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GENETIC AND EPIDEMILOGICAL STUDIES OF DYSTOCIA – DIFFICULT LABOUR

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Bronze sculpture “Väntan” (*Expecting*) by Åsa Melin.

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Nog finns det mål och mening i vår färd
men det är vägen, som är mödan värd.

K. Boye

To my dear and beloved family:

Katarina, Andreas, Elinor, Lovisa and *Philippa*

ABSTRACT

Objective: To explore the epidemiological characteristics, the influence on reproductive health and the genetic basis of dystocia – prolonged and difficult labour.

Material and methods: The thesis has a retrospective design and is mainly based on a material from an entire cohort of women, extracted from the Swedish Medical Birth Register, who had their first delivery during the years 1973 to 1997. This includes totally 2 539 534 deliveries. The number of dystocia diagnoses at the first and second delivery was counted. Frequencies of operative deliveries and neonatal diagnoses of the child were compared between groups of women with or without dystocia. Interdelivery interval and number of children was also analyzed. Relative risk for dystocia was calculated and model-fitting (Mx) was used to estimate the relative contribution of genetic and environmental factors for the liability to experience dystocia. In addition a group of 23 women from Huddinge University Hospital and Västervik County Hospital were analyzed in detail with mutational screening of the candidate genes steroid-5-α-reductase (*SRD5A1*), endothelin 1 (*EDN1*), prostaglandin F 2α-receptor (*PTGFR*). The Swedish Multi-generation register was used to establish family connections and studies of medical records and telephone interviews were made in another group of 104 women, mainly sister pairs, with a history of dystocia. Single nucleotide polymorphism (SNP) whole genome scanning and non-parametric linkage (NPL) analysis was used in the families with a high occurrence of dystocia. Re-sequencing of the candidate genes oxytocin (*OXT*) and oxytocin receptor (*OXTR*) was performed in cases with dystocia.

Results: In the entire material the incidence of the diagnosis of dystocia was 8.0%. At the first delivery with term singletons and head presentation there was an incidence of the diagnosis of dystocia of 11.8%. The dystocia group had 12.5% caesarean and 51.6% instrumental deliveries vs. 6.6% and 6.8% in the non-dystocia group. In the group with dystocia 25.2% of the children had a neonatal diagnosis such as asphyxia, cerebral functional disturbances etc. vs. 14.7% in the group with women without. Maternal age and height, BMI, gestational length, fetal gender, weight and head circumference were all independently related to an increased risk of dystocia. The interval to the second delivery was 41.7 months in women with dystocia and they had significantly fewer children; 2.19 vs. 42.6 and 2.30 respectively in non-dystocic women. There was an increase in the diagnosis of dystocia over the study period and a great variation between different delivery wards. Measures of familial similarity (relative risks and correlation of liability) for dystocia were higher in monozygotic than in dizygotic twins, other sibling pairs and mother-daughter pairs. Correlation of liability was also higher in full-sisters than in half-sisters. Model-fitting suggested that genetic effects accounted for 28 % of the susceptibility for dystocia.

The genotyping of sister pairs showed a trend towards linkage with suggestive NPL-score (3.15) on chromosome 12p12. No mutations were found in the candidate genes *SRD5A1*, *EDN1*, *PTGFR*, *OXT*, or *OXTR*.

Conclusion: Dystocia is a complex condition that influences the reproductive health of Swedish women. Women with a diagnosis of dystocia at their first delivery have an increased risk of operative delivery. Their newborns have more complications regarding general condition, circulation, and respiratory function. Women with dystocia have significantly fewer children, and this was most pronounced in women who were delivered instrumentally.
The phenotype is quite poorly defined even though the clinical entity is very well recognized among obstetricians and midwives. There is clearly a genetic component in the susceptibility of experiencing dystocia but the exact origin has not been identified. Even though several polymorphisms were found it seems unlikely that mutations in the genes of steroid-5-α-reductase (*SRD5A1*), endothelin 1 (*EDN1*), prostaglandin F 2α-receptor (*PTGFR*), oxytocin (*OXT*) or oxytocin receptor (*OXTR*) are main causes of this condition.
The increases of the diagnosis of dystocia as well as the frequency of caesarean sections present major challenges for the obstetric care of today and in the future.

Key words: Dystocia, epidemiology, genetics, labour, obstetric complications, parturition, susceptibility, uterus

LIST OF PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the text by their roman numerals.

I. Algovik M, Lagercrantz J, Westgren M, Nordenskjold A. **No mutations found in candidate genes for dystocia.** Hum Reprod. 1999;14(10):2451-4.

