alcohol consumption during pregnancy may be underestimated when the assessment relies on the patients' self-reporting. Any alcohol consumption may also increase the exposure of women to traumas and assaults during pregnancy, since in one third of cases of intimate partner violence, alcohol use is involved (Lipsky *et al.* 2005, Coputo *et al.* 2007).

Alcohol crosses the placenta easily and concentrates in the amniotic fluid and in the fetus. Neurotoxic and teratogenic effects in the fetus may be mediated directly through toxicity of alcohol, but also indirectly through alcohol-induced alterations in the feto-maternal hormone balance, which can disturb fetal and placental growth (Gabriel *et al.* 1998; Burd *et al.* 2007). The placentas of alcohol users more often show signs of villous infarction and intervillous thrombosis, which may

enhance fragility in vessels and together with alcohol-promoted vasoconstriction predispose pregnant women to placental abruption (Burd *et al.* 2007).

According to a large meta-analysis, **cocaine abuse** increases the risk of PA with a RR of 4.55 (95% CI 3.19–6.50) compared with drug-free pregnant women (Addis *et al.* 2001). This is consistent with the results of an earlier meta-analysis that showed a higher risk of PA with an OR of 3.92 (95% CI 2.77–5.46) compared with non-users (Hulse et al. 1997). Cocaine is a central nervous system stimulant that causes hypertension by increasing the level of norepinephrine in the blood circulation, leading to peripheral vasoconstriction in arterial beds. This vasoconstriction exposes the placenta and also the fetus to hypoxia, and may lead to ischaemic damage in both, enhancing the risk of PA (Plessinger and Woods, 1998; Little *et al.* 1999). Mooney and associates (1998) found more chorionic villus haemorrhage and oedema even in the absence of clinical placental abruption in the placentas of women using cocaine. Differences in the responses to cocaine have been observed within a single rat strain, which could be explained by genetic factors (Church and Subramanian, 1997). It is reasonable to speculate that there may also be genetic variations in susceptibility to the damaging effects of cocaine among pregnant women (Plessenger and Woods, 1998).

M**ethamphetamine abuse** also seems to increase the risk of placental abruption, and the mechanisms are speculated to be similar to those of cocaine (Stewart and Meeker, 1997; Kuczkowsky, 2007; Winslow *et al.* 2007). Drug abuse is an enormous problem among pregnant women in many Western countries, since up to 10% of pregnant women have been suggested to be exposed to cocaine in some urban areas in the USA and Canada (Little *et al.* 1999) and 3% of all pregnant women in the USA had used illicit substances in the preceding month in 2002 (Winslow et al. 2007). Moreover, 8.2% of pregnant women presenting with PROM had a positive cocaine urine screen result in a study by Delaney and associates (1997).

2.1.5.3. Mechanical risk factors

Traumas complicate 5–7% of US pregnancies (Connolly *et al.* 1997; Mattox and Goetzl, 2005). Both severely injured women (17-to 23-fold risk of PA) as well as non-severely injured pregnant women were at an increased risk of PA (Schiff *et al.* 2002; El Kady *et al.* 2004). Mainly blunt abdominal traumas, such as traffic accidents, which account for more than 50% of all traumas during pregnancy (Aitokallio-Tallberg and Halmesmäki, 1997; Mattox and Goetzl, 2005; Schiff and Holt, 2005), falls and assaults (Dahmus and Sibai, 1993; Leone Pak *et al.* 1998; Rachana *et al.*

2002; El Kady *et al.* 2004) result in placental abruption as well as other adverse pregnancy outcomes.

2.1.5.4. Obstetric risk factors

Multiparity, especially grandmultiparity, seems to increase the incidence of PA (Ananth *et al.* 1996). Babinszki and associates (1999) found an incidence of PA of 1.5% among great-grand multipara (more than nine deliveries) Jewish women in New York, compared with a 1.0% incidence found in multiparous women with similar socioeconomic, age and racial background characteristics. Yasmeen and associates (2005) observed an association between PA and grandmultiparity (OR 1.3, 95% CI 1.2–1.5) compared with multiparity, in a large study consisting 25,512 grandmultiparas and of 265,060 multiparas.

Stillbirth in an earlier pregnancy has been associated with a significantly higher frequency of placental abruption in subsequent pregnancy (5.4% *vs.* 0.7%) according to Heinonen and Kirkinen (2000), and, in other studies, a substantially increased risk of PA in subsequent pregnancies, with ORs of 9.4 to 13.1 compared with women without previous stillbirth (Linqvist and Happach, 2006; Black *et al.* 2008). **Recurrent spontaneous abortions** are also associated with a higher risk of PA with an OR of 1.6 (95% CI 1.3–2.0) compared with non-affected women (Paterson *et al.* 1979; Sheiner *et al.* 2005).

Bleeding in the first and/or second trimester (Sipilä *et al*. 1992) or retroplacental haemorrhage detected in the first trimester together with bleeding suggest an increased risk of PA (RR 5.6, 95% CI 2.8–11.1) as well as other adverse pregnancy outcomes (Nagy *et al.* 2003). Salafia and associates (1995) found histological evidence of old intrauterine bleeding in 64% of the placentas of women who delivered prematurely and had PA. Ananth and colleagues concluded that vaginal bleeding early in pregnancy and later-found placental lesions were evidence of placental abruption as a chronic process (2006a).

Premature rupture of membranes (PROM) has been associated with a 3.6-fold increased risk of PA and also an inverse correlation to gestational length (Holmgren and Olofsson, 1997; Ananth *et al.* 2004). Major *et al.* (1995) found an association between preterm premature rupture of membranes (PPROM) and PA, with an OR of 6.1 (95% CI 4.1–9.0) compared with women with intact membranes. They also highlighted the importance of bleeding as an indicator of increased risk of PA in pregnancies with PPROM. In women affected by PPROM, PA appeared significantly

more frequently in those with vaginal bleeding (12%) than in those without bleeding (3.5%)(Hnat *et al.* 2005).

Chorioamnionitis has been reported to increase the risk of PA significantly (OR 2.61, 95% CI 1.65–4.12) compared with non-infectious cases (Kramer *et al.* 1997). Nath and associates (2007) discovered that histological chorioamnionitis was significantly more often present in preterm PA cases (30.8%) than in controls (12.5%), with an OR of 3.6 (95% CI 1.7–10.5). The corresponding rates as regards term PA were 34.6% and 20.4% (OR 2.8, 95% CI 1.3–6.1), respectively. This was also confirmed in a recent Finnish study by Tikkanen and associates (2006a). Ananth and associates (2004) stated that PPROM, intrauterine infections and oligohydramnios are independent risk factors, but that they also have cumulative effects on the risk of PA. According to the results of a recent study, respiratory infections requiring hospitalisation, especially as regards chronic bronchitis, also have an association with the development of PA (Getahun *et al.* 2006b). Han and associates proposed that even oral cavity bacteria may have an association with the development of PA, via MIAC and PPROM (Han *et al.* 2006).

Table 5. Maternal hypertensive disorders in pregnancy and the risk of placental abruption.

Study	***n* of cases/(*n* matched pairs) /*n* of controls (PA%)**	**Essential hypertension OR (95% CI)**	**Pregnancy induced hypertension OR (95% CI)**	**Pre-eclampsia OR (95% CI)**
Kramer *et al.* 1997, Canada	415/36,875 (1.1%)	1.59 (1.02-2.50)	1.59 (1.02-2.50)	2.32 (1.58-3.42)
Rasmussen *et al.* 1999, Norway	2,974/523,372 (0.66%)		1.96 (1.59-2.41)	
Kyrklund-Blomberg *et al.* 2001, Sweden	4,003/795,459 (0.51%)	1.9 (1.2-3.2)	1.5 (1.1-2.0)	2.2 (1.9-2.7)* 5.6 (4.7-6.6)#
Zetterström *et al.* 2005, Sweden	3,331/681,515 (0.49%)	2.3 (1.6-3.4) (38/ 3,374 =1.13%)	na	na
Lindqvist and Happach, 2006, Sweden	175/(2,371)/24,207 (0.7%)	na	na	3.4 (1.4-8.2)
Tikkanen *et al.* 2006a, Finland	198/(396)/46,742 (0.42%)	ns	ns	2.7 (1.3-5.4)
Ananth *et al.* 2007b, USA	173,865/29,968,545 (0.58%)	aRR 2.4 (2.3-2.5) (3,445/ 221,404 =1.56%)	aRR 2.5 (2.4-2.6) (15,611/ 1,153,761 =1.36%)	na

*mild, #severe; na, not applicable; ns, not statistically significant; aRR, adjusted relative risk.

There is discordancy between different authors about the effect of **maternal hypertension,** especially chronic hypertension, on PA (Zetterström *et al.* 2005). Direct comparison between studies is difficult because of varying study criteria and adjustments for confounding factors. Moreover, chronic hypertension in combination with smoking or superimposed pre-eclampsia (PE) has been associated with additive effects on the risk of PA (Ananth *et al.* 1999b). In one historical study, up to 45% of women with severe PA were hypertensive (Pritchard *et al.* 1970).

Pre-eclampsia seems to have a considerable impact on the aetiology of placental abruption, with ORs of 2.2 to 3.4 (Kyrklund-Blomberg et al. 2001; Lindqvist and Happach, 2006; Tikkanen et al. 2006b) compared with non-affected women in Nordic countries. PE in the first pregnancy seems to double the risk of PA in the second pregnancy (OR 1.9, 95% CI 1.51–2.38) (Ananth et al. 2007a).

Gestational diabetes mellitus (GDM) has not been significantly associated with PA. Some investigators have found an association between pre-gestational type-1 diabetes mellitus (DM1) and PA (Kyrklund-Blomberg *et al.* 2001; OR 3.4, 95% CI 1.4–8.2) but not all (Tikkanen *et al.* 2006a).

External cephalic version (ECV) does not seem to result in PA when protocols follow current recommended guidelines (Collaris and Oei, 2004; Nassar *et al.* 2006). **Amniocentesis** and chorionic villus sampling for prenatal karyotyping (Eriksen *et al.* 1991; Cederholm *et al.* 2003) and third trimester amniocentesis have not been associated with an increased risk of PA (Hodor *et al.* 2006).

2.1.5.5. Uterine risk factors

Previous Caesarean section considerably increases the risk of PA and the mechanism is suggested to be connected to impaired placental attachment and reduced blood supply in the scarred low segment of the uterus (Hemminki and Meriläinen, 1996; Rasmussen *et al.* 1999; Lydon-Rochelle *et al.* 2001; Juntunen *et al.* 2004; Getahun *et al.* 2006a). A short inter-pregnancy interval after Caesarean delivery also seems to increase the risk of PA (RR 1.5, 95% CI 1.1–2.3) according to Getahun *et al.* (2006). These connections with PA are noteworthy, since the rates of Caesarean sections in developed countries still seem to be rising; in Norway the rate has risen from 1.8% in 1967 to 16.4% in 2006 (Daltveit *et al.* 2008) and in the USA from 20.7% in 1996 to 31.1% in 2006 (MacDorman *et al.* 2008).

Table 6. History of Caesarean section and association with placental abruption compared with women with vaginal first birth.

	n	**PA in Case %/ controls %**	**OR (95% CI)**
Hemminki and Meriläinen, 1996,	8,250mp	3.8/1.6	2.4 primiparas ($p < 0.01$)
Finland	8,642mp	7.6/2.0	3.9 multiparas ($p < 0.01$)
Rasmussen *et al.* 1999, Norway	523,327	0.82/0.57	1.39 (1.22-1.60)
Lydon-Rochelle *et al.* 2001, USA*	95,630	1.37/1.09	1.3 (1.1-1.5)
Juntunen *et al.* 2004, Finland £	149mp	3.4/ 0	na ($p < 0.024$)
Getahun *et al.* 2006, USA*	156,475	0.95/0.74	RR 1.5 (1.1-2.3)
Tikkanen *et al.* 2006a, Finland *#	46,742	0.35/ 0.26	1.7 (1.1-2.8)
Yang *et al.* 2007, USA	5,146,742	0.68/0.48	1.40 (1.36-1.45)
Daltveit *et al.* 2008, Norway *	637,497	0.84/0.48	1.7 (1.6-1.9) 2.0 (1.8-2.2) PA not excluded

mp, matched pairs; na, not applicable;*cases with placental abruption in the first delivery excluded; #placenta previa cases included, £ repeated 4 to 10 Caesarean births, with next similar indication Caesarean section selected as a control .

Women with **uterine leiomyomas** seem to have an increased risk of PA with an OR of 2.6 (95% CI 1.6–4.2) (*n=105,909; PA=690 (0.65%))* according to Sheiner *et al.* (2004) and an OR of 3.87 (95% CI 1.63–9.17) according to Coronado *et al.* (2000). Placentation on a leiomyoma may decrease placental blood supply and thus predispose the placenta to ischaemia and PA.

Tikkanen and associates (2006a) found women with **uterine malformations** to bear a very high risk of PA (OR 8.1, 95% CI 1.7–40) compared with women with no known uterine malformations. On the other hand, women with uterine malformations have been shown to have elevated maternal serum α-fetoprotein concentrations as well (Heinonen *et al.* 1996a), which in turn have been shown to increase the risk of PA. Moreover, the amount and severity of uterine malformations in the "normal" obstetric population has not been well assessed, thus making it difficult to evaluate their significance in the development of PA.

2.1.5.6. Assisted reproduction treatments (ART)

Assisted reproduction treatments are known to increase the risk of PA partly, as a result of the risk of multifetal pregnancies with their associated risks. However, according to Pandian *et al.* (2001) unexplained infertility *per se* also carries a high risk of PA with an aOR of 3.05 (95% CI 1.4–6.2) even after adjusting for age, parity and fertility treatments in singleton pregnancies. In vitro fertilisation (IVF) has been shown to double the risk of PA in comparison with normal pregnancies

(Källen *et al.* 2005; Shevell *et al.* 2005). This increase may partly be explained by higher rates of PE and PPROM. Källen and associates (2005) suggested that twin pregnancies would also bear a higher risk of incidences of thromboembolic complications because of the higher oestrogen levels compared with singleton pregnancies, and thus higher risk of PA.

Table 7. ART and its association with placental abruption.

Study	Källen *et al.* 2005, Sweden	Shevell *et al.* 2005*, USA	Schieve *et al.* 2007*, USA
***n* study population/*n* ART**	2,013,633/ 13,264	36,062/ 1,776	157,066/ 1400mp
PA% controls	0.6%	0.7%	0.74%
***n* Ovulation induction/** PA%	na	1,222/1,4%	na
OR (95% CI)		2.4 (1.3-4.2)	
***n* IVF** /(PA%)	9,063/	554 /2.2%	na
aOR (95% CI)	2.3 (1.7-2.7)	2.4 (1.1-5.2)	
***n* ICSI /PA**	4,162/	na	na
aOR (95% CI)	1.6 (0.91-2.9)		
***n* IVF + ICSI +**	13,261/	na	
gamete or zygote transfer/	2.2 (1.7-2.7)		1.85%, 2.5 (1.9-3.2) mp
PA%,	*1.9 (1.4-2.5)		*1.56%, 2.2 (1.4-3.2) mp
OR (95% CI)	**1.5 (0.91-2.4)		¤1.65%, 3.8 (1.6-9.4) mp

*singleton pregnancies only; ** multiples; mp, matched pairs; na, not applicable; ¤, highly selected population

2.1.5.7. Fetal risk factors

Male gender has been shown to be associated with an increased risk of PA according to several investigators, with relative risks of 1.3 to 1.4 (Kåregård and Gennser, 1986; Raymond and Mills, 1993; Kramer *et al.* 1997; Linqvist and Happach, 2006). Nielsen and associates stated recently that recurrent PA in particular is almost exclusively preceded by the birth of a boy and followed by a dysregulated immune response against male-specific minor histocompatibility (HY) antigens (2007).

In several studies PA has been found to be associated with **intrauterine growth retardation** (IUGR). Raymond and Mills (1993) found a RR of 2.32 (95% CI 1.68–3.20) (13.7%/6.3%) as regards placental abruption and Lindqvist and Happach reported (2006) an OR of 3.2 (95% CI 1.8–5.5) in association with IUGR. In a recent Finnish study 25% of newborns with PA were growth-restricted compared with 4% of the controls (OR 7.9, 95% CI 4.4–14.3) (Tikkanen *et al.* 2006a). Women with incipient preterm births and fetal growth disorders have an increased tendency to have

their pregnancies ending in abruption (Ananth *et al.* 1999a; Rasmussen *et al.* 1999; Ananth and Wilcox, 2001; Kyrklund-Blomberg *et al.* 2001; Tikkanen *et al.* 2006b). However, Infante-Rivard and associates (2002) found no association between thrombophilia polymorphisms and intrauterine growth restriction.

Pregnancies with **fetal congenital anomalies** are associated with a higher rate of PA compared with normal pregnancies. Linhart *et al.* (2000) found an OR of 1.34 (95% CI 0.58–3.10) as regards congenital anomalies in preterm births and placental abruptions. Eriksen and colleagues (1991) more often (8.0%) found congenital malformations in pregnancies with PA compared with normal pregnancies (3.2%)(OR 2.7, 95% CI 1.2–5.9). This result was in accordance with the findings of Raymond and Mills (1993), who showed an increased risk of PA (4.4% *vs.* 1.7 %; RR 2.57, 95% CI 1.48–4.44) among women with fetal congenital anomalies compared with women without anomalous fetuses. This increase was especially noted in cases of congenital heart defects (3.3% *vs.* 0.7%; RR 4.63, 95% CI 2.49–8.55). Usually, pregnancies with congenital fetal anomalies are excluded from studies, in which the risk factors of PA are evaluated, because these pregnancies are known to carry an over all increased risk of miscarriages, stillbirths and adverse pregnancy outcomes (Linhart *et al.* 2000).

Non-vertex presentation of the fetus has been found to have an association with PA (OR 1.5, 95% CI 1.1–2.0) in preterm births compared with fetuses with vertex presentation, but the causes behind this association are not fully known (Sheiner *et al.* 2002).