II. Algovik M, Nilsson E, Cnattingius S, Lichtenstein P, Nordenskjold A, Westgren M. **Genetic influence on dystocia.** Acta Obstet Gynecol Scand. 2004 Sep;83(9):832-7.

III. Algovik M, Kivinen K, Peterson H, Kere J, Westgren M. **Genetic evidence of multiple loci in dystocia – difficult labour.** *(submitted)*

IV. Algovik M, Brudin L, Westgren M. **Perinatal outcome and reproductive health in mothers affected by dystocia in their first delivery.** *(submitted)*

CONTENTS

Publications

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CAM	Cell adhesion molecules
CI	Confidence interval
COX	Cyclooxygenase
GPCR	G-protein coupled membrane receptors
CRH	Corticotropin-releasing hormone
cGMP	Cyclic guanosine monophosphate
ECM	Extracellular matrix
EDN1	Endothelin 1 gene
ERα	Estrogen receptor alfa
ERβ	Estrogen receptor beta
F5	Coagulation factor V gene (van Leiden)
ICD	International classification of diseases
IL-1, 6, 8	Interleukins 1, 6 and 8
KEGG	Kyoto Encyclopedia of Genes and Genomes
MAPK	Mitogen activated protein kinase
MBR	Swedish Medical Birth Registry
mRNA	Messenger ribonucleic acid
NO	Nitrogen monoxide
NPL	Non parametric linkage
OR	Odds ratio
OXT	Oxytocin gene
OXTR	Oxytocin receptor gene
PCR	Polymerase chain reaction
PGE_2	Prostaglandin E2
$PGF_{2\alpha}$	Prostaglandin F2 alfa
PR-A	Progesterone receptor A
PR-B	Progesterone receptor B
PTGFR	Prostaglandin F2 alfa receptor gene
SRD5A1	Steroid 5-alfa reductase type 1 gene
SNP	Single nucleotide polymorphism
TGF-β	Transforming growth factor beta
TNF-α	Tumour necrosis factor alfa
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation

1 INTRODUCTION

1.1 BACKGROUND

Dystocia is derived from the Greek dys- (difficult) and tokos (labour) and it is a complex condition that is influenced by many different factors. The clinical knowledge and recognition of dystocia is widespread but really trying to define the condition has proved to be a futile search.

Dystocia, defined as prolonged and difficult labour, is a common obstetric problem affecting 8-10% of all deliveries. It is a major global health problem due to the increased risk of intrauterine asphyxia of the fetus, need for operative delivery and subsequently increased risk of both fetal and maternal morbidity such as haemorrhages, infections, and neurological disabilities (Saunders, Paterson et al. 1992; Chelmow, Kilpatrick et al. 1993; Macara and Murphy 1994). Dystocia followed by instrumental delivery is also a major maternal psychological trauma (Ryding, Wijma et al. 1998; Nystedt, Hogberg et al. 2005). The increase in caesarean section rates during the past 20 years has at least partly been attributed to an increase in the incidence of dystocia (Florica, Stephansson et al. 2006; Lowe 2007).

Numerous studies have suggested a genetic component in several other obstetric complications such as gestational hypertension and preeclampsia (Arngrimsson, Bjornsson et al. 1990; Mogren, Hogberg et al. 1999; Salonen Ros, Lichtenstein et al. 2000; Laivuori 2007), preterm birth and low birth weight (Clausson, Lichtenstein et al. 2000), prolonged pregnancy (Mogren, Stenlund et al. 1999), as well as other conditions (Ward 2008).

Animal studies have implicated that mutations in certain genes could be responsible for a deficiency of normal parturition, for example testosterone 5-α reductase type 1 gene (*SRD5A1*) (Mahendroo, Cala et al. 1996), and prostaglandin F2α receptor gene (*PGF2αR*) (Sugimoto, Yamasaki et al. 1997). An early review has suggested several candidate genes to be associated with labour problems (Dizon-Townson and Ward 1997). Studies of humans has not, so far, revealed any significant associations except from one study that has shown an association with preterm labour and the Factor V gene (*F5*) in women (Hao, Wang et al. 2004).

Previous epidemiological studies have also detected an increased risk of dystocia in a woman with affected mother or sister (Varner, Fraser et al. 1996; Berg-Lekas, Hogberg et al. 1998). These findings have triggered the search for the genetic background of dystocia. To be able to discuss dystocia a thorough understanding of the regulation of the complicated process of parturition is useful. In the study of genetic effects during pregnancy matters are more complicated by the fact that maternal, paternal and fetal genes have to be accounted for.

1.2 HUMAN PARTURITION

1.2.1 Anatomy and physiology

To fully appreciate the fantastic abilities of the uterus it is important to understand its dualistic way of functioning during pregnancy. In a strict anatomic sense the uterus is one organ but functionally it is separated into the cervix and the corpus. These two parts develop quite differently during pregnancy and are joined by the isthmus (Fig. 1).

The cervix is to the greatest fraction made up of a fibrous connective tissue with extracellular matrix (ECM) such as collagen while the corpus is mainly made of smooth muscle embedded in the ECM. During pregnancy there is a continuous remodelling of both the cervix and the corpus and the isthmus is developed into a 10 cm long connective lower uterine segment. In the first part of pregnancy the uterus just grows in a continuous fashion to protect and nourish the growing fetus. The smooth muscle cells increase both in number and size. The interface between the "self" of the uterine wall and the "non-self" of the placenta and fetus is very fascinating but the immunological regulation is not discussed in this thesis (Trowsdale and Betz 2006). Throughout pregnancy the corpus uteri remains quiescent except from a few practicing contractions, the Braxton-Hicks contractions that all pregnant women experience. Early loss of this quiescence can lead to premature delivery.

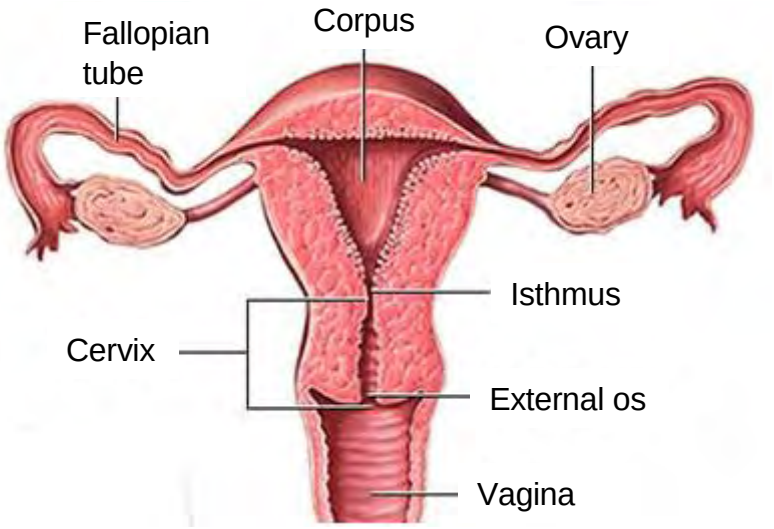

Figure 1. Anatomy of the uterus.

Progesterone plays an important part in maintaining this quiescence and affects myometrial contractility through genomic and non-genomic pathways (Mesiano 2001). The genomic pathways are mediated through the nuclear progesterone receptors A and B (PR-A, PR-B) where PR-B is the principal mediator and PR-A has a more regulatory function and represses the expression of PR-B. Progesterone, for example, decreases myometrial oxytocin and prostaglandin $F_{2\alpha}$ responsiveness by inhibiting expression of their receptors. Estrogen, on the other hand, has a more stimulating action and increases uterine contractility and oxytocin responsiveness. While estrogen levels are increased during most of the pregnancy the decreased sensitivity to estrogen before parturition is probably due to low levels of estrogen receptors α and β (ERα, ERβ). There are some studies indicating that progesterone via interaction with PR-B inhibits myometrial ERα expression and causes the myometrium to be refractory to circulating estrogens (Mesiano 2001). Nitrogen monoxide (NO) plays a part in relaxing the uterus during pregnancy through cGMP and NO is found in increasing amounts in the myometrium to prevent premature contractions and preterm birth (Carbillon, Seince et al. 2001). The enzyme nitrogen monoxide synthetase is diminished at the time of delivery to facilitate labour.

While the cervix is very hard and unyielding during the first part of pregnancy it turns into a soft and flexible tissue at delivery. This is due to a change of the arrangement of

the collagen fibrils where their interconnections are reduced. There is quite convincing evidence that this maturation and remodelling of the cervix is an inflammatory process (Sennstrom, Ekman et al. 2000; Romero, Espinoza et al. 2006). Prostaglandins PGE_2 and $PGF_{2\alpha}$ have a central part in this maturation process (Lopez Bernal 2003). During pregnancy the innervation of the cervix seems to be dense and unaltered while the corpus turns almost denervated (Tingaker, Johansson et al. 2006).
The exact mechanisms that initiate delivery in humans are not known. In most mammals parturition is preceded by a fall in the levels of progesterone but this is not the case in humans (Rezapour, Backstrom et al. 1997). There are suggestions that instead there is a relative reduction of the progesterone due to a reduced sensitivity to progesterone (Mesiano, Chan et al. 2002; Zakar and Hertelendy 2007). There is also an exponential increase in the levels of corticotrophin-releasing hormone (CRH) produced by the placenta that induces changes in fetal cortisol concentrations, lung maturation and myometrial receptor expression (Smith 2007). It has also been suggested that there is a placental clock working through this increase of CRH (McLean, Bisits et al. 1995). When delivery is imminent the uterus starts to contract regularly and the contractions increase both in intensity and frequency during labour. Normally the contractions start with a pace maker in either of the corners of the uterus and are then propagated in a wavelike pattern toward the cervix to push the infant in that direction. The muscle cells are connected by the gap junctions that quickly transmit the electric impulses. Myometrial maturation is characterized by the synthesis of oxytocin receptors and estrogen-enhanced gap junctions (Carbillon, Seince et al. 2001) and the formation of gap junctions is stimulated by estrogen but depressed by progesterone.