Women with multifetal gestations seem to have an approximately doubled risk of PA compared with those with single fetus pregnancies, but the pathophysiological processes may differ from those associated with abruptions in singleton pregnancies (Ananth *et al.* 2001b; Campbell and Templeton, 2004; Salihu *et al.* 2005). As plurality (the number of fetuses in a pregnancy) increases from 1 to 3, the incidence of PA rises (single 0.62%, twins 1.22% and triplets 1.59%), whereas the risk of abruption-associated perinatal mortality declines (Salihu *et al.* 2005). In addition, the birth weight discordancy between twins increases the risk of PA even in the lowest weight discordancy groups compared with twins with appropriate growth (Ananth *et al.* 2003). In the same study by Ananth and associates (2003), it was observed that approximately one fifth of the twin pregnancies without placental abruption, but more than two thirds of twin pregnancies with placental abruption were born before H32. Moreover, PA itself in twin pegnancies increases the risks of SGA infants (RR 1.3, 95% CI 1.2–1.4) and preterm birth (RR 1.5, 95% CI 1.4–1.6) in twin pregnancies compared with twin pregnancies without placental abruption (Ananth *et al.* 2005b). Some researchers have found no increased incidence of PA in twin pregnancies (Geipel *et al.* 2001;

Tikkanen *et al.* 2006b), but this may have been due to small numbers of multifetal pregnancies in these studies.

Genetic risk factors are discussed in section 2.2. and are depicted in Table 10, where candidate gene association studies in connection with PA are presented.

2.1.6. Clinical outcomes

2.1.6.1. Maternal outcome

Placental abruption has significant acute and long-term complications as regards both mother and infant. Maternal outcome is mainly dependent on the severity of placental abruption. PA bears **peripartal risks** as regards obstetric haemorrhage, disseminated intravascular coagulopathy (DIC), renal failure, emergency hysterectomy and maternal mortality. The most common causes of **maternal death** are massive haemorrhage and DIC (Oyelese and Ananth, 2006). According to Atrash and associates approximately 6% of maternal deaths in the USA in 1979–86 were associated with PA (1990)**.** Fortunately, maternal mortality rates have decreased by as much as 99% during the last century in developed countries, mainly as a result of the use of antibiotics and development in intensive care, but acute profuse haemorrhage still represents a major clinical peril for pregnant women.

Severe **haemorrhage** is suggested to occur in about 25% of PA cases and the blood loss is often underestimated (Konje and Taylor, 2006). Partly this is a result of concealed haemorrhage into the myometrium and difficulties in interpreting the indirect signs of haemorrhage, and this may lead to maternal hypovolaemic shock. Hypovolaemia in turn may produce renal or multi-organ failure together with **DIC**, which is present in about 10% of abruption cases (Clark, 2004) and approximately 35% of severe abruptions (Konje and Taylor, 2006). PA is the most common associated condition among the causes of acute obstetric DIC (Matsuda *et al.* 2005). In cases of placental abruption with a stillborn fetus, Witlin and Sibai (2001) found DIC in 28.3% and renal failure in 5.0% and acute respiratory distress syndrome in 5.0% of the mothers. Patients with DIC have an imbalance between coagulation, anticoagulation and fibrinolysis which cause simultaneous widespread clotting and bleeding problems that may end with impaired microcirculatory perfusion and multiple organ failure (Clark, 2004; Sivula *et al.* 2005; Konje and Taylor, 2006). High concentrations of tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in the placenta and

myometrium (nearly 8000 times and 6.5 times higher than plasma concentrations, respectively) may be significant aetiological factors of DIC in PA cases (Kuczynski *et al.* 2002).

Placental abruption complicates about 12% of **HELLP** (haemolysis, elevated liver enzymes and low platelets) syndrome pregnancies and 3% of pre-eclampsia pregnancies (Vigil-De Garcia, 2001; Bhattacharya and Campbell, 2005; Cavkaytar *et al.* 2007).

Peripartal emergency **hysterectomy** takes place at an incidence of 0.2–0.8 per 1000 deliveries and this is partly caused by PA (Engelsen *et al.* 2001; Whiteman *et al.* 2006). According to Engelsen and associates (2001) 2 out of 11 peripartal hysterectomies in Norway (1981–1996) were performed because of PA. In a national estimate study in the USA, placental abruptions led to 6.4% of all peripartal hysterectomies (Whiteman *et al.* 2006). In many studies of obstetric emergencies PA is included in the general category of obstetric haemorrhages and thus it cannot be distinguished from the data on its own. Placental abruptions also cause delayed maternal discharge from hospital (Sheiner *et al.* 2002).

Caesarean delivery is performed in up to 91% of deliveries in which PA is involved (Kåregård and Gennser, 1986; Tikkanen *et al.* 2006b). It carries its own perioperative and postoperative risks, and there are substantially increased risks of recurrent Caesarean delivery and placentation abnormalities in following pregnancies (Juntunen *et al.* 2004; Tikkanen *et al.* 2006a).

The likelihood of another pregnancy after PA is lower than normal, since women with placental abruption are more reluctant to become pregnant again compared with other women (Rasmussen *et al.* 1997). Among women with PA and a surviving newborn, 59% had a subsequent delivery, compared with 71% of women without PA. Corresponding rates after a perinatal loss were 83% and 85%. Rasmussen *et al.* (1997) concluded that this reflects the distress and anxiety caused by placental abruption. However, mothers seem to continue smoking regardless of previous adverse pregnancy outcome (Cnattingius *et al.* 2006).

Feto-maternal bleeding in PA may produce **isoimmunisation** in Rh-negative women in following pregnancies unless treated with Rh-immune globulin (Mattox and Goetzl, 2005; Konje and Taylor, 2006).

Placental abruption may have **long-term implications for the health of mothers**, since stillbirth (late fetal death) seems to increase the risk of premature maternal mortality twofold (crude hazard ratio (HR) 2.08, 95% CI 1.65–2.61) as rgards cardiovascular diseases (CVDs) among parous women (Calderon-Margalit *et al.* 2007). In addition, premature delivery (RR 1.7, 95% CI 1.5–1.9) as well as the delivery of a low birth-weight infant has a strong association with maternal CVD mortality (Smith *et al.* 2000). In other studies maternal placental syndromes (defined as pre-eclampsia, PA and IUGR) have been shown to increase the risk of later maternal cardiovascular

diseases (adjusted HR 2.0, 1.7–2.2). Moreover, the combined presence of stillbirth (adjusted HR 4.4, 95% CI 2.4–7.9) or IUGR (adjusted HR 3.1, 95% CI 2.2–4.5) with 'maternal placental syndromes' add to the risk of CVD considerably (Ray *et al.* 2005).

2.1.6.2. Fetal outcome

Fetal outcome is strongly dependent on both the severity and the gestational age at which PA occurs (Oyelese and Ananth 2006). Placental abruption complicates up to 5.1% of preterm births and only about 0.3% of term deliveries (Rasmussen *et al.* 1996a; Ananth and Wilcox, 2001; Sheiner *et al.* 2002 and 2003; Ananth *et al.* 2006b). In other words, the vast majority, up to 68.8% of children born after pregnancies complicated by PA are premature (Oyelese and Ananth, 2006; Tikkanen *et al.* 2006b; Nath *et al.* 2007). Fortunately, the PA-associated perinatal mortality rate has shown a declining trend in recent decades, from 27.5% in 1979 (Paterson *et al.* 1979; Saftlas *et al.* 1991) to as low as 5.3% 1999 (Ananth *et al.* 1999a; Ananth and Wilcox, 2001). As the number of fetuses in pregnancy increases from 1 to 3, the risk of PA rises, whereas the risk of abruption-associated perinatal mortality declines (Salihu *et al.* 2005).

Prematurity accounts for a great deal of PA-associated **perinatal mortality**, especially as regards very preterm births (H22–28/32) (Allred and Batton, 2004; Salihu 2005), whereas in term pregnancies PA increases the stillbirth rate significantly, being indeed the strongest risk factor of perinatal mortality in term pregnancies with an OR of 50.5 (95% CI 32.2–79.1) (Sheiner *et al.* 2003). Placntal abruption is the presumed explanation for stillbirths in 16% to 19% of all fetal deaths (Petterson *et al.* 2002; Black *et al.* 2008) and for 12 % of perinatal deaths (Ananth and Wilcox, 2001). Abruptions that involve more than 50% of the placental surface are often associated with fetal death (Oyelese and Ananth, 2006).

The incidence of **premature** deliveries (< 37 gestational weeks) in Finland is 5.2% of all deliveries, whereas it is as high as 12.7% in the USA (Jakobsson *et al.* 2008). Preterm infants are at increased risk of short- and long-term disorders of the lungs (respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD)), brain lesions (intraventricular haemorrhage (IVH), periventricular leucomalasia (PVL) and white matter damage (WMD)), and intestinal lesions (necrotising enterocolitis (NEC)) and long-term ophthalmic lesions (retinopathy of prematurity (ROP)), plus other problems in hearing and neurological and cognitive development (Allred and Batton, 2004; Dammann *et al.* 2005). In one report, only 26% of extremely low birth weight (ELBW) infants (birth weight < 1000 g) were classified as normally developed at the age of 5 (Mikkola *et al.* 2005). The prognosis of premature infants is now much better than that it was two

decades ago, reflecting evolved obstetric and neonatal practice, such as corticosteroid therapy prior to birth, surfactant administration and progress in availability of neonatal intensive care unit (NICU) services in developed countries (Huisjes *et al.* 1979; Mikkola *et al.* 2005).

The association between PA and **low birth weight** (60.3% *vs*. 11.2% in controls, OR 13.7, 95% CI 7.4–25.2) is proposed to be chiefly mediated by prematurity, as it has been found to be significant in both SGA (OR 17.4, 95% CI 4.6–64.9) and appropriate-for-gestational-age (AGA) fetuses (OR 15.8, 95% CI 8.4–29.8), and moreover this association appeared not to be modified by maternal thrombophilia status (Nath *et al.* 2008). In a recent Finnish study IUGR was found in 25% of newborns with PA compared with 4% of the controls (OR 7.9, 95% CI 4.4–14.3) (Tikkanen *et al.* 2006b).

Heinonen and Saarikoski (2001b) found PA to be the strongest risk factor of fetal **asphyxia** (umbilical artery blood base deficit >12 mmol/L) at delivery with OR (3.74, 95% CI 2.15–6.51). In general, fetal asphyxia affects 2.5% of structurally normal singleton births and seems to be associated with cerebral palsy (CP) and placental abruption. There is strong evidence for a causal link between acute profound hypoxic ischaemia and dyskinetic tetraplegic cerebral palsy. Hemiplegic CP, on the other hand, is usually seen in extremely preterm children with PVL due to a focal cerebral infarction or stroke (Rennie *et al.* 2007). Characteristically, diplegic CP is caused by a perinatal hypoxic ischaemic insult at term, when hypoxia produces intraventricular haemorrhage and thus CP (Spinillo *et al.* 1994; Kayani *et al.* 2003). Placental abruption was found to be associated with children born with CP (OR 8.6, 95% CI 5.6–13.3) in a large Swedish study by Thorngren-Jerneck *et al.* (2006).

Koyama and associates (2005) identified pathological findings in the temporal bones of newborn infants with neonatal asphyxia that later produced problems with hearing and balance. Childhood survivors of perinatal hypoxic ischaemia are at risk of cognitive deficits even in the absence of functional motor disorders (Rennie *et al.* 2007). Moreover, Becher and associates (2006) found in their neuropathology study of stillborns that most of the fetuses stillborn because of placental abruption had clinicopathological signs of recent brain damage that occurred in the period immediately before death, suggesting suboptimal placental function. An animal model mimicking PA with antenatal hypoxia-ischaemia has broadened our knowledge of perinatal brain injuries such as CP (Derric *et al.* 2004).

It has been shown that children born after pregnancies complicated by PA seem to be at nearly a twofold risk of sudden infant death syndrome (**SIDS**), according to Li and Wi (1999)(OR 1.9, 95% CI 1.1–3.3) and Getahun *et al.* (2004)(OR 1.57, 95% CI 1.24–1.89).

Moreover, the place of birth, distance to a hospital, the care level of the obstetric unit, the availability of obstetrician and anaesthetic and pediatric services seem strongly to affect the neonatal outcome of the children born after placental abruption. Kayani *et al*. (2003) concluded that the interval of 20 min or less from the decision to preceed with Caesarean section to delivery substantially reduced neonatal morbidity and mortality in severe PA cases complicated by fetal bradycardia.

Table 8. Fetal outcome in connection with placental abruption.

Study	**cases/(controls)/ deliveries incidence %**	**perinatal mortality % OR (95% CI)**	**prematurity %, OR (95% CI) *H>32* H32-36**	**mean GA at birth**	**SGA % OR (95% CI)**
Eriksen *et al.* 1991, Denmark	30/ (87)/5,727 0.5%	19.5%/ 0.9%	54%/ 3.7%	na	na
Ananth *et al.* 1999a, USA	530/53,371 1.0%	aRR 8.9 (6.0-13.0)	aRR 3.9 (3.5-4.4) 39.6%/ 9.1%	na	14.3%/ 8.1% aRR 2.0 (1.5-2.4)
Kyrklund-Blomberg *et al.* 2001, Sweden	4,003/795,459 0.51%	10.6%/ 0.52%	55.7%/ 5.62% *50.2 (46.2-54.4)* 15.6 (14.5-16.8)	na	11.1%/ 3.1% 4.1 (3.8-4.6)
Salihu *et al.* 2005 USA	93,968/15,051,872 0.62% [5,051/413,619 1.22%] {353/22,585 1.56%}	12.5%/0.8% [5.8%/4.0%] {20.0%/ 7.4%}	na	34.8%/38.9% [32.6%/36.0%] {29.1%/32.1}	na
Tikkanen *et al.* 2006, Finland a,b	198/(396)/46,742 0.42%	9.2%/1% 10.1 (3.4-31.1)	59%/19% 12.9 (8.3-19.8)	na	25%/ 4% ¤ 7.9 (4.4-14.3)

[twins]; {triplets}; ¤ <10th growth percentile; GA, gestational age; SGA, small for gestational age.

2.1.7. Management

Timely clinical diagnosis is the cornerstone of optimal management in cases of PA. Management should be individualized according to the severity of the abruption, gestational age and the maternal

and fetal condition (Oyelese and Ananth, 2006). Early delivery, amniotomy, adequate blood and crystalloid transfusion, adequate analgesia for pain relief, monitoring of maternal condition and reassessment of fetal condition are the essentials of the management of PA (Neilson, 2003). In cases of acute obstetric DIC, which is present in approximately in 35% of severe PA cases (Konje and Taylor, 2006), management should begin promptly and aggressively (Kobayashi *et al.* 2001). Contemporaneous syndromes such as pre-eclampsia and HELLP should be treated accordingly. In acute PA cases tocolysis is considered to be contraindicated (Neilson, 2003).

In cases of mild PA in the early gestational weeks with reassuring maternal and fetal conditions, management can be expectant, with close monitoring and possible administration of antibiotics and corticosteroids for fetal lung maturation at 24–34 gestational weeks (Holmgren and Olofsson, 1997). Moreover, antenatal care should be arranged so that intensive care units are within reach if necessary and adequate obstetric services are available (Hladky *et al.* 2002; Oyelese and Ananth, 2006). In selected cases of preterm placental abruption, tocolysis with atosiban, β-sympathomimetics or magnesium sulphate may be used (Bond *et al.* 1989; Towers *et al.* 1999; Konje and Taylor, 2006).

Caesarean birth is often the method of choice to save the infant (Rasmussen *et al.* 1996b). According to a study by Kayani *et al.* (2003) a decision to operate so that delivery took place in 20 min or less was associated with substantially reduced neonatal morbidity and mortality in cases of severe placental abruption. In cases of fetal demise, vaginal delivery is recommended as long as the maternal condition does not necessitate immediate birth (Neilson, 2003). Fetal death increases the risk of maternal DIC remarkably (Clark, 2004).

In cases of severe **trauma**, such as motor vehicle accidents, pregnant patients should have 24-hour close monitoring (fetal heart rate tracing) if there are any signs of frequent uterine activity (> 5 contractions per hour), abdominal or uterine tenderness, non-reassuring fetal heart rate patterns in vaginal bleeding or evidence of hypovolaemia during the first 4 hours after trauma (Dahmus and Sibai, 1993). Pregnant women hospitalised following motor vehicle accidents are at an increased risk of adverse pregnancy outcome, regardless of the presence or severity of injuries (Schiff and Holt, 2002). Seat-belt use considerably reduces the severity of the risk of adverse pregnancy outcomes (Pearlman *et al.* 2000; Schiff *et al.* 2005). The Kleithauer-Betke test is not indicative of PA in pregnant trauma patients and should not be routinely used in evaluating pregnant trauma patients (Dhanraj and Lambers, 2004). Cahill *et al.* (2008) also added fibrinogen levels and coagulation studies in the list of tests not recommended for patients presenting with minor trauma.

Falls and motor vehicle crashes have been reported to be the leading mechanisms of injury requiring hospitalisation during pregnancies in the USA. Assaults represented the third most

common mechanism of injury during pregnancy (El Kady *et al.* 2004) and in minor trauma cases nearly 20% of the patients were victims of assaults (Cahill *et al.* 2008). The possibility of assault should be identified and closely monitored in every case of 'fall' because of the increased risk of PA, since up to 17% to 32% of pregnant women in the USA are prone to assaults (Lipsky *et al.* 2005; Mattox and Goetzl, 2005). Cahill and associates (2008) suggested that an appropriate protocol for pregnant women presenting with minor trauma, i.e. the absence of substantial maternal injury or any other clinically worrisome signs, would comprise a physical examination, a brief assessment of fetal wellbeing, and patient counselling on the warning signs and symptoms of abruption.

In order to prevent isoimmunization, all Rhesus negative pregnant women should recieve anti-D-immunoglobulin in the cases of placental abruption and the same should be applied to pregnant trauma patients, regardless of the severity of the trauma (Dahmus and Sibai, 1993; Mattox and Goetzl, 2005; Konje and Taylor, 2006).

Congenital or acquired **thrombophilias** (FV Leiden, antithrombin III, prothrombin gene mutation, protein S and C deficiency, MTHFR deficiency, lupus anticoagulant and anticardiolipin antibodies) should be excluded by means of **screening** tests two to six months after the birth if there are no other obvious causes of PA (Alfirevic *et al.* 2001; Oyelese and Ananth 2006). Even if nothing decisive has been proven to date, there are recommendations to use low-molecular weight heparin or aspirin in subsequent pregnancies with screening positive women (Oyelese and Ananth, 2006). Use of **folic acid and multivitamin supplements** also seems to reduce the risk for PA in MTHFR defective women according to Nilsen and associates (2008).

2.1.8. Prediction and prevention of PA

Doppler ultrasonographic measurements of uterine artery blood flow are non-specific for PA, but in general, persistent notching of of uterine artery wave forms is suggested to predict risk pregnancies as regards placental dysfunction (Harrington *et al.* 1996). Screening for **biochemical markers**, such as AFP, β-hCG, PAPP-A, PlGF, sFlt-1, fibronectin and thrombomodulin, has been suggested to identify women who might develop PA, but the evidence is weak so far. Tikkanen and her colleagues (2007) found that second trimester measurement of angiogenic factors and AFP lacks the sensitivity and specificity to predict PA. Specific genetic screening tests are still not yet feasible either. A **history of PA** in a previous pregnancy adds considerably to the risk of recurrent

abruption and is discussed more specifically in section 2.1.9. Today we have no tools at hand to target prenatal care and prevention strategies in risk pregnancies.

2.1.8.1. Risk estimation

A risk estimation score for PA (Lindqvist and Happach, 2006) as well as mathematical modelling to predict PA (Baumann, 2000) may prove useful in the future in high risk pregnancies, but both of these are based on the results of retrospective studies, with no evidence of their usefulness in prospective clinical situations.

2.1.8.2. Primary prevention

In high risk pregnancies as regards PA and premature delivery, optimal delivery planning should consist of referring the birth to a tertiary level hospital to maximise the availability of adequate obstetric and NICU services for better prognosis (Stephansson *et al.* 2003; Rautava *et al.* 2007). Prevention of recurrent premature delivery by administration of 17-alpha-hydroxyprogesterone caproate in selected risk pregnancies might reduce some premature placental abruption cases (Meis *et al.* 2003).

Cessation of smoking at the beginning of pregnancy, and preferably beforehand, would probably be the most effective way to prevent PA in up to 7–20% of cases according to several investigators (Ananth *et al.* 1999b; Oyelese and Ananth, 2006; Högberg *et al.* 2007). Strategies to **reduce alcohol consumption** during pregnancy have been proved to be effective (Halmesmäki, 1988; Floyd *et al.* 2006; Bailey and Sokol, 2008) and should be more actively implemented in perinatal care. Clustering of social problems also seems to be correlated to adverse pregnancy outcomes (Raatikainen *et al.* 2007a,b) and this small marginal group of pregnant women might benefit from early intervention, possibly preventing some PAs as well. **Maternal use of folic acid and multivitamin supplements** seems to reduce risk of PA in MTHFR-defective women according to Nilsen *et al.* (2008). **Seat belt use** would considerably diminish the possibility of placental abruption in traffic accidents (Schiff and Holt, 2005).

2.1.9. Recurrence risk

The incidence of recurrent PA is substantial, ranging from 2.8% to 16.6% in several studies, and so a previous PA remains the most predictive risk factor as regards a new abruption (Clark, 2004; Tikkanen *et al.* 2006a; Ananth *et al.* 2007a). Pritchard and associates (1970) found that 7% of women with PA ending in stillbirth had the same outcome in a subsequent pregnancy. Recurrence after two abruptions is as great as up to 25% (Clark, 2004; Lindqvist and Happach, 2006). Rasmussen and associates (2001) suggested intense biweekly follow-up of a pregnancy subsequent to PA, beginning six weeks prior to the gestational age of the initial PA. In addition, maternal immunological responses against male-specific minor histocompatibility (HY-) antigens have been suggested to play a role in recurrent PA (Nielsen *et al.* 2007).

Table 9. Recurrence of placental abruption.

Study, year ***n* PA/ *n* controls (PA incidence %)**	***n* of recurrent PA cases/ PA cases** **(PA incidence %)**	**Recurrence risk**
Kåregård and Gennser, 1986 3,959/ 894,619 (0.44%)	42/ 864 (4.9%)	10.2 (7.4-13.5)
Ylä-Outinen *et al.* 1987 180/ 85,177 (0.21%)	4/ 130 (3.1%)	11 fold risk
Rasmussen *et al.* 1997* (0.64%)	218/ 4,951 (4.4%) *Third PA 10/52*	6.4 (5.6-7.4) *Third PA 19.2%,* *OR 36.5 (21.2-62.7)*
Furuhashi *et al.* 2002 81/ 20,322 (0.4%)	6/81 (7.4%) 6/22 (27.3%)	
Tikkanen *et al.* 2006a 198/46,742 (0.42%) [114/ 209]	[10/114(8.8%)/ 3/209 (1.4%)]	OR 7.0 (1.9-26) AOR 4.5(1.1-18)
Lindqvist and Happach, 2006 175/ 24,207 (0.7%) [112/2,363]	[9/112 (8%)/ 8/2,363 (0.3%)]	OR 25.8 (9.8-68.3)
Ananth *et al.* 2007a 1,076/153,734 (0.7%)	30(2.8%)/ 1,076(0.7%)	OR 3.16 (2.18-4.58)

* gestation at least 16 weeks; [Case control study]

In some of these studies the recurrence rate has been calculated by comparing the recurrent cases with all PA cases, and some by looking at only those PA cases who became pregnant again, and so these figures are not directly comparable with each other (Furuhashi *et al*. 2002).

2.2. Genetic epidemiology

The human genome consists of approximately 23,000 genes and nearly 3 billion base pairs (bp). The first complete genome sequence of a single human individual was published in 2007 by Levy and associates. Moreover, the genomes of several hundred other organisms have been sequenced (www.ebi.ac.uk/genomes/index.html). This genomic information has dramatically changed the process of identifying disease genes. It provides new, invaluable tools for understanding the basic human genetic make-up and how variations in the genomic sequence result in disease (Peltonen and McKusick, 2001; http://genomics.energy.gov).

One of the major goals of the post-genome era is to understand the role of genetics in human health and diseases according to the International Human Genome Sequencing Consortium 2001 (Venter *et al.* 2001). The goal of genetic research is the discovery of susceptibility genes which will inform understanding of the pathophysiology of diseases, such as PA. This will later enhance the development of new techniques for prevention, diagnosis and treatment of diseases (Chappell and Morgan, 2006; Özgür *et al.* 2008). While fewer than 100 gene-disease associations were known before the HUGE project started in 1990, currently more than 1400 have been identified (**http://www.genome.gov/1106929**). The availability of a complete human genome sequence will enormously facilitate the identification of the genetic components of more complex and more common disorders in which multiple genetic and environmental factors interact.

The amount of biomedical information concerning identification of disease genes is growing rapidly. The challenge for scientists is that relevant information about new discoveries should not remain hidden in unstructured texts of published papers. New tools have been proposed for the otherwise laborious process of identification of new candidate genes for experimental studies by integrating automatic text mining and network analysis methods to extract known disease genes and to predict unknown disease genes. For example, there is now a web-based system under development for browsing disease-specific gene-interaction networks at http://gin.ncibi.org. (Özgür *et al.* 2008).

The Finnish population is one of the best-studied genetic isolates (Peltonen *et al.* 1995, 1999). It is particularly suitable for genetic studies, since there is exceptional genealogical data available from a well-established church record system which dates back to 1640, and now there are unique personal identification numbers that can be used to bring together different data bases (such as the birth or cancer registers). The influences of founder effect, genetic drift and isolation of the gene pool have created a 'Finnish disease heritage' in which a single mutation causes the majority of disease cases. This provides excellent opportunities for gene hunts, with special study designs for

the identification of rare disease genes and also major loci which contribute to complex diseases (Peltonen *et al.* 1995). Willer *et al.* (2006) suggested that the four-population-based tag SNP selection of the CEPH (Centre d'etude du polymorphisme humain) Utah HapMap (CEU) database provides an adequate basis for tag-SNP selection in Finnish individuals.

Genetic background can be studied by way of genome-wide approach or by candidate gene-based association studies. Linkage studies have been mainly used to detect single-gene disorders in families and pedigrees that are inherited in a Mendelian mannor (Daly and Day, 2001). Nowadays, genome-wide snipping can be used in polygenic diseases and in case-control settings. Genome-wide screens can suggest novel hypotheses, and candidate gene studies can be used to test hypotheses suggested by pathophysiology or global screening strategies (Chappell and Morgan, 2006).

Candidate gene studies are based on testing previously suspected susceptibility genes. Furthermore genetic effects can be explored by using animal models which either over-express the gene of interest or are deficient in the selected gene.

2.2.1. Evidence for genetic basis of PA

There are no sib-pair- or twin-studies concerning placental abruption. In a study by Lindqvist and Happach (2006) 5% of the PA patients reported their first-degree relatives to have had PA. Plunkett *et al.* (2008) estimated the increased risk of PA among siblings compared with the population risk (0.8%) when an individual born after an affected pregnancy was the proband. They calculated the sibling risk ratio (*Lambda s*) to be 4.0 (95% CI 2.6–5.3) for PA and the sibling-sibling odds ratio (sib-sib OR) adjusted for known risk factors to be 3.8 (95% CI 2.6–5.5). Thus they interpreted their results as suggesting that placental abruption aggregates in families, which may be explained partly by genetics.

Placental abruption shares some common risk factors and possibly aetiological features with pre-eclampsia, which has been proven to have a strong familial component (Chappell and Morgan, 2006). An increased incidence of thrombophilia has been found in patients with adverse obstetric outcomes, and **FV Leiden mutation** has been associated with PA in some studies (Kupferminc *et al.* 1999; Järvenpää, 2006; Procházka *et al.* 2007). The high **recurrence** rate of placental abruptions, discussed in section 2.1.9, refers to the genetic background of this adverse obstetric outcome.

2.2.2. Genetic studies in families

In family studies, refining the phenotype would be the ideal study method, but the low incidence of placental abruption in general population limitates this strategy. Moreover, with a transient and rare disorder such as PA, difficulties arise in finding multicase-families, as susceptibility is apparent only during pregnancy and as there are no known male phenotypes (Chappell and Morgan, 2006). Thus there are no families for linkage studies to date.

2.2.3. Candidate gene studies

Candidate gene studies are based on genes that are selected because of a hypothesis about their role in the aetiology of the disease. Potential candidate susceptibility genes are chosen by studying biochemical or physiological pathways that may be involved in the pathophysiology of the disease. Functional polymorphisms of the candidate gene or polymorphisms that may be in linkage disequilibrium with functional changes are selected. Candidate gene studies are usually conducted by using a case-control approach, where differences in allele frequencies of the selected polymorphisms are compared in a population-based sample of affected and unaffected unrelated individuals (Risch, 2000; Tabor *et al.* 2002).

To date, candidate genes studied in relation to PA are mainly based on the theory of defective trophoblast invasion and incomplete remodelling of the spiral arteries. The candidate genes involved in vasculopathy can be classified in three groups: Thrombophilias, hemodynamic changes and oxidative stress. Hypotheses of immunological rejection and inflammatory processes as etiologic basis of PA have produced some new candidate genes to be studied.

Candidate gene studies in placental abruption are presented in Table 10.

Table 10. Candidate gene studies in placental abruption.

Gene and mutation, polymorphism, allele or genotype	Chromosome location	association +/-	p-value OR (95%CI)	*n* PA/ controls	Population	Study, year,
Thrombophilias						
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	+	11.8 (1.36-102)	27/29	Israel, mixed	Wiener-Megnagi *et al.* 1998 (*APCR)*
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	+	4.9 (1.4-17.4)	20/110	Israel, Jews	Kupfermic *et al.* 1999
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	-	na	23/44	UK, mixed	Alfirevic *et al.* 2001
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	+	18 (2.2-159)	7/ 100	Greece, Greeks	Agorastos *et al.* 2002
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	-	na	100/ 217	South Africa, blacks	Hira *et al.* 2002
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	+	9.12 (2.18-31.7)	50/ 100	Italy, whites	Faccinetti *et al.* 2003
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	-	1.5 (0.9-2.6)	102/ 2366	Sweden, Caucasians	Procházka *et al.* 2003
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	-	0.7 (0.15-3.28)	116/ 112	Finland, Caucasians	Jääskeläinen *et al.* 2004
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	-	*p*=1.0	31/ 4436	USA, mixed	Dizon-Townson *et al.* 2005
FV Leiden G1691A Arg506Gln	1q24.2 Exon10	+	15.57 (1.9-127)	9/ 111	Finland, Caucasians	Järvenpää *et al.* 2006
FV Leiden G1691A Arg506Gln	1q23 Exon10	+	3.0 (1.4-6.7)	142/ 196	Czech Republic	Procházka *et al.* 2007
FV G1628A Arg485Lys	1q24.2 Exon 10	-	1.4 (0.6-3.5)	116/ 112	Finland, Caucasians	Jääskeläinen *et al.* 2004
FV T1335C Met385Thr	1q24.2 Exon 8	-	1.90 (0.17-21.8)	116/ 112	Finland, Caucasians	Jääskeläinen *et al.* 2004
F2 G20210A	11p11.2 3'-utr	+	8.9 (1.82-43.6)	20/ 110	Israel, Jews	Kupferminc *et al.* 1999 *prothrombin*
F2 G20210A	11p11.2 3'-utr	+	6.86 (1.83-25.63)	27/ 156	Israel, Jews	Kupferminc *et al.* 2000
F2 G20210A	11p11.2 3'-utr	-	1.0 (0.08-11.12)	22/ 44	UK, mixed	Alfirevic *et al.* 2001
F2 G20210A	11p11.2 3'-utr	-	na	7/100	Greece, Greeks	Agorastos *et al.* 2002
F2 G20210A	11p11.2 3'-utr	-	na	100/ 217	South Africa, blacks	Hira *et al.* 2002
F2 G20210A	11p11.2 3'-utr	+	12.25 (2.36-29.6)	50/ 100	Italy, whites	Faccinetti 2003
F2 G20210A	11p11.2 3'-utr	-	na	9/ 111	Finland, Caucasians	Järvenpää *et al.* 2006
F2 G20210A	11p11.2 3'-utr	-	na	180/ 196	Czech Republic	Procházka *et al.* 2007
THBD C1418T Ala455Val	20p11.21 Exon 1	-	na	100/ 217	South Africa, blacks	Hira *et al.* 2002 *Thrombomodulin*
MTHFD1 G1958A Arg653Gln	14q23.2 Exon 20	+	2.85 (1.47-5.53)	64/ 184	Ireland, Caucasians	Parle-McDermott *et al.* 2005
MTHFR A1298C Glu429Ala	1q36.22 Exon 5	+	3.18 (1.01-10.37)	18/ 114	South Africa, blacks	Gebhardt *et al.* 2001
MTHFR A1298C Glu429Ala	1q36.22 Exon 5	-	0.98 (0.37-2.61)	64/ 184	Ireland, Caucasians	Parle-McDermott *et al.* 2005
MTHFR A1298CGlu429Ala	1q36.22 Exon 5	-	2.32 (0.93-5.78)	195/ 198	USA, mixed	Ananth CV *et al.* 2007d
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	2.0 (0.5-8.1)	20/110	Israel, Jews	Kupferminc *et al.* 1999
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	na	23/ 44	UK, mixed	Alfirevic *et al.* 2001
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	0.99 (0.28-3.30)	18/ 114	South Africa, blacks	Gebhardt *et al.* 2001
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	na	100/ 217	South Africa, blacks	Hira *et al.* 2002
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	0.64 (0.23-1.76)	64/ 184	Ireland, Caucasians	Parle-McDermott *et al.* 2005
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	0.96 (0.30-3.05)	117/ 112	Finland Caucasians	Jääskeläinen *et al.* 2006*
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	1.09 (0.09-12.12)	155/ 338	South Africa, blacks	Naidu *et al.* 2006
MTHFR C677T Ala222Val	1p36.22 Exon 5	-	na	9/ 111	Finland, Caucasians	Järvenpää *et al.* 2006
MTHFR	1p36.22	-	0.72	195/ 189	USA, mixed	Ananth *et al.* 2007d

C677T Ala222Val	Exon 5		(0.41-1.27)			
MTRR	5p15.3-p15.2	-	1.18	136/ 136	USA,	Ananth *et al.* 2007c
A66G Ile22Met	Exon 2		(0.62-2.24)		mixed	*unknown*
BHMT G742A Arg239Gln	5q13.1-q13.2	+	2.82	136/	USA,	Ananth *et al.* 2007c
	Exon 6		(1.84-4.97)	136	mixed	*unknown, homocysteine*
RFC-1	Exon 2	-	1.1	196/191	USA,	Ananth *et al.* 2008,
A80G His27Arg			(0.6-2.2)		mixed	*reduced folate carrier*
Endothelial function and hemodynamics						
eNOS	7q36.1	+	4.06	35/ 170	Japan	Yoshimura *et al.* 2001
G894T Glu298Asp	Exon 7		(1.98-8.71)			
eNOS	7q36.1	+	*p*=0.006	47/42	South Africa	Hillermann *et al.* 2005
G894T Glu298Asp	Exon 7		3.51 (1.76-9.98)			
AGT	1q42-43	-	ns	48/45	South Africa	Hillermann *et al.* 2005
C704T Met235Thr	Exon 2					*Angiotensinogen*
AGT	1q42-43	+	3.30	62/ 240	USA,	Zhang *et al.* 2006
C704T Met235Thr	Exon 2		(1.81-6.04)		mixed	
Immune function						
HLA-DRB1*15	6p21.3	+	na	8	Denmark	Nielsen *et al.* 2007
-DRB3*0301				recurrent		(major HY)*
-DQB1*05						
CTLA-4**	2q33	+	*AA-genotype* 1.94 (1.09-2.07)	117/ 112	Finland	Jääskeläinen *et al.* 2008a
IL 1Ra	2q14.2	ns	*p*=0.29	116/ 112	Finland	Jääskeläinen *et al.* 2008b

*HLA-class II alleles to restrict CD4+ T-cell responses against male-specific minor histocompatibility (HY) antigens.

** negative regulator of T-cell responses; na, not applicable; NA, not available; ns, not statistically significant.

Overall, Leiden mutation has been found to have an association with PA, but not in all populations, and no link between other FV mutations and PA has been detected. Conflicting results have also been found with F2 mutations. Homozygosity of the C677T MTHFR mutation has been associated with pregnancy complications such as recurrent pregnancy loss and SGA infants (Kupferminc *et al.* 1999). However, the results of studies of F2 mutation association with PA are again conflicting (Table 10). Homocysteine inhibits endothelial cell proliferation, induces trophoblast apoptosis and reduces hCG secretion *in vitro*. Thus trophoblast cell death may be the pathogenic mechanism by which homocysteine causes pregnancy complications related to placental diseases (Di Simone *et al.* 2003).

The results of candidate gene studies in PA to date have been inconsistent and have not always been replicable in other studies. Since the background of PA seems to be complex (polygenic) the contribution of individual polymorphisms is likely to be modest and variable across populations. The population sample size may be insufficient, and there are often difficulties in defining the exact phenotype and selecting the population studied, which can bias the results. Complex diseases can also vary in their aetiological mechanisms in different individuals (Tabor *et al.* 2002; GOPEC Consortium 2005). Thrombophilias confined to the fetal side *per se* do not seem to increase the risk of PA (Ariel and Anteby, 2004).

Candidate genes (and biological mechanisms) studied in relation to PA are of low penetrance and so the likelihood of a woman carrying an allelic variant and presenting with clinical manifestations is relatively low (Zdoukopoulos and Zintzaras, 2008). It is likely that numerous polymorphisms in many genes affect the risk of placental abruption, and several genes and allelic variants in different genes may have additive or contrasting effects (Risch, 2000). Association studies are likely to be more effective than linkage studies for studying complex diseases because they have a greater statistical power to detect several genes with small effects (Tabor *et al.* 2002).

2.2.4. Candidate genes in the present study

2.2.4.1. The microsomal epoxide hydrolase (EPHX) gene

EPHX gene is located in chromosomal region 1q42.1 and it is expressed in many organs, including the ovaries and placenta (Hartsfield *et al.* 1998; Vogel-Bindel *et al.* 1982; Seidegård and DePierre, 1983). The encoded protein is involved in the phase I hydrolysis of epoxides and is thought to be involved in detoxification processes, in the metabolism of endogenous compounds in the sodium-dependent uptake of bile acids into hepatocytes and in oxidative stress (Nam *et al.* 1997, Pang *et al.* 2002; Zhu *et al.* 2003). Hassett and associates (1994) described two amino acid-altering single nucleotide polymorphisms (SNPs) in the coding region of the human EPHX1 gene and showed that both were associated with alterations in protein activity during *in vitro* expression studies. The Tyr113His variant was associated with a 40% decrease in enzymatic activity, whereas the His139Arg variant was assocated with an increase of 25%. These alterations are thought to be linked to protein stability.

Polymorphism in the EPHX gene has been shown to have roles in the reproductive system and to modify the susceptibility to pre-eclampsia (Zusterzeel *et al.* 2001; Laasanen *et al.* 2002), spontaneous abortions (Wang *et al.* 1998), ovarian cancer (Lancaster *et al.* 1996), and polycystic ovary syndrome (Korhonen *et al.* 2003), the high activity genotype beeing associated with ovarian cancer, pre-eclampsia, and protection against spntaneous abortion and polycystic ovary syndrome. The speculative mechanisms behind these observations may be associated with alterations in steroid metabolism and the response to oxidative stress. Interestingly, keeping in mind the plausible role of EPHX gene in female reproduction and suggested involvement in the development of pathologic processes in the placenta, like it has been shown in pre-eclampsia (Zusterzeel *et al.* 2001; Laasanen *et al.* 2002) and in frequent spontaneous abortions (Wang *et al.* 1998)**,** both are known risk factors

of placental abruption. The possible association between EPHX gene polymorphisms and placental abruption has not been investigated previously in Finnish women.

In animal studies, Miyata *et al.* (1999) found that EPHX-null mice were fertile and had no phenotypic abnormalities.

2.2.4.2. The endothelial nitric oxide synthase (eNOS) gene

eNOS gene (encoding NOS3 protein) at gene map locus 7q36.1 is involved in the synthesis of nitric oxide (NO) in endothelial cells from L-arginine, as are other isoforms of nitric oxide synthase (NOS): inducible NOS (iNOS encoding NOS2 protein) and neuronal NOS (nNOS encoding NOS1 protein). Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor (EDRF), which regulates vasomotor tone and blood flow by inhibiting smooth muscle contraction and platelet aggregation (Ji et al. 2007) and by limiting the oxidation of atherogenic low-density lipoprotein (Furchgott and Zawadzki, 1980; Hogg *et al*. 1993). Yoshimura *et al.* (1998) found eNOS Glu298Asp variant to have association with coronary spasm susceptibility in Japanese population, but this has not been confirmed in other populations. There are considerable inter-ethnic differences in the distribution of NOS3 variants and in the estimated haplotype frequencies (Tanus-Santos *et al.* 2001). Decreased NO synthesis in pre-eclampsia (Arngrimsson *et al.* 1997; Napolitano *et al.* 2000) may have a crucial involvement in the pathophysiology of pregnancy-induced hypertension (PIH)(Granger *et al.* 2001).

Yoshimura *et al.* (2001) found a strong assosiation between the eNOS Glu298Asp variant (Glu298Asp homo- and heterozygotes) and placental abruption in a Japanese population. This association has not been investigated previously in Caucasian populations.

Nisoli and associates (2003) found that eNOS-null mutant mice had a reduced metabolic rate and showed accelerated weight gain compared with wild-type mice and they concluded that NO plays a fundamental role in controlling body energy balance. These NOS3 mutant mice were hypertensive (Huang *et al.* 1995). Furthermore, transgenig mice over-expressing NOS3 have been reported to have significantly lower blood pressure compared with control littermates (Ohashi *et al.* 1998), which also provides several insights into the pathogenesis of nitrate tolerance.

2.2.5. Animal models

To date there are no naturally occurring animal models of placental abruption. Khatun *et al.* (2001) found in their rat exposure model that local cold stress to the soles induced retroplacental haemorrhage and they suggested that something similar might account for some cases of human placental abruption. Derric *et al.* (2004) concluded that their rabbit model for human cerebral palsy (with preterm fetal hypoxia-ischaemia causing hypertonia and motor deficits in neonatal rabbits) might mimic the fetal damages associated with placental abruptions. Although PIH has been simulated in rats by reducing placental perfusion (Granger *et al.* 2001), there is no proper animal model for placental abruption.

So far, genetically modified animal models, such as knock-out or transgenic thrombophilic mice, have not been created for studying placental abruption. There are several complicating factors in creating animal models for PA, such as the facts that knock-out gene mutation may be lethal, placental abruptions may be of maternal and/or fetal genetic origin, and the placentas of animals may be very different as regards anatomy in comparison with human placentas. Hence the results could not be applied straightforwardly in humans.

2.2.6. Clues to novel candidate genes

Clues from **pathophysiology** that have so far merited detailed genetic analysis are mainly related to impaired placentation and thrombophilias, and only some to endothelial function and hemodynamics or immune functions of the placenta (Kupferminc *et al.* 2000, Yoshimura *et al.* 2001, Hira *et al.* 2002, Järvenpää *et al.* 2006, Ananth *et al.* 2007c and 2007d, Jääskeläinen *et al.* 2008a and 2008b; Zdoukopoulos and Zintzaras, 2008).

Transcriptomics with microarray techniques allows simultaneous investigation of thousands of genes and comparison of gene expression in healthy and diseased tissues may reveal clues to the causes of the diseases and thus indicate specific genes or pathways for further research. Microarray techniques are based on selected complementary DNA (cDNA) or oligonucleotide probes that are immobilized on chips and hybridization is then detected according to the labelling of samples, with radioactive tags or fluorescent dyes (Chappell and Morgan, 2006).

Proteomics or metabolomics is considered to hold great promises as regards revealing possible biomarkers in early stages of placental problems and enlightening causative mechanisms of disease when integrated with genetic approaches. Multiple protein expression in tissues or biofluids can be

seen screened and candidate genes then suggested for further research (Chappell and Morgan, 2006).

3. AIMS OF THE STUDY

The aims of the present studies were:

To define phenotypic diversity of placental abruption and its clinical consequences by an epidemiological approach (Studies I and II)

To find genetic evidence to be used in candidate gene studies by assessment of patient-specific recurrence and familial risks in the Finnish population (Studies II and III)

To test two individual susceptibility genes as regards placental abruption (Studies IV and V)

4. SUBJECTS AND METHODS

4.1. Kuopio University Hospital Birth Register

The information source for these studies is the Kuopio University Hospital Birth Register, which is a computerised database starting from April 1989 and containing information on maternal pregnancy characteristics, pregnancy complications and outcome and information on the newborn until the age of seven days as regards all pregnancies proceeding beyond 22+0 gestational weeks or birth weight more than 500 g. The database includes information required for the national Birth Register (Stakes, 2006) and a large quantity of additional information used for clinical purposes. The validity of the data has been manually checked for some specific pregnancy complications, such as perinatal death, velamentous umbilical cord insertion and umbilical cord knots (Heinonen *et al.* 1996b; Airas and Heinonen, 2002), as well as now for placental abruptions.

The data were collected from self-administered questionnairies returned to maternity centres by 22 weeks of pregnancy and women's maternity case notes that they carry with them and the questions concerned marital status, employment, previous operations and illnesses, contraceptive use, obstetric history, smoking, alcohol consumption and paternal characteristics. The data was completed and updated by way of nurse and midwife interviews at visits or at delivery at Kuopio University Hospital. Real time information on pregnancy complications, pregnancy outcome and the neonatal period was electronically filed in a systematic manner as part of the clinical work undertaken by the nurses and midwives taking care of delivery and neonatal care.

Informed consent was obtained from all the child-bearing women at the time of data collection according to the protocol approved by the Committee for Ethical issues in Human Research at the University of Kuopio.

4.2. Definition and subjects

4.2.1. Placental abruption

Diagnosis of placental abruption was based on clinical examination, and occasionally on ultrasonography carried out by the attending physician who took care of the delivery. At least two of the following criteria were required for the diagnosis: 1) vaginal bleeding in late pregnancy, 2) uterine tenderness with increased baseline uterine tone monitored externally, 3) fetal distress or death, and 4) a blood clot behind the placenta. Furthermore, in a few cases placental abruption was

histologically confirmed by pathological examination after delivery. Possible mild forms of placental abruption with spontaneous resolution in patients in a stable condition and with no increased uterine activity were not enrolled in the study. In cases of fetal demise the placentas were examined by experienced pathologists to rule out the possibility of abruption.

4.2.2. Subjects

Information was collected prospectively from March 1989 until December 2002 from 23 075 women who gave singleton birth at Kuopio University Hospital (KUH), which is the tertiary level perinatal centre of the area. Information was retrieved retrospectively from 170 women who had earlier had PA from the hospital documents and Birth Registry. They were contacted and interviewed by telephone or face to face. Those who responded (*n*=140) were interviewed and their pedigrees were assessed. They were asked to sign an informed consent document for these studies and requested to give a blood sample for DNA analysis (*n*=117). At the same time, blood samples were collected from controls (*n*=115) who gave birth at Kuopio University Hospital after uncomplicated singleton pregnancies and who had had at least two normal pregnancies, including the current one. From the controls, blood was drawn at enrolment to the labour room. The controls originated from a regional population in the area, they had no clinical signs of any pregnancy disorder, and were enrolled by random selection (convenience sample) in these case-control studies to ensure homogeneity of the genetic background of the study population.

Table 11. Clinical characteristics

Characteristic	**Placental abruption *n* = 170**	**Non-PA pregnancies *n* = 22,905**	***p***
Mean maternal age (years)	30.3 (+/-6.0 SD)	28.8 (+/-5.3 SD)	0.001
Mean birth weight at term (g)	3398 (+/-573 SD)	3606+/-492	< 0.001
Gestational age at delivery (weeks)	36.0 (35.2-36.8 SD)	39.8 (39.5-40.1 SD)	< 0.001
Pre-eclampsia *n* (%)	22 (12.9)	763 (3.3)	< 0.001
Caesarean delivery *n* (%)	126 (74.1)	3699 (16.2)	< 0.001

*=X^2-test

Table 12. Study population.

Subgroups	*n*/controls	Time of recruitment	Main outcome
I	170/22,905	1989–1999	phenotype
II	59/14,267	1990–2000	recurrence rate
III	index patients 129 mothers 129 sisters 214	1989–2001	familial risk
IV	117/115	1994–2002	two polymorphisms in EPHX genes
V	116/113	1994–2002	polymorphism in eNOS gene

4.3. Methods

4.3.1. Genetic analyses

Selection of the candidate genes, EPHX and eNOS, was based on the fact that they have been associated with oxidative stress, and endothelial dysfunction and vascular regulation of haemodynamics, these being plausibly important in the pathophysiology of PA. Both of these genes have also been studied previously in Kuopio as regards genetic association with pre-eclampsia (Laasanen *et al.* 2002; Häkli *et al.* 2003).

The genes studied, their functions, polymorphisms/SNPs and locations in genes and chromosomes are presented in Table 13. The candidate gene association method was used, in which genotypes and alleles were compared with PA and control groups.

Table 13. The genes studied.

Study	Gene	Function of the gene	Polymorphism /SNP	Location in the gene	Chromosome location
IV	EPHX*	Detoxification	T → C Tyr113His	Exon 3	1q42.1
IV	EPHX*	Detoxification	A → G His139Arg	Exon 4	1q42.1
V	eNOS#	Hemodynamics	G894T Glu298Asp	Exon7	7q36

* = microsomal epoxide hydrolase, # = endothelial Nitric Oxide Synthase

4.3.2. DNA extraction and polymerase chain reaction (PCR) analyses

Genomic DNA was extracted from peripheral (cubital venous) blood lymphocytes by using a standard phenol-chloroform extraction method (Vandenplas *et al.*1984). Mutation analysis for a G894T (Glu298Asp) polymorphism in exon 7 of the eNOS gene was performed as described earlier by Yoshimura *et al.* (2001). The polymerase chain reaction (PCR) primers and annealing temperatures used in Studies IV and V are presented in Table 14. The sizes of the fragments and the digestion products are given in table 15.

Table 14. PCR primers and annealing temperatures used in PCR amplification analyses.

Study	Primers	Annealing temperature(^{o}C)
IVA	Exon 3: F:5'-GGG GTC CTG AAT TTT GCT CC-3'	55
	Exon 3: R:5'-CAA TCT TAG TCT TGA AGT GAC GGT-3'	55
IVB	Exon 4: F:5'-TCT GGT GCC AGA GCC TGA CCG TGC-3'	64
	Exon 4: R:5'-ATG GAA CCT CTA GCA GCC CCG TAC C-3'	64
V *sense*	Exon 7: F :5'-AAG GCA GGA GAC AGT GGA TGG A-3'	69
antisense	Exon 7: R: 5'-CCC AGT CAA TCC CTT TGG TGC TCA-3'	69

Table 15. PCR methods, restriction enzymes and products.

Study	Method	Reference	Size of the PCR amplification product (bp)	Restriction enzyme	Sizes of the products after digestion (bp)
IVA	PCR-RFLP	Hassett *et al.* 1994	198	Tth111 I	198 or 175+23
IVB	PCR-RFLP	Hassett *et al.* 1994	322	RsaI	295+27 or 174+121+27
V	PCR-RFLP	Yoshimura *et al.* 1998	248	BanII	163+85 or 248

RFLP = restriction fragment length polymorphism.

Table 16. Determination of alleles and genotypes (bp numbers are in parentheses).

Study	Allele	Genotype
IVA	T=Tyr113 (198) or C=His113 (175+23)	TT, TC, CC
IVB	A=His139 (296+27) or G=Arg139 (174+121+27)	AA, AG, GG
V	G=Glu298 (163+85) or T=Asp298 (248)	GG,TG,TT

4.4. Statistical analyses

Differences between study subjects and controls were tested for significance by X^2 statistics (dichotomous variables), and, where the minimal estimated expected value was < 5, Fisher's exact test was applied. A p value of < 0.05 was considered statistically significant. Two-tailed pooled t-tests were used to analyse continuous variables. Maternal serum AFP multiples of the median (MoM) in affected and unaffected pregnancies were compared by using the two-tailed pooled t-test after $\log_{10}$ transformation of maternal serum AFP concentrations. Logarithmic transformation of the data allowed the use of parametric tests, since the serum marker levels seemed to fit (log) Gaussian distributions.

Possible confounding variables were identified from background data, obstetric risk factors and health behaviour. Multivariate analysis of significant or nearly significant effects (p <0.1) was based on multiple logistic regression analysis (BMDP Statistical Software Inc., LosAngeles, CA).
Statistical analyses for comparing single-point allele and genotype frequencies were performed using Pearson's X^2 test (two-sided asymptotic p values) with SPSS 9.0 software (SPSS Inc., Chicago, IL, USA). Odds ratios (ORs), as the estimates of relative risk of disease, were calculated using 95% confidence intervals (CIs).

We used an expectation-maximization (EM) algorithm to obtain maximum-likelihood estimates of haplotype frequencies with standard deviations (SDs) (each haplotype consisting of a pair of SNPs, and unknown gametic phase; Arlequin version 2.000 software, Genetics and Biometry Lab, Department of Anthropology, University of Geneva, Geneva, Switzerland). Thus, haplotype frequencies were derived from mathematical modelling and not direct determination. Haplotype frequency comparisons between the women who had experienced placental abruption, and the control group, with absolute chromosome numbers, were performed using Fisher's exact test (two-sided p values with Monte Carlo estimation at the 99% confidence level). Hardy–Weinberg distributions of genotypes in women who had experienced placental abruption and the control group, as well as pair-wise linkage disequilibrium (LD) analyses, were assessed using GenePop option 1 (http://wbiomed.curtin.edu.au/genepop/genepop_op1.html).
An RxC program employing the Metropolis algorithm was used for analysis of contingency tables to obtain unbiased estimates of exact p values with standard errors (SEs) in haplotype analyses. Sample size and power determinations were performed using nQuery Advisor 4.0 software (Statistical Solutions, Saugus, Mass., USA).

5. RESULTS

The main outcomes of these studies are shown in Table 17.

Table 17. Main outcomes

Study	Main outcomes	Incidence of PA in cases%/controls% or Frequency of genetic markers (%)	RR or OR (CI 95%)
I	Adverse effects:		RR
	fetal death	6.5/ 0.4	18.5 (11.8-29.1)
	prematurity	59.4/ 5.9	10.1 (8.6-11.8)
	birth weight < 2500 g	44.7/ 4.3	10.3 (8.6-12.4)
	SGA (< 10^{th} percentile)	11.2/ 9.4	1.2 (0.77-1.83)
	low Apgar score (< 7 in 1 min)	40.6/ 4.8	8.5 (7.0-10.3)
	low Apgar score (< 7 in 5 min)	25.9/ 1.7	15.2 (12.1-19.2)
	fetal venous pH < 7.15 at birth	2.9/ 1.1	2.7 (1.2-6.3)
	neonatal death	3.5/ 0.2	17.2 (9.3-31.9)
II	Recurrence	11.9/ 0.7	16.9 (8.2-34.9)
III	1^{st} degree relatives of index patients without recurrence	0.4	ns
	1^{st} degree relatives of index patients with recurrence	4.0	OR 5.6 (1.36-23.2)
IV	Protection against PA associated with the haplotype of the *EPHX* gene	C-A (His13-His139)(19) *vs.* T-A (Tyr113-His139)(63) T-G (Tyr113-Arg139) (9) C-G (His113-Arg139) (9)	OR 0.55 (0.36-0.85)
V	Susceptibility to PA associated with the eNOS gene Glu298Asp	TT (13.8) *vs.* TG (38.8) GG (47.4)	OR 1.34 (0.58-1.64)

ns = not statistically significant

5.1. Epidemiologic studies (Studies I, II and III)

In the clinical study (Study I) the overall incidence of placental abruption was 0.74%. We found pre-eclampsia to be the strongest risk factor of PA (OR 4.39, 95% CI 2.78–6.94) and grand multiparity (OR 3.60, 95% CI 1.23–10.5), velamentous umbilical cord insertion (OR 2.53, 95% CI 1.23–5.21), cigarette smoking (OR 2.46, 95% CI 1.53–3.96), prior fetal demise (OR 2.02, 95% CI 1.00–4.05), advanced maternal age (> 35 years; OR 1.62, 95% CI 1.10–2.41) and previous miscarriage (OR 1.55, 95% CI 1.09–2.22) to be independent reproductive risk factors. Of the newborns, 59.4% were premature and the perinatal mortality (PNM) rate was 10%. This means an excess of 17 neonatal deaths and 102 premature infants in this ten-year birth cohort. Hence, in the KUH referral area, there were 15 to 20 PAs a year, the birth of every 15th premature infant was associated with PA, and every year one or two infants died neonatally as a result of PA.

The studies on recurrence and family risk (Studies II and III) showed that the recurrence rate of PA was as high as 11.9%. If the PA was not recurrent, obstetric prognosis was comparable to that in the general obstetric population. Specifically, there were not significantly more cases of SGA infants, fetal distress or premature birth, but the incidence of pre-eclampsia was increased compared with the control group. In the ten-year cohort we found seven women with recurrent PA. In Study III we found that only recurrent placental abruption in the index patients increased the risk of abruption in first-degree relatives (sisters and mothers). We found four such families in this obstetric population in ten years.

5.2. Genetic studies (Studies IV and V)

In **Study IV** genetic variability in exons 3 (T →C (Tyr113His)) and 4 (A →G (His139Arg)) of the microsomal epoxide hydrolase (EPHX) gene showed that the low activity haplotype of the gene (C-A (His113-His 139)) was protective against placental abruption. The estimated frequency of this haplotype was 0.188 +/- 0.03 in the women with PA and 0.297 +/- 0.03 in the controls.

In **Study V** we found no association between Glu298Asp polymorphism in the eNOS gene and PA. The frequency of the TT genotype was 13.8% in the cases and it was 10.6% in unaffected women. Power analysis showed that 14,500 women would have been needed to be enrolled in the study in order to reach statistical significance.

6. DISCUSSION

6.1. General aspects

The clinical picture and incidence of placental abruption in our study were similar to those in other recent studies on PA, presenting with excess smoking, older maternal age and more pre-eclamptic pregnancies (Tikkanen *et al.* 2006a, b). In this maternity cohort, problem clustering (drug abuse, poor participation in prenatal care etc.) seemed to be minimal. Phenotypically, placental abruptions were associated with prematurity, but seemed not to be associated with intrauterine growth retardation, contrary to the results of some previous studies (Raymond and Mills, 1993; Linqvist and Happach, 2006; Tikkanen *et al.* 2006b). Of the newborns, 59.4% were premature and the perinatal mortality rate (PNM) was 10%, in line with the outcomes of other studies conducted in the Nordic Countries (Kyrklund-Blomberg *et al.* 2001; Tikkanen *et al.* 2006b). Caesarean section was performed in 74.1% of PA patients and direct visualisation of PA was successful in 20% of the cases, figures which are also comparable to those in previous studies.

The recurrence rate of 11.9% was in line with other studies as well. In contrast to the results of an epidemiological birth registry study in Norway (Rasmussen *et al.* 2000), we did not find the next pregnancies, without recurrent PA, to have subsatantially worse outcomes compared with those in the normal obstetric population. Unlike some other researchers (Kåregård and Gennser, 1986; Linqvist and Happach, 2006), we did not find a difference in fetal gender in our study either.

This was the first familial risk assessement of PA based on drawn pedigrees. There have been no previous studies on twins or sib pairs as regards placental abruption. An increased risk of PA seemed to concern only first-degree relatives, i.e. sisters and daughters of patients with recurrent abruption, which strongly suggested a genetic basis of the disorder.

Genetic susceptibility studies showed that in this Finnish population the eNOS gene did not have a decisive role in the pathogenesis of placental abruption and that the low-activity haplotype of the EPHX gene seemed to be protective against PA. It is likely, however, that there are considerable inter-ethnic differences in the distribution of genetic variants and in estimated haplotype frequencies.

6.2. Validity of the results − strengths and limitations

The nurses and midwives wrote meticulously detailed and structured information during antenatal and delivery visits and thus the data were accurate. This hospital-based cohort was a representative sample of the general obstetric population of this area. Since the index patients were contacted personally and their family histories and medical records were scrutinized, the collected data are to be considered reliable and consistent with those in the hospital birth registry. The diagnosis of PA is reliable, as the description of PA in the patient records had to fulfil the inclusion criteria of the study. In addition, many cases PA diagnoses were confirmed histologically. Possibly, the incidence of PA might have been underestimated, because some milder cases may have been missed.

Unfortunately, randomised trials concerning PA are not feasible. Because our cohort studies were retrospective, the birth register data were prone to some degree of under-reporting of some behavioural variables, such as smoking, alcohol and/or drug abuse and violence during pregnancy. On the other hand, most variables here were collected during pregnancy, thus evading the bias of misclassification of data collected after delivery. When interviewed by telephone, people may have a tendency to forget or be reluctant to report all details.

The poor neonatal outcome in our study has merely been overestimated, since milder cases of placntal abruption may have been missed. Moreover, a possible source of bias is that Kuopio University Hospital served as a tertiary referral centre for four central hospitals and two local hospitals as regards obstetric patients during the period of data collection, and thus some adverse outcomes may be over- represented.

Exclusion of multiple pregnancies and cases of placenta praevia helped us to focus on genetically susceptible cases rather than on cases at high risk of PA because of mechanical reasons. However, inclusion of trauma patients may lead to underestimation of the genetic basis of PA. Data on such patients may not be suitable inclusion criteria when searching for evidence of genetic risk factors of PA.

In our genetic studies (Studies IV and V) the gene bands have been sequenced with specific probes and therefore the nucleotide sequences are exactly those expected. We used valid statistical analyses and methods. Moreover, the power analysis gave us insight into the validity of the negativity of the eNOS association study results. Haplotype analysis, a method that we used in assessment of the role of the *EPHX* gene in PA, is considered advantageous compared with analyses based on individual SNPs, particularly in complex diseases (Morris and Kaplan, 2002). The genetic component in placental abruption is likely to be multifactorial in nature and environmental factors probably modify the disease risk individually. Furthermore, as the placenta is fetal tissue and

therefore contains fetal DNA, fetal genetic variants may also play a role in PA, and the maternal-fetal interaction should be considered in future studies. The number of PA cases was limited and the statistical power to show clear associations between individual polymorphisms and a disease as rare as PA is weak with a study population of this size.

6.3. Clinical and statistical significance

Placental abruption contributes to a great many cases of prematurity and perinatal asphyxia and these have extensive and expensive short- and long-term effects on the affected infants, their families and on society. For most women PA is a worrying experience and many of them are reluctant to become pregnant again (Rasmussen *et al.* 1997). We found that if there is no recurrence of PA the obstetric prognosis in following pregnancies is nearly comparable to that of the general obstetric population. This should be comforting information for women who have had such a traumatic obstetric experience and who are considering pregnancy again. On the other hand, the sisters of index patients with recurrent placental abruption have an increased risk of PA and thus their pregnancies may warrant close surveillance as well.

Since the recurrence rate of PA is as high as 11.9%, these patients should be identified, and according to speculations by Salim *et al*. (2008), subsequent pregnancies should be closely followed up to reduce possible adverse outcomes and minimize the damage. Preferably, at two to six weeks before the gestational time at which the previous abruption occurred, these mothers should be located near the obstetric hospital, with adequate paediatric and anaesthesiological services available and a NICU within reach (Rasmussen *et al*. 2001).

Although information about the genetic risk factors of PA might be important to the individuals who carry susceptibility genes, this is of limited significance in terms of public health, so far. Phenotypic diversity and multiple and large spectra of differing symptoms already make the diagnosis of PA difficult. Moreover, the recognition of fetal compromise within low-risk pregnancies is especially challenging.

Should there be any behavioural risk factors to be avoided, such as smoking, pregnant women should be encouraged to change their habits to diminish the risk and recurrence of PA. However, since 10 to15% of women smoke during their pregnancies, these cannot be considered risk pregnancies for PA just because of smoking, unless they carry other risk factors as well. On the other hand, a bleeding pregnant woman with risk factors such as smoking or pre-eclampsia should make the clinician think about the increased possibility of PA.

Power analysis as regards the eNOS gene showed that more than 14,500 placental abruption cases should have been enrolled to achieve statistical significance with a power of 80% and an α-value of 0.05. This means that it is highly unlikely that our results were false-negative, since in order to find proof for contrary results, more than ten thousand cases would have been needed in the study. The significance of this genetic research is to improve general scientific knowledge of genetic epidemiology of placental abruption as a basis for further research.

6.4. Generalizability

The results of the population-based cohort analysis (Study I) were well applicable to the general Finnish population of reproductive-aged women and their offspring. The data as regards PA cases in other Finnish tertiary referral hospitals are similar to our findings at Kuopio University Hospital (Tikkanen *et al*. 2006a), since there seemed not to be great variation in the incidences of PE, Caesarean section or PNM in these recent Finnish studies. Not all studies carried out in the USA or the UK are applicable to the Finnish population because of different life styles and heterogeneity of populations compared with the mainly homogeneous Caucasian population of Finland. Moreover, the recurrence risk in the families is relatively low.

There are no clinical applications so far as regards the genetic studies (Studies IV and V) of PA. As yet, there are no feasible specific DNA tests for genetic susceptibility to PA.

6.5. Future perspectives – suggestions for future research and clues to novel candidate genes

In a clinical setting there may be an increase in the number of PA cases in the future, since there seems to be an increasing trend as regards contributing risk factors, such as advanced maternal age, assisted reproduction treatments and increased Caesarean section rates, together with more smoking among fertile-aged women and an increasing number of illicit drug abusers.

To gain more statistical power to show clear associations between individual polymorphisms and a disease as rare as PA, considerably larger study populations would be needed than that have been used previously. Careful patient selection might also produce more relevant results. Recruitment at the time of diagnosis would be more likely to minimize phenotypic heterogeneity than retrospectively retrieved data from possibly incomplete or unreliable medical records. Allele frequencies of many polymorphisms vary between patients of different ethnicity (Chappell and Morgan, 2006; Zdoukopoulos and Zintzaras, 2008). The results of genome-wide screens can

suggest novel hypotheses, and candidate gene studies can be used to test hypotheses suggested by pathophysiology or global screening strategies. Determining gene-disease associations may prove to be a target for therapeutic and/or preventative strategies (Chappell and Morgan 2006).

There have been no genome-wide scanning studies on placental abruption so far. Placental abruption seems to be genetically heterogeneous and not inherited in a simple Mendelian manner. The inconsistency of results of genetic association studies on PA may imply that the associations are likely to be modest for individual polymorphisms. Considering the multifactorial nature of PA and gene-gene and gene-environment interactions, larger studies are needed for power improvement and/or pooling of data using meta-analysis (Chappell and Morgan, 2006; Zdoukopoulos and Zintzaras, 2008). Genome-wide scans to date have been focused mainly on maternal genotype, and more research involving both the maternal and fetal genotypes and genotypic interactions at multiple loci is needed in the future (Chappell and Morgan, 2006).

Studies of gene expression profiles (with microarray techniques) are considered to hold great promise. Yet the question concerns transcriptomics or proteomics with transient disorders that are apparent only during pregnancy, since the genes expressed in the placenta may be the cause or consequence of the disease. In addition, obtaining matched control samples without confounding factors, such as infection, may be difficult (Chappell and Morgan, 2006). So far there have been no studies of placental abruption conducted by using microarray techniques.

To gain statistical power for genetic studies, the study populations should consist of hundreds of thousands of individuals to obtain enough cases. For example, collecting recurrent PA cases from all the Nordic countries based on national birth registers could offer a wider pool to be studied via genome-wide screening. Multicentre collaboration of clinicians, geneticists, epidemiologists, bioinformaticians and biostatisticians is needed to establish large enough DNA resources to conduct meaningful meta-analyses and solve complex problems of haplotype estimation, gene-gene and fetal-maternal genotype interactions (Chappell and Morgan, 2006).

7. SUMMARY AND CONCLUSIONS

I. The overall incidence of placental abruption was low (0.74%). Significant reproductive risk factors were pre-eclampsia, grand multiparity, velamentous umbilical cord insertion, cigarette smoking, prior fetal demise, advanced maternal age (> 35 years) and previous miscarriage. The outcome of PA remains relatively poor: the prematurity rate was ten times higher and the perinatal mortality rate was fifteen times higher than in the normal obstetric population.

II. The recurrence rate was considerable, 11.9%, about one in ten placental abruptions.

III. The risk of PA in the first-degree relatives of index patients appeared to cluster in the families with recurrent placental abruption.

IV. The low-activity haplotype of the microsomal epoxide hydrolase gene (C-A His113-His139) was protective against placental abruption.

V. The Glu298Asp polymorphism in the eNOS gene was not associated with PA in the Finnish population.

The genetic effect is probably most prominent in women without clinical risk factors, i.e. young and non-smoking women without pre-eclampsia. Those women with recurrent PA appeared to carry a high inherited liability, probably representing the group which is most likely to carry susceptibility genes for PA. In future studies considerable efforts should be made to minimize phenotypic heterogeneity to enable the identification of genetic associations among promising candidate genes.

Most cases of placental abruption seemed to be sporadic, with no family history and no recurrence. The genetic component appeared to be modest. Despite the assessement of risk factors, much remains unclear about the underlying disease mechanisms of PA and the disease is still unpredictable and unpreventable.

8. REFERENCES

Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A. Transfer of proinflammatory cytokines across term placenta. Obstet Gynecol 2005;106:802-7.

Addis A, Moretti M E, Syed F A, Einarson T R, Koren G. Fetal effects of cocaine: an updated meta-analysis. Reprod Toxicol 2001;15:341-369.

Agorastos T, Karavida A, Lambropoulos A, Constantinidis T, Tzitzimikas S, Chrisafi S, Saravelos H, Vavilis D, Kotsis A, Bontis J. Factor V Leiden and prothrombin G20210A mutations in pregnancies with adverse outcome. J Matern Fetal Neonatal Med 2002;12:267-273.

Aitokallio-Tallberg A, Halmesmäki E. Motor vehicle accident during the second or third trimester of pregnancy. Acta Obstet Gynecol Scand 1997;76:313-317.

Allred L S, Batton D. The effect of placental abruption on the short-term outcome of premature infants. Am J Perinatol 2004;3:157-62.

Alfirevic Z, Mousa H A, Martlew V, Briscoe L, Perez-Casal M, Toh C H. Postnatal screening for thrombophilia in women with severe pregnancy complications. Obstet Gynecol 2001;79:753-759.

Ananth C V, Berkowitz G S, Savitz D A, Lapinski R H. Placental abruption and adverse perinatal outcomes. JAMA 1999a;282:1646-51.

Ananth C V, Demissie K, Hanley M L. Birth weight discordancy and adverse perinatal outcomes among twin gestations in the United States: The effect of placental abruption. Am J Obstet Gynecol 2003;188:954-60.

Ananth C V and Cnattingius S. Influence of maternal smoking on placental abruption in successive pregnancies: a population-based prospctive cohort study in Sweeden. Am J Epidemiol 2007;166:289-295.

Ananth C V, Elsasser D, Kinzler W L, Peltier M R, Getahun D, Leclerc D, Rozen R R. Polymorphisms in methionine synthase reductase and betaine-homocysteine S-methyltransferase genes: Risk of placental abruption. Mol Genet Metab 2007c;91:104-110.

Ananth C V, Getahun D, Peltier M R, Smulian J C. Placental abruption in term and preterm gestations – evidence for heterogeneity in clinical pathways. Obstet Gynecol 2006b;107:785-92.

Ananth C V, Oyelese Y, Prasad V, Getahun D, Smulian J C. Evidence of placental abruption as a chronic process: associations with vaginal bleeding early in pregnancy and placental lesions. Eur J Obstet Gynecol Reprod Biol 2006a;128:15-21.

Ananth C V, Oyelese Y, Srinivas N, Yeo L, Vintzileos A M. Preterm premature rupture of membranes, intrauterine infection, and oligohydramnios: Risk factors for placental abruption. Obstet Gynecol 2004;104:71-7.

Ananth C V, Oyelese Y, Yeo L, Pradhan A, Vintzileos A M: Placental abruption in the United States, 1979 through 2001: temporal trends and potential determinants. Am J Obstet Gynecol 2005a; 192:191-8.

Ananth C V, Peltier M R, Chavez M R, Kirby R S, Getahun D, Vintzileios A M. Recurrence of ischemic placental disease. Obstet Gynecol 2007a;110:128-33.

Ananth C V, Peltier M R, De Marco C, Elsasser D A, Getahun D, Rosen R, Smulian J C for the New Jersey-Placental Abruption Study Investigators. Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption. Am J Obstet Gynecol 2007d;197:385.e1-385.e7.

Ananth C V, Peltier M R, Kinzler W L, Smulian J C, Vintzileos A M. Chronic hypertension and risk of placental abruption: is the association modified by ischemic placental disease? Am J Obstet Gynecol 2007b; 197:273.e1-273.e7.

Ananth C V, Peltier M R, Moore D F, Kinzler W L, Leclerc D, Rozen R R for the New Jersey-Placental Abruption Study Investigators. Reduced folate carrier 80A→G polymorphism, plasma folate, and risk of placental abruption. Hum Genet 2008;124:137-145.

Ananth C V, Smulian J C, Demissie K, Vintzileios A M, Knuppel R A: Placental Abruption among Singleton and Twin Births in the United States: Risk Factor Profiles. Am J Epidemiol 2001b;153: 771-8.

Ananth C V, Smulian J C, Vintzileos A M. Incidence of placental abruption in relation to cigarette smoking and hypertensive disorders during pregnancy: a meta-analysis of observational studies. Obstet Gynecol 1999b;93:622-8.

Ananth C V, Smulian J C, Srinivas N, Getahun D, Salihu H M. Risk of infant mortality among twins in relation to placental abruption: contributions of preterm birth and restricted fetal growth. Twin Res Hum Genet 2005b;8:524-531.

Ananth C V, Wilcox A J. Placental abruption and perinatal mortality in theUnited States. Am J Epidemiol 2001a;153:332-7.

Ananth C V, Wilcox A J, Savitz D A, Bowes W A, Luther E R. Effect of maternal age and parity on the risk of uteroplcental bleeding disorders in pregnancy. Obstet Gynecol 1996;88:511-6.

Ariel I, Anteby E, Hamani Y, Redline R W. Placental pathology in fetal thrombophilia. Hum Pathol 2004;35:729-33.

Arngrimsson R, Hayward C, Nadaud S, Baldursdottir A, Walker J J, Liston W A, Bjarnadottir R I, Brock D J H, Geirsson R T, Connor J M, Soubrier F. Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region. Am J Hum Genet 1997;61:354-362.

Atrash H K, Koonin M L, Lawson H W, Franks A L, Smith J C. Maternal mortality in the United States 1979-1986. Obstet Gynecol 1990;76:1055-60.

Babinszki A, Kerenyi T, Torok O, Grazi V, Lapinski R H, Berkowitz R L. Perinatal outcome in grand and great-grand multiparity: Effects of parity on obstetric risk factors. Am J Obstet Gynecol 1999;181:669-74.

Bailey B A, Sokol R J. Pregnancy and alcohol use: evidence and recommendations for prenatal care. Clin Obstet Gynecol 2008;51:436-44.

Bardy A H, Seppälä T, Lillesunde P, Kataja J M, Koskela P, Pikkarainen J, Hiilesmaa V K. Objectively measured tobacco exposure during pregnancy: neonatal effects and relation to maternal smoking. BJOG 1993;100:721-726.

Barron F, Hill W C. Placenta previa, placenta abruption. Clin Obstet Gynecol 1998;41:527-32.

Baumann P, Blackwell S C, Schild C, Berry S M, Friedrich H J. Mathematic modelling to predict abruptio placentae. Am J Obstet Gynecol 2000;183:815-22.

Becher J-C, Bell J E, Keeling J W, Liston W A, McIntosh N, Wyatt B. The Scottish Perinatal Neuropathology study – clinicopathological correlation in stillbirths. BJOG 2006; 113:310-317.

Bhattacharya S, Campbell DM. The incidence of complications of pre-eclampsia. Hypertens Pregnancy 2005;24:181-90.

Black M, Shetty A, Bhattacharya S. Obstetric outcomes subsequent to intrauterine death in the first pregnancy. BJOG 2008;115:269-274.

Bond A L, Edersheim T G, Curry L, Druzin M L, Hutson J M. Expectant management of abruptio placentae before 35 weeks of gestation. Am J Perinatol 1989;2:121-123.

Burton G J, Jauniaux E. Placental oxidative stress: from miscarriage to pre-eclampsia. J Soc Gynecol Investig 2004;11:342-52.

Cahill A G, Bastek J A, Stamilio D M, Odibo A O, Stevens E, Macones G A. Minor trauma in pregnancy – is the evaluation unwarranted? Am J Obst Gynecol 2008;198:208-9.

Calderon-Margalit R, Friedlander Y, Yanetz R, Deutsch L, Manor O, Harlap S, Paltiel O. Late stillbirths and long-term mortality of mothers. Obstet Gynecol 2007;109:1301-8.

Campbell D M, Templeton A. Maternal complications of twin pregnancy. Int J Gynaecol Obstet 2004;84:71-73.

Cavkaytar S, Ugurlu E N, Karaer A, Tapisiz O L, Danisman N. Are clinical symptoms more predictive than laboratory parameters for adverse maternal outcome in HELLP syndrome? Acta Obstet Gynecol Scand 2007;86:648-51.

Cederholm M, Haglund B, Axelsson O. Maternal complications following amniocentesis and chorionic villus sampling for prenatal karyotyping. BJOG 2003;110:392-9.

Chandra S, Scott H, Dodds L, Watts C, Blight C, Van Den Hof M. Unexplaned elevated maternal serum alpha-fetoprotein and/or human chorionic gonadotropin and the risk of adverse outcomes. Am J Obstet Gynecol 2003; 189:775-781.

Chang Y L, Chang S D, Cheng P J. Perinatal outcome in patients with placental abruption with and without antepartum haemorrhage. Int J Gynaecol Obstet 2001;75:193-194.

Chappell S, Morgan L. Searching for genetig clues to the causes of pre-eclampsia. Clin Sci 2006;110:443-458.

Church M W, Subramanian M G. Cocaine's lethality increases during late gestation in the rat: A study of "critical periods" of exposure. Am J Obstet Gynecol 1997:176.901-6.

Clark S L. Placenta previa and abruptio placentae. In: Maternal Fetal Medicine: Principles and practice, 5th ed, 2004. Creasy R K, Resnik R (Eds), W B Saunders Company.

Cnattingius S. Maternal age modifies the effect of maternal smoking on intrauterine growth retardation but not on late fetal death and placental abruption. Am J Epidemiol 1997;145:319-23.

Cnattingius S, Akre O, Lambre M, Ockene J, Granath F. Will an adverse pregnancy outcome influence the risk of continued smoking in the next pregnancy? Am J Obstet Gynecol 2006;195:1680-6.

Collaris R J, Oei S G. External cephalic version: a safe procedure? A systematic review of version-related risks. Acta Obstet Gynecol Scand 2004;83:511-518.

Connolly A, Katz V L, Bash K L, McMahon M J, Hansen W F.Trauma and pregnancy. Am J Perinatol 1997;14:331-6.

Coputo F, Trevisani F, Bernardi M. Alcohol misuse and traffic accidents. Lancet 2007;10;369:463-4.

Coronado G D, Marshall L M, Schwartz S M. Complicatins in pregnancy, labour, and delivery with uterine leiomyomas: a population-based study. Obstet Gynecol 2000;9:764-9.

Cunningham F G, Gant N F, Leveno K L, Gilstrap III L C, Hauth J C, Wensrtom K D. Williams Obstetrics. 21st ed, 2001. McGraw Hill.

Dahmus M A, Sibai B M. Blunt abdominal trauma: Are there any predictive factors for abruptio placentae or maternal-fetal distress? Am J Obstet Gynecol 1993;169:1054-9.

Daly A K, Day C P.Candidate gene case-control association studies: advantages and potential pitfalls. Br J Clin Pharmacol 2001;52:489-99.

Daltveit A K, Tollånes M C, Pihlström H, Irgens L M. Caesarean delivery and subsequent pregnansies. Obstet Gynecol 2008;111:1327-34.

Dammann O, Leviton A, Gappa M, Dammann C E L. Lung and brain damage in preterm newborns, and their association with gestational age, prematurity subgroup, infection/inflammation and long term outcome. BJOG 2005;112:Suppl 1, S4-S9.

Derric M, Luo N L, Bregman J C, Jilling T, Ji X, Fisher K, Gladson C L, Beardsley D J, Murdoch G, Back S A, Tan S. Preterm fetal hypoxia-ischaemia causes hypertonia and motor deficits in the neonatal rabbit: a model for human cerebral palsy? J Neurosci 2004;24:24-34.

Dhanraj D, Lambers D: The incidences of positive Kleihauer-Betke test in low-risk pregnancies and maternal trauma patients. Am J Obstet Gynecol 2004;190:1461-3.

Di Simone N, Maggiano N, Caliandro D, Riccardi P, Evangelista A, Carducci B, Caruso A. Homocysteine induces trophpblast cell death with apoptotic features. Biol Reprod 2003;69:1129-34.

Dizon-Townson D, Miller C, Sibai B, Spong C Y, Thom E, Wendel Jr G, Wenstrom K, Samuels P, Cotroneo M A, Moawad A, Sorokin Y, Meis P, Miodovnik M, O'Sullivan M J, Conway D, Wapner R J, Gabbe S G. The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus. Obstet Gynecol 2005;106:517-24.

Dugoff L, Hobbins J C, Malone F D, Porter T F, Luthy D, Comstock C H, Hankins G, Berkowitz R L, Merkatz I, Craigo S D, Timor-Tritsch I E, Carr S R, Wolfe H M, Vidaver J, D'Alton M E. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER trial). Am J Obstet Gynecol 2004;191:1446-51.

Dupre A R, Morrison J C, Martin J N Jr, Floyd R C, Blake P G. Clinical application of the Kleiheuer-Betke test. J Reprod Med 1993;38:621-4.

El Kady D, Gilbert W M, Anderson J, Danielsen B, Towner D, Smith L H. Trauma during pregnancy: An analysis of maternal and fetal outcomes in a large population. Am J Obstet Gynecol 2004;190:1661-8.

Emery C L, Morway L F, Chung-Park M, Wyatt-Ashmead, Sawady J, Beddow T D. The Kleihauer-Betke test; clinical utility, indication, and corrreltion in patients with placental abruption and cocaine use. Arch Pathol Lab Med 1995;119:1032-7.

Engelsen I B, Albrechtsen S, Iversen O E. Peripartum hysterectomy-incidence and maternal morbidity. Acta Obstet Gynecol Scand 2001;80:409-412.

Eriksen G, Wohlert M, Ersbak V, Hvidman L, Hedegaard M, Skajaa K. Placental Abruption. A case-control investigation. BJOG 1991;98:448-452.

Eskes T K A B. Clotting disorders and placental abruption: Homocysteine – a new risk factor. Eur J Obstet Gynecol Reprod Biol 2001;95:206-12.

Faccinetti F, Marozio L, Grandone E, Pizzi C, Volpe A, Benedetto C. Thrombotic mutations are a main risk factor for placental abruption. Haematologica 2003;88:785-788.

Faye-Petersen O M, Heller D S, Joshi V V, editors. Second edition, 2006. Gross abormalities of the placenta: lsions due to disturbances of maternal and of fetal blood flow.In: Handbook of placental pathology. Oxon,UK: Taylor & Francis.

Floyd R L, Ebrahim S, Tsai J, O'Connor M, Sokol R. Strategies to reduce alcohol-exposed pregnancies. Matern Child Health J 2006;10:S149-S151.

Furchgott R F, Zawadzki J V. The obligatory role of endithelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-376.

Furuhashi M, Kurauchi O, Suganuma N. Pregnancy following placental abruption. Arch Gynecol Obstet 2002;267:11-13.

Gabriel K, Hofmann C, Glavas M, Weinberg J. The hormonal effects of alcohol use on the mother and fetus. Alcohol Health Res World 1998;22:170-7.

Geipel A, Ludwig M, Germer U, Katalinic A, Diedrich K, Gembruch U. Uterine artery Doppler velocimetry and the outcome of pregnancies resulting from ICSI. Hum Reprod 2001;16:1397-402.

Gebhardt G S, Scholtz C L, Hillermann R, Odendaal H J. Combined heterozygosity for methylenetetrahydrofolate reductase (MTHFR) mutations C677T and A1298C is associated with abruptio placentae but not with intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol 2001;97:174-177.

Getahun D, Ananth C V, Peltier M R, Smulian J C, Vintzileios A M. Acute and chronic respiratory diseases in pregnancy: associations with placental abruption. Am J Obstet Gynecol 2006b;195:1180-4.

Getahun D, Oyelse Y, Salihu H M, Ananth C V. Previous Caesarean delivery and risks of placenta previa and placental abruption. Obstet Gynecol 2006a; 107:771-8.

Ghidini A, Salafia C M. Gender differences of placental dysfunction in severe prematurity. BJOG 2005;112:140-144.

Glantz C, Purnell L. Clinical utility of sonography in the diagnosis and treatment of placental abruption. J Ultrasound Med 2002;21:837-40.

Granger J P, Alexander B T, Bennett W A, Khalil R A. Pathophysiology of pregnancy induced hypertension. Am J Hypertens 2001;14:178S-185S.

Halmesmäki E. Alcohol councelling of 85 pregnant problem drinkers: effect on drinking and fetal outcome. BJOG 1988;95:243-7.

Han Y W, Ikegami A, Bissada N F, Herbst M, Redline R W, Ashmead G G. Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. J Clin Microbiol 2006;44:1475-83.

Harrington K, Cooper D, Lees C, Hecher K, Campbell S. Doppler ultrasound of the uterine spiral arteries: the importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby. Ultrasound Obstet Gynecol 1996;7:182-8. O

Harris B A, Gore H, Flowers C E. Peripheral placental separation: a possible relationship to premature labour. Obstet Gynecol 1985;66:774-8.

Hartsfield J K Jr, Sutcliffe M J, Everett E T, Hassett C, Omiecinski C J, Saari J A. Assignment of microsomal epoxide hydrolase (EPHX) to human chromosome 1q42.1 by in situ hybridization. Cytogenet Cell Genet 1998;83:44-45.

Hassett C, Aicher L, Sidhu JS, Omiecinski C J. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. Hum Mol Genet 1994;3:421-428.

Heinonen S, Ryynänen M, Kirkinen P, Saarikoski S. Uterine malformation: a cause of elevated maternal serum alpha-fetoprotein concentrations. Perinat Diagn 1996a;16:635-9.

Heinonen S, Ryynänen M, Kirkinen P, Saarikoski S. Velamentous umbilical cord insertion may be suspected from maternal serum α-fetoprotein and hCG. Br J Obstet Gynaecol 1996b;103:209-13.

Heinonen S, Kirkinen P. Pregnancy outcome after previous stillbirth resulting from causes other than maternal conditions and fetal abnormalities. Birth 2000;27:33-37.

Heinonen S, Saarikoski S. Reproductive risk factors of fetal asphyxia at delivery: a population based analysis. J Clin Epidemiol 2001b; 54(4):407-10.

Heinonen S, Taipale P, Saarikoski S. Weights of placentae from small-for-gestational age infants revisited. Placenta 2001a;22:399-404.

Hemminki E, Meriläinen J. Long-term effects of Caesarean sections: Ectopic pregnancies and placental problems. Am J Obstet Gynecol 1996;174:1569-74.

Herman A, Weintraub Z, Bukovsky Y, Arieli S, Zabow P, Caspi E, Ron-El R. Dynamic ultrasonographic imaging of the third stage of labor: New perspective into third stage mechanism. Am J Ogstet Gynecol 1993;168:1496-9.

Herman A, Zimerman A, Arieli S, Tovbin Y, Bezer M, Bukovsky I, Panski M. Down-up sequential separation of the placenta. Ultrasound Obstet Gynecol 2002;19:278-281.

Hillermann R, Carelse K, Gebhart G S. The Glu298Asp variant of the endothelial nitric oxide synthase gene is associated with an increased risk for abruptio placentae in pre-eclampsia. J Hum Genet 2005;50:415-9.

Hira B, Pegoraro R J, Rom L, Govender T, Moodley J. Polymorphisms in various coagulation genes in black South African women with placenal abruption. BJOG 2002;109:574-5.

Hladky K, Yankowitz J, Hansen W F. Placental Abruption. Obstet Gynecol Surv 2002;57:299-305.

Hnat M D, Mercer B M, Thurnau G, Goldenberg R, Thom E A, Meis P J, Moawad A H, Iams J D, Van Dorsten J P. Perinatal outcomes in women with preterm rupture of membranes between 24 and 32 weeks of gestation and a history of vaginal bleeding. Am J Obstet Gynecol 2005;193:164-8.

Hodor J G, Poggi S H, Spong C Y, Goodwin K M, Vink J S, Pezzulio J C, Ghidini A. Risk of third-trimester amniocentesis: a case-control study. Am J Perinatol 2006;23:177-80.

Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S. Inhibition of low-density lipoprptein oxidation by nitric oxide. Potential role in atherogenesis. FEBS Lett 1993;334:170-174.

Holmgren P Å, Olofsson J I. Preterm premature rupture of membranes and the associated risk for placental abruption. Inverse correlation to gestational length. Acta Obstet Gynecol Scand 1997;76:743-747.

Huisjes H J, Touwen B C L, van Doorm J M. Perinatal mortality and late sequelae in children born after abruptio placentae. Eur J Obstet Gynecol Reprod Biol 1979;1:45-49.

Hulse G K, Milne E, English D R, Holman C D. Assessing the relationship between maternal cocaine use and abuptio placentae. Addiction 1997;92:1547-51.

Hung T H, Hsieh C C, Hsu J J, Lo L M, Chiu T H, Hsieh T T. Risk factors for placental abruption in an Asian population. Reprod Sci 2007;14:59-65.

Huang P L, Huang Z, Mashimo H, Bloch K D, Moskowitz M A, Bevan J A, Fishman M C. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 1995;377:239-242.

Häkli T, Romppanen E L, Hiltunen M, Helisalmi S, Punnonen K, Heinonen S. Endothelial nitric oxide synthase polymorphism in pre-eclampsia. J Soc Gynecol Investig 2003;10:154-7.

Høgberg V, Rasmussen S, Irgens L M. The effect of smoking and hypertensive disorders on abruptio placentae in Norway 1999-2002. Acta Obstet Gynecol Scand 2007;86:304-309.

Infante-Rivard C, Rivard G-E, Yotov W V, Génin E, Guiguet M, Weinberg C, Gauthier R, Feoli-Fonseca J C. Absence of association of thrombophilia polymorphisms with intrauerine growth restriction. NEJM 2002;347:19-25.

Jaffe M H, Schoen W C, Silver T M, Bowerman R A, Stuck K J. Sonography of abruption placentae. AJR 1981;137:1049-54.

Jakobsson M, Gissler M, Paavonen J, Tapper AM. The incidence of preterm deliveries decreases in Finland. BJOG 2008;115:38-43.

Ji Y, Ferracci G, Warley A, Ward M, Leung K-Y, Samsuddin S, Leveque C, Queen L, Reebye V, Pal P, GkaliagKousi E, Seager M, Ferro A. Beta-actin regulates platelet nitric oxide synthase 3 activity through interaction with heat shock protein 90. Proc Nat Acad Sci 2007;104:8839-8844

Joseph K S, Allen AC, Dodds L, Turner L A, Scott H, Liston R.The perinatal effects of delayed childbearing. Obstet Gynecol 2005;105:1410-8.

Juntunen K, Makarainen L, Kirkinen P. Outcome after a high number (4-10) of repeated Caesarean sections. BJOG 2004;111:561-3.

Järvenpää J, Pakkila M, Savolainen E-R, Perheentupa A, Järvelä I, Ryynänen M. Evaluation of Factor V Leiden, Prothrombin and Methylenetetrahydrofolate reductase gene mutations in patients with sever pregnancy complications in Northern Finland. Gynecol Obstet Invest 2006;62:28-32.

Jääskeläinen E, Keski-Nisula L, Toivonen S, Romppanen E-L, Helisalmi S, Punnonen K, Heinonen S. MTHFR C677T polymorphism is not associated with placental abruption or pre-eclampsia in Finnish women. Hypertens Pregnancy 2006;25:73-80.

Jääskeläinen E, Toivonen S, Keski-Nisula L, Paattiniemi E-L, Helisalmi S, Punnonen K, Heinonen S. CTLA-4 polymorphism 49-G is associated with placental abruption and pre-eclampsia in Finnish women. Clin Chem Lab Med 2008a;46:169-173.

Jääsleläinen E, Keski-Nisula L, Toivonen S, Paattiniemi E-L, Helisalmi S, Punnonen K, Heinonen S. Polymorphism of the interleukin 1 receptor antagonist (IL1Ra) gene and placental abruption. J Reprod Immunol 2008b;79:58-62.

Kaminsky L M, Ananth C V, Prasad V, Nath C, Vintzileios A M.The influence of maternal cigarette smoking on placental pathology in pregnancies complicated by abruption. Am J Obstet Gynecol 2007;197:275-277.

Kaminski M, Rumeau C, Schwartz D. Alcohol consumption in pregnant women and the outcome of pregnancy. Alcohol Clin Exp Res 1978;2:155-63.

Kayani S I, Walkinshaw SA, Preston C: Pregnancy outcome in severe placental abruption. BJOG 2003; 110:679-683.

Keeling J W. Fetal and Neonatal Pathology, 3rd edition, 2002, Springer-Verlag.

Khatun S, Kobayashi T, Belayet H, Sumimoto K, Iwaki T, Kanayama N. Local cold stress to the soles induces retroplacental haemorrhage in rats. Semin Thromb Hemost 2001;27:79-86.

Khong T Y. Placental vascular development and neonatal outcome. Semin Neonatol 2004;9:255-63.

Kikutani M, Ishihara K, Araki T: Value of Ultrasonography in the Diagnosis of Placental Abruption. J Nippon Med Sch 2003; 70:227-233.

Kiss H, Schneeberger C, Tschugguel W, Lass H, Huber J C, Husslein P, Knöfler M. Expression of endothelial (type III) nitric oxide synthase in cytotrophoblastic cell lines: regulation by hypoxia and inflammatory cytokines. Placenta 1998;19:603-11.

Kobayashi T, Terao T, Maki M, Ikenoue T. Diagnosis and management of acute obstetrical DIC. Semin Thromb Hemost 2001;21:161-167.

Konje J C, Taylor D J. Bleeding in late pregnancy. In: James D K, Steer P J, Weiner C P, Gonik B, editors. High risk pregnancy: management options, 3rd ed, 2006. Elsevier Saunders Co.

Korhonen S, Romppanen E-L, Hiltunen M, Helisalmi S, Punnonen K, Hippeläinen M Heinonen. Two exonic single nucleotide polymorphisms in the microsomal epoxide hydrolase gene are associated with polycystic ovary syndrome. Fertil Steril 2003;6:1353-7.

Koyama S, Kaga K, Sakata H, Iino Y, Kodera K: Pathological findings in the temporal bone of newborn infants with neonatal asphyxia. Acta Otolaryngol 2005;125:1028-32.

Kramer M S, Usher R H, Pollack R, Boyd M, Usher S. Etiologic determinants of abruptio placentae. Obstet Gynecol 1997;89:221-6.

Kuczkowsky K M. The effects of drug abuse on pregnancy. Curr Opin Obstet Gynecol 2007;19:578-85.

Kuczynski J, Uszynski W, Zekanowska E, Soszka T, Uszynski M. Tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in the placenta and myometrium. Eur J Obstet Gynecol Reprod Biol 2002;105:15-19.

Kupfermic M J, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing J B. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med. 1999:340:9-13.

Kupfermic M J, Peri H, Zwang E, Yaron Y, Wolman I, Eldor A. High prevalence of the prothrombin gene mutation in women with intrauterine growth retardation, abruptio placentae and second trimester loss. Acta Obstet Gynecol Scand 2000;79:963-967.

Kyrklund-Blomberg N B, Gennser G, Cnattingius S: Placental abruption and perinatal death. Paediatr Perinat Epidemiol 2001;15:290-297.

Kåregård M, Gennser G. Incidence and recurrence rate of abruption placentae in Sweden. Obstet Gynecol 1986;67:523-8.

Källén B, Finnström O, Nygren K G, Otterblad Olausson B, Wennerholm U-B: In vitro fertilisation in Sweden: obstetric characteristics, maternal morbidity and mortality. BJOG 2005; 112:1529-35.O

Laasanen J, Romppanen E-L, Hiltunen M, Helisalmi S, Mannermaa A, Punnonen K, Heinonen S. Two exonic single nucleotide polymorphisms in the microsomal epoxide hydrolase gene are jointly associated with pre-eclampsia. Eur J Hum Genet 2002;10:569-73.

Lancastr J M, Brownlee H A, Bell D A, Futreal P A, Marks J R, Berchuck A, Wiseman R W, Taylor J A. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. Mol Carsinog 1996;17:10-2.

Leone Pak L, Reece E A, Chan L: Is adverse pregnancy outcome predictable after blunt abdominal trauma? Am J Obstet Gynecol 1998;179:1140-4.

Levy S, Sutton G, Ng P C, Feuk L, Halpern A L, Walenz B P, Axelrod N, Huang J, Kirkness E F, Denisov G, Lin Y, MacDonald J R, Wing Chun Pang A, Shago M, Stockwell T B, Tsiamouri A, Bafna V, Bansal V, Kravitz S A, Busam D A, Beeson K Y, McIntosh T C, Remington K A, Abril J F, Gill J, Borman J, Rogers Y-H, Frazier M E, Scherer S W, Strausberg R L, Venter J C. The diploid genome sequence of an individual human. PloS Biol 2007;5:e254.

Li D K, Wi S. Maternal placental abnormality and the risk of sudden infant death syndrome. Am J Epidemiol 1999;149:608-11.

Lindqvist P G, Happach C. Risk and risk estimation of placental abruption. Eur J Obstet Gynecol Reprod Biol 2006;126:160-4.

Linhart Y, Bashiri A, Maymon E, Shoham-Vardi I, Furman B, Vardi H, Mazor M. Congenital anomalies are an independent risk factor for neonatal morbidity and perinatal mortality in preterm birth. Eur J Obstet Gynecol Reprod Biol 2000;90:43-49.

Lipsky S, Caetano R, Field C A, Larkin G A. Is there a relationship between victim and partner alcohol use during an intimate partner violence event? Findings from an urban emergency department study of abused women. J Stud Alcohol 2005;66:407-412.

Little B B, Snell L M, Trimmer K J, Ramin S M, Ghali F, Blakely C A, Garret A. Peripartum cocaine use and adverse pregnancy outcome. Am J Hum Biol 1999;11:598-602.

Lockwood C J, Toti P, Arcuri F, Paidas M, Buchwalder L, Krikun G, Schatz F. Mechanisms of abruption-induced premature rupture of the fetal membranes. Thrombin-enhanced interleukin-8 expression in term deciduas. Am J Pathol 2005;167:1443-9.

Lydon-Rochelle M, Holt V L, Easterling T R, Martin D P. First-birth Caesarean and placental abruption or previa at second birth. Obstet Gynecol 2001;97:765-9.

MacDorman M F, Menacer F, Declerq E. Caesarean birth in United States: Epidemiology, trends and outcomes. Clin Perinat 2008;2:293-308.

Magriples U, Chan D W, Bruzec D, Copel J A, Hsu C-D: Thrombomodulin: A New Marker for Placental Abruption. Thromb Hemost 1999;81: 32-4.

Major C A, de Veciana M, Lewis D F, Morgan M A. Preterm premature rupture of membranes and abruption placentae: Is there an association between these pregnancy complications? Am J Obstet Gynecol 1995;172:672-6.

Matsuda Y, Maeda T, Kouno S. Fetal/Neonatal outcome in abruptio placentae during preterm gestation. Semin Thromb Hemost 2005;l31:327-333.

Mattox K L, Goetzl L. Trauma in pregnancy. Crit Care Med 2005;33 [Suppl.]:S385-S389.

Mayhew TM. Villous trophoblast of human placenta: a coherent view of its turnover, repair and contributions to villous development and maturation. Histol Histopathol 2001;16:1213-1224.

Meis P J, Klebanoff M, Thom E, Dombrowski M P, Sibai B, Moawadi A H, Spong C Y, Hauth J C, Miodovnik M, Varner M W, Leveno K J, Caritis S N, Iams J D, WapnerR J, ConwayD, O'Sullivan M J, Carpenter M, Mercer B, Ramin S M, Thorp J M, Peaseman A M, Gabbe S; National institute of child health and human development maternal-fetal medicine units network. Prevention of recurrent premature delivery by 17-alpha-hydroxyprogesterone caproate, NEJM 2003;348:2379-2385.

Merz E. Ultrasound in Gynecology and Obstetrics, Textbook and Atlas. Thieme Medical Publishers, Inc. Stuttgart 1991.

Mikkola K, Tommiska V, Salokorpi T, Lehtonen L, Tammela O, Pääkkönen L, Olsen P, Korkman M, Fellman V: Neurodevelopmental outcome at 5 years of age of a national cohort of extremely low birth weight infants who were born in 1996-1997. Pediatrics 2005; 116:1391-1400.

Miyata M, Kudo G, Lee Y-H, Yang T J, Gelboin H V, Fernandez-Salguero P, Kimura S, Gonzalez F J. Targeted disruption of the microsomal epoxide hydrolase gene: Microsomal epoxide hydrolase is required for carsinogenic activity of 7,12-dimethylbenz[alpha]anthrasene. J Biol Chem 1999;274:23963-8.

Mooney E E, Boggess K A, Herbert W N P, Layfield L J. Placental pathology in patients using cocaine: an observational study. Obstet Gynecol 1998;91:925-9.

Morris R W, Kaplan N L. On the advantage of haplotype analysis in the presens of multiple disease susceptibility alleles. Genet Epidemiol 2002;23:221-233.

Nagayama C, Suzuki S. Influence of position on outcome in patients with placental abruption. J Nippon Med Sch.2006;73:351-3.

Nagy S, Bush M, Stone J, Lapinski R H, Gardó S. Clinical significance of subchorionic and retroplacental haematomas detected in the first trimester of pregnancy. Obstet Gynecol 2003;102:94-100.

Naidu C A, Moodley J, Pegoraro R. Methylenetetrahydrofolate (MTHFR) reductase gene polymorphism in African women with abruptio placentae. Eur J Obstet Gynecol Reprod Biol. 2007;132:241-243.

Nakatsuka M, Asagiri K, Kimura Y, Kamada Y, Tada K, Kudo T. Generation of peroxynitrite and apoptosis in placenta of patients with chorioamnionitis: possible implications in placental abruption. Hum Reprod 1999;14:1101-6.

Nam S Y, Kim J H, Cho C K, Yoo S Y, Kim S G. Enhancement of radiation-induced hepatic microsomal epoxide hydrolase gene expression by Oltipraz in rats. Radiat Res 1997;147:613-20.

Nassar N, Roberts C L, Barratt A, Bell J C, Olive E C, Peat B: Systematic review of adverse outcomes of external cephalic version and persisting breech presentation at term. Paediatr Perinat Epidemiol 2006; 20: 163-171.

Nath C A, Ananth C V, Smulian J C, Shen-Schwarz S, Kaminsky L. Histologic evidence of inflammation and risk of placental abruption. Am J Obstet Gynecol 2007;197:319-21.

Nath C A, Ananth C V, DeMarco C, Vintzileos A M. Low birthweight in relation to placental abruption and maternal thrombophilia status. Am J Obstet Gynecol. 2008;198:293.e1-293.e5.

Neiger R, Krohn H J, Troflatter M O. Plasma fibrin D-dimer in pregnancies complicated by partial placental abruption. Tennessee Medicine 1997;10:403-5.

Neilson J P. Interventions for treating placental abruption .The Cochrane Database of Systematic Reviews 2003, Issue 1.Art. No.: CD003247. DOI: 10.1002/14561858.CD003247.

Nilsen R M, Vollset S E, Rasmussen S A, Ueland P M, Daltveit A K. Folic acid and multivitamin supplement use and risk of placental abruption: A population-based registry study. Am J Epidemiol 2008;167:867-874.

Nielsen H S, Mogensen M, Steffensen R, Kruse C, Christiansen OB. Indications of anti-HY immunity in recurrent placental abruption. J Reprod Immunol 2007;75:63-9.

Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba M O. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science 2003;299:896-899.

Nolan T E, Smith R P, Devoe L D. A rapid test for abruptio placentae: Evaluation of a D-dimer latex agglutination slide test. Am J Obstet Gynecol 1993;169:265-9.

Nyberg D A, Cyr D R, Mack L A, Wilson D A, Shuhman W P. Sonographic spectrum of placental abruption. AJR 1987b:148:161-164.

Nyberg D A, Mack L A, Benedetti T J, Cyr D R, Schuman W P. Placental abruption and placental haemorrhage: correlation of sonographic findings with fetal outcome. Radiology 1987a;164:357-61.

Oyelese Y, Ananth C V. Placental abruption. Obstet Gynecol. 2006;108:1005-16.

Pandian Z, Bhattacharaya S, Templeton A. Review of unexplained infertility and obstetric outcome: a 10 year review. Hum Reprod 2001;12:2593-7.

Pang S-T, Dillner K, Wu X, Pousette Å, Norstedt G, Flores-Morales A. Gene expression profiling of androgen deficiency predicts a pathway of prostate apoptosis that involves genes related to oxidative stress. Endocrinology 2002;143:4897-906.

Parle-McDermott A, Mills J L, Kirke P N, Cox C, Signore C C, Kirke S, Molloy A M, O'Leary V B, Pangilinan F J, O'Herlihy C, Brody L C, Scott J M. MTHFD1 R653Q polymorphism is a maternal genetic risk factor for severe abruption placentae. Am J Med Genet. 2005;132A:365-368.

Paterson M E. The atiology and outcome of abruption placentae. Acta Obstet Gyecol Scand 1979;58:31-35.

Pearlman M D, DeSantis Klinich K, Schneider L W, Rupp J, Moss S, Ashton-Miller J. A comprehenshive program to improve safety for pregnant women and fetuses in motor vehicle crashes: A preliminary report. Am J Obstet Gynecol 2000;182:1554-64.

Peltier M R. Immunology of term and preterm labor. Reprod Biol Endocrinol 2003;1:122.

Peltonen L, Pekkarinen P, Aaltonen J. Messages from an isolate: lessons from the Finnish gene pool. J Biol Chem 1995;376:679-704.

Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. Hum Mol Genet 1999;10:1913-23.

Petterson K, Bremme K, Bottinga R, Hofsjö A, Hulthén-Varli I, Kublickas M, Norman M, Papadogiannakis N, Wånggren K, Wolff K. Diagnistic evaluation of intrauterine fetal deaths in Stockholm 1998-99. Acta Obstet Gynecol Scand 2002;81:284-92.

Plessenger M A, Woods J R.Cocaine in pregnancy; recent data on maternal and fetal risks. Obstet Gynecol Clin North Am 1998;25:99-118.

Plunkett J, Borecki I, Morgan T, Stamilio D, Muglia L J. Population-based estimate of sibling risk for preterm birth, preterm premature rupture of membranes, placental abruption and pre-eclampsia. BMC Genetics 2008;9:44.

Pritchard J A, Mason R, Corley M, Pritchard S. Genesis of severe placental abruption. Am J Obstet Gynecol 1970;1:22-27.

Prochazka M, Happach C, Marsal K, Dahlbäck B, Lindqvist P G. Factor V Leiden in pregnancies complicated by placental abruption. BJOG 2003;110:462-6.

Prochàska M, Lubuský M, Slavík L, Hrachovec P, Zielina M, Kudela M, Lindqvist P G. Frequency of selected trombophilias in women with placental abruption. Aust N Z J Gynaecol 2007;47:297-301.

Raatikainen K, Heiskanen N, Heinonen S. Under-attending free antenatal care is associated with adverse pregnancy outcomes. BMC Public Health 2007;7:268

Raatikainen K, Huurinainen P, Heinonen S. Epub 2006 Sep 7 Smoking in early gestation or through pregnancy: a decision crucial to pregnancy outcome. Prev Med 2007;44:59-63.

Pugh M B (Ed).(Phelan S T) Stedman's Medical Dictionary, 27th Edition, 2000, Lippincott Williams & Wilkins. (Baltimore, Maryland, USA).

Rachana C, Suraiya K, Hisham A-S, Abdulaziz A-M, Hai A: Prevalence and complications of physical violence during pregnancy. Eur J Obstet Gynecol Reprod Biol 2002; 103: 26-29.

Rasmussen S, Irgens L M, Albrechtsen S, Dalaker K. Women with the history of placental abruption: when in a subsequent pregnancy should special surveillance for a recurrent placental abruption be initiated. Acta Obstet Gynecol Scand 2001;80:708-12.

Rasmussen S, Irgens L M, Bergsjoe P, Dalaker K. The occurrence of placental ablations in Norway 1967-1991. Acta Obstet Gynecol Scand 1996a;75:222-8.

Rasmussen S, Irgens L M, Bergsjoe P, Dalaker K. Perinatal mortality and case fatality after placental abruption in Norway 1967-1991. Acta Obstet Gynecol Scand 1996b;75:229-34.

Rasmussen S, Irgens L M, Dalaker K. The effect on the likelihood of further pregnancy of placental abruption and the rate of its recurrence. BJOG 1997;104:1292-5.

Rasmussen S, Irgens L M, Dalaker K. A history of placental dysfunction and risk of placental abruption. Pediatr Perinat Epidemiol 1999;13:9-21.

Rasmussen S, Irgens L M, Dalaker K. Outcome of pregnancies subsequent to placental abruption: a risk assessment. Acta Obstet Gynecol Scand. 2000;79:496-501.

Rathore S S, McMahon M J. Racial variation in the frequency of intrapartum haemorrhage. Obstet Gynecol 2001;97:178-83.

Rautava L, Lehtonen L, Peltola M, Korvenranta E, Korvenranta H, Linna M, Hallman M, Andersson S, Gissler M, Leipälä J, Tammela O, Häkkinen U; PERFECT Preterm Infant Study Group. The effect of birth in secondary- or tertiary-level hospitals in Finland on mortality in very preterm infants: a birth-register study. Pediatrics 2007;119:e257-63.

Ray J G, Laskin C A. Folic acid and Homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: A systematic review. Placenta 1999;20:519-529.

Ray J G, Vermeulen M J, Schull M J, Redelmeier D A. Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. Lancet 2005;366:1797-803.

Raymond E G, Mills J L. Placental abruption; Maternal risk factors and associated fetal conditions. Acta Obstet Gynecol Scand 1993;72:633-9.

Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, Fisher S J. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin Invest. 2004;114:744-54.

Redline R W. Thrombophilia and placental pathology. Clin Obstet Gynecol 2006;49:885-94.

Redman C W G and Sargent I L. Placental depris, oxidative stress and pre-eclampsia. Placenta 2000;21:597-602.

Redman C W G and Sargent I L. Latest advances in understanding pre-eclampsia. Science 2005;308:1592-94.

Rennie J M, Hagman C F, Robertson N J. Outcome after intrapartum hypoxic ischaemia at term. Semin Fetal Neonatal Med 2007;12:398-407.

Rigby E. An Essay on the Uterine Haemmorrhage, which Precedes the Delivery of the Full Grown Foetus: Illustrated with Cases. London: J Johnson, 1775.

Risch N J. Searching for genetic determinants in the new millenium. Nature 2000;405:847-856.

Rosen T, Schatz F, Kuczynski E, Lam E, Koo A B, Lockwood C J. Thrombin-enhanced matrix metalloproteinase-1 expression: a mechanism linking plaental abruption with premature rupture of the membranes. J Matern Fetal Neonatal Med 2002;11:11-17.

Ryynänen M. Kehityshäiriöidenseulonta odottavan äidin seerumin alfa-fetoproteiinitutkimuksella Itä-Suomessa. Väitöskirja. Kuopion korkeakoulu, Kuopio 1982.

Sadler T W. Langman's medical embryology. 10th ed, 2006. Lippincott Williams & Wilkins.

Saftlas A F, Olson D R, Atrash H K, Rochat R, Rowley D. National trends in the incidence of abruptio placentae 1979-1987. Obstet Gynecol 1991;78:1081-6.

Salafia C M, López-Zeno J A, Sherer D M, Whittington S S, Minior V K, Vintzileos A M. Histologic evidence of old intrauterine bleeding is more frequent in prematurity. Am J Obstet Gynecol 1995;173:1065-70.

Salihu H M, Bekan B, Aliyu M H, Rouse D J, Kirby R S, Alexander G R: Perinatal mortality associated with abruptio placenta in singletons and multiples. Am J Obstet Gynecol 2005; 193: 198-203.

Salihu H M, Lynch O, Alio A P, Kornosky J L, Clayton H B, Mbah A K. Extreme obesity and risk of placental abruption. Hum Reprod. 2009;24:438-44.

Salim R, Czarnowicki T, Nachum Z, Shalev E. The impact of surveillance on pregnancy outcoe among women with a prior history of antepartum complications attributed to thrombosis: a cohort study. Reprod Biol Endocrinol 2008;6:55.

Schieve L A, Cohen B, Nannini A, Ferre C, Reynolds M A, Zhang Z, Jeng G, Macaluso M, Wright V C, for the Massachusetts Consortium for Assisted Reproductive Technology Epodemiologic Research (MCARTER). Matern Child Health J 2007;11:517-25.

Schiff M A, Holt V L, Daling J R. Maternal and infant outcomes after injury during pregnancy in Washington State from 1989 to 1997. J Trauma 2002;53:939-45.

Schiff M A, Holt V L. The injury severity score in pregnant trauma patients: predicting placental abruption and fetal death. J Trauma 2002;53:946-9.

Schiff M A, Holt V L. Pregnancy outcomes following hospitalization for motor vehicle crashes in Washington State from 1989 to 2001. Am J Epidemiol 2005;161:503-510.

Schwarzler P, Holden D, Nielsen S, Hahlin M, Sladkevicius P, Bourne T H. The conservative management of first trimester miscarriages and the use of colour Doppler sonography for patient selection. Hum Reprod 1999;14:1341-5.

Seidegård J, DePierre. Microsomal epoxide hydrolase. Properties, regulation and function. Biochim Biophys Acta 1983;695:251-270.

Shah S, Miller P R, Meredith J W, Chang M C. Elevated admission white blood cell count in pregnant trauma patients: An indicator of ongoing placental abruption. Am Surg 2002;68:644-7.

Sheiner E, Bashiri A, Levy A, Hershkovitz R, Katz M, Mazor M. Obstetric characteristics and perinatal outcome of pregnancies with uterine leiomyomas. J Reprod Med 2004;49:182-6.

Sheiner E, Levy A, Katz M, Mazor M. Pregnancy outcome following recurrent spontaneous abortions. Eur J Obstet Gynecol Reprod Biol 2005;118:61-65.

Sheiner E, Shoham-Vardi I, Hadar A, Hallak M, Hackmon R, Mazor M. Incidence, obstetric risk factors and pregnancy outcome of preterm placental abruption: a retrospective analysis. J Matern Fetal Neonatal Med 2002;11:34-9.

Sheiner E, Shoham-Vardi I, Hallak M, Hadar A, Gortzak-Uzan L, Katz M, Mazor M. Placental abrption in term pregnancies: clinical significance and obstetric risk factors. J Matern Fetal Neonatal Med 2003;13:45-9.

Shevell T, Malone F D, Vidaver J, Porter T F, Luthy D A, Comstock C H, Hankins G D, Eddelman K, Dolan S, Dugoff L, Craigo S, Timor I E, Carr S R, Wolfe H M, Bianchi D W, D'Alton M E: Assisted Reproductive Technology and Pregnancy Outgcome. Obstet Gynecol 2005; 106:1039-45.

Signore C, Mills J L, Qian C, Yu K, Lam C, Epstein F H, Karumanchi S A, Levine R J. Circulating angiogenic factors and placental abruption. Obstet Gynecol 2006;108:338-44.

Sipilä P, Hartikainen-Sorri AL, Oja H, Von Wendt L. Perinatal outcome of pregnancies complicated by vaginal bleeding. Br J Obstet Gynaecol 1992;99:959-63.

Sivula M, Tallgren M, Pettilä V. Modified score for disseminated intravascular coagulation on the critically ill. Intensive Care Med 2005;31:1209-1214.

Smith G D, Whitney E, Gissler M, Hemminki E. Birth dimensions of offspring, premature birth, and the mortality of mothers. Lancet 2000;356:2066-7.

Spinillo A, Fazzi E, Stronati M, Ometto A, Capuzzo E, Guaschino S: Early morbidity and neurodevelopmental outcome in low-birth weight infants born after third trimester bleeding. Am J Perinatol 1994; 11:85-90.

Stakes. Synnyttäjät, synnytykset ja vastasyntyneet 2006 -Föderskor, förlossningar och nyfödda 2006 -Parturients, Deliveries and Births 2006. Suomen virallinen tilasto, Terveys 2007.

Stakes. Synnyttäjät, synnytykset ja vastasyntyneet 2007-Föderskor, förlossningar och nyfödda 2007 -Parturients, Deliveries and Births 2007. Suomen virallinen tilasto, Terveys 2008.

Steinborn A, Rebmann V, Scharf A, Sohn C, Grosse-Wilde H. Soluble HLA-DR levels in the maternal circulation of normal and pathologic pregnancy. Am J Obstet Gynecol 2003;188:473-9.

Steinborn A, Seidl C, Sayehli C, Sohn C, Seifried E, Kaufmann M, Schmitt E. Anti-fetal immune response mechanisms may be involved in the pathogenesis of placental abruption. Clin Immunol 2004;110:45-54.o

Stephansson O, Dickman P W, Johansson A L V, Kieler H, Cnattingius S. Time of birth and risk of intrapartum and early neonatal death. Epidemiology 2003;14:218-222.

Stewart J L, Meeker J E. Fetal and infant deaths associated with maternal methamphetamine abuse. J Anal Toxicol 1997;21:515-7.

Tabor H K, Risch N J, Myers R M. Candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002;3:391-7.

Tanus-Santos J E, Desai M, Flockhart D A. Effects of ethnicity on the distribution of clinically relevant endothelial nitric oxide variants. Pharmacogenetics 2001;11:719-725.

Thorngren-Jerneck K, Herbst A. Perinatal factors associated with cerebral palsy in children born in Sweden. Obstet Gynecol 2006;108:1499-1505.

Tikkanen M, Nuutila M, Hiilesmaa V, Paavonen J & Ylikorkala O. Prepregnancy risk factors for placental abruption. Acta Obstet Gynecol Scand 2006a, 85 ;40-44.

Tikkanen M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Clinical presentation and risk factors of placental abruption. Acta Obstet Gynecol Scand 2006b;85:700-705.

Tikkanen M, Stenman U-H, Nuutila M, Paavonen J, Hiilesmaa V, Ylikorkala O. Failure of second-trimester measurement of soluble endoglin and other angiogenic factors to predict placental abruption. Prenat Diagn 2007;27:1143-1146.

Towers C V, Pircon R A, Hepard M. Is tocolysis safe in the management of third-trimeste bleeding? Am J Obstet Gynecol 1999;180:1572-8.

Van Rijn M, Van der Schouw Y T, Hagenaars A M, Visser G H A, Christiaens G C M L. Adverse obstetric outcome in low- and high-risk pregnancies: Predictive value of maternal serum screening. Obstet Gynecol 1999;94:929-934.

Vigil-De Garcia P. Pregnancy complicated by pre-eclampsia – eclampsia with HELLP syndrome. Int J Gynaecol Obstet 2001;72:17-23.

Venter J C *et al.*. The sequence of the human genome. Science 2001;291:1304-1351.

Vogel-Bindel U, Bentley P, Oesch F. Endogenous role of microsomal epoxide hydrolase. Ontogenesis, induction, inhibition, tissue distribution, immunological behaviour and purification of microsomal epoxide hydrolase with 16α,17α-epoxyandrostene-3-one as substrate. Eur J Biochem 1982;126:425-431.

Wang X, Wang M, Niu T, Chen C, Xu X. Microsomal epoxide hydrolase polymorphism and risk of spontaneous abortion. Epidemiology 1998;9:540-4.

Whiteman M K, Kuklina E, Hills S D,Meikle S F, Posner S F, Marchbanks P A. Incidence and determinants of peripartum hysterectomy. Obstet Gynecol 2006;108:1486-92.

Wiener-Megnagi Z, Ben-Sholomo I, Goldberg Y, Shalev E. Resistance to activated protein C and the Leiden mutation: High prevalence in patients with abruptio placentae. Am J Obstet Gynecol 1998;179:1565-7.

Williams M A, Hickok D E, Zingheim R W, Zebelman A M. Maternal serum CA 125 levels in the diagnosis of abruptio placentae. Obstet Gynecol 1993;82:808-12.

Willer C J, Scott L J, Bonnycastle L L, Jackson A U, Chines P, Pruim R, Bark C W, Tsai Y Y, Pugh E W, Doheny K F, Kinnunen L, Mohlke K L, Valle T T, Bergman R N, Tuomilehto J, Collins F S, Boehnke M. Tag SNP selection for Finnish individuals based on the CEPH Utah HapMap database. Genet Epidemiol 2006;30:180-190.

Winslow B T, Voorhees K I, Pehl K A. Methamphetamine abuse. Am Fam Physician 2007 15;76:1169-74.

Witlin A G and Sibai B M. Perinatal and maternal outcome following abruptio placentae. Hypertens Pregn 2001;20:195-203.

Yang Q, Wen S W, Oppenheimer L, Chen X K, Gao J, Walker MC. Association of Caesarean delivery for first birth with placenta praevia and placental abruption in second pregnancy. BJOG 2007;114:609-613.

Yasmeen S, Danielsen B, Moshesh M, Gilbert W M. Is grandmultiparity an independent risk factor for adverse perinatal outcomes? J Matern Fetal Neonat Med 2005;17:277-280.

Ylä-Outinen A, Palander M, Heinonen P K. Abruptio placentae – risk factors and outcome of the newborn. Eur J Obstet Gynecol Reprod Biol 1987;25:23-28.

Yoshimura T, Yoshimura M, Tabata A, Yasue H, Okamura H. The missense Glu298Asp variant of the endothelial nitric oxide synthase gene is strongly associated with placental abruption. Hum Genet 2001;108:181-183.

Zetterström K, Norden Lindberg S, Haglund B, Hanson U. Maternal complications in women with chronic hypertension: a population-based cohort study. Acta Obstet Gynecol Scand 2005;84:419-424.

Zdoukopoulos N, Zintzaras E. Genetic riskfactors for placental abruption: a HuGE review and meta-analysis. Epidemiology 2008;19:309-323.

Zhang X Q, Craven C, Nelson L, Varner M W, Ward K J. Placental abruption is more frequent in women with the angiotensinogen Thr235 mutation. Placenta 2007;28:616-9.

Zhu Q, Xing W, Qian B, von Dippe P, Schneider B L, Fox V L, Levy D. Inhibition of human m-epoxide hydrolasegene expression in case of hypercholanemia. Biochim Biophys Acta 2003;1638:28-16.

Zusterzeel P M L, Peters W H M, Visser W, Hermsen K J M, Roelofs H M J, Steegers E A P. A polymorphism in the gene for microsomal epoxie hydrolase is associated with pre-eclampsia. J Med Genet 2001: 38:234-237.

Özgür A, Vu T, Erkan G, Radev D R. Identifying gene-disease associations using centrality on a literature mined gene-interaction network. ISMB 2008;24:i277-i285.

9. APPENDIX: ORIGINAL PUBLICATIONS I - V

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Kuopio University Publications D. Medical Sciences

D 427. Saarelainen, Soili. Immune Response to Lipocalin Allergens: IgE and T-cell Cross-Reactivity.
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2008. 104 p. Acad. Diss.

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2008. 168 p. Acad. Diss.

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2008. 83 p. Acad. Diss.

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D 440. Hyvönen, Juha. Suomen psykiatrinen hoitojärjestelmä 1990-luvulla historian jatkumon näkökulmasta. 2008. 279 p. Acad. Diss.

D 441. Mäkinen, Heidi. Disease activity and remission in rheumatoid arthritis: comparison of available disease activity measures and development of a novel disease sctivity indes: the mean overall index for rheumatoid arthritis (MOI-RA).
2008. 129 p. Acad. Diss.

D 442. Kousa, Anne. The regional association of the hardness in well waters and the incidence of acute myocardial infarction in rural Finland.
2008. 92 p. Acad. Diss.