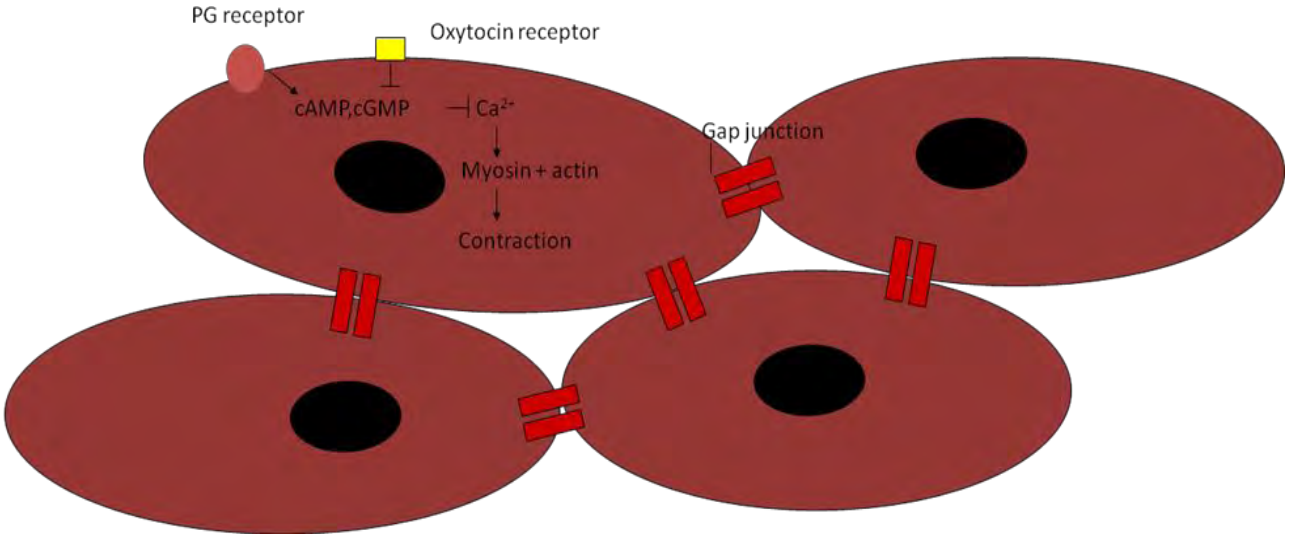

Figure 2. Physiology of myometrial cell contraction.

Prostaglandins play an important part both by stimulating the ripening of the cervix but also by promoting the contractions (Olson 2003). $PGF_{2\alpha}$ mobilizes Ca^{2+} from intracellular depots and seems to work synergistically with oxytocin in increasing the calcium-influx into the smooth muscle cells (Wray, Kupittayanant et al. 2001; Lopez Bernal 2003). PGE_2 has a more complex role and can have both a relaxing and stimulating effect on the myometrium. To regulate the contractility and relaxation of the myometrium there is crosstalk between these pathways to control calcium dynamics

(Sanborn 2007). The dramatic increase in prostaglandin concentration is stimulated by the inflammatory cytokines IL-1, IL-6, IL-8, VEGF and TNF-α (Osman, Young et al. 2003). The levels of these cytokines are increased due to inflammatory cascades initiated by recruitment of leucocytes and macrophages. To be able to produce the increasing amounts of prostaglandins the expression of the enzyme cyclooxygenase (COX) is greatly augmented (Sparey, Robson et al. 1999; Osman, Young et al. 2006). In addition endothelin 1, which has a contractile effect on smooth muscle both in vessels and myometrium (Wolff, Nisell et al. 1993), is found in increasing amounts in the uterus. Endothelin seems to be a more powerful stimulator of contraction than oxytocin.

During labour there is an increase of lactate that can cause acidosis and studies have shown that blood sampled from the uterine wall of women with dysfunctional labour had a significantly lower pH and higher lactate than that of women without signs of uterine dysfunction (Quenby, Pierce et al. 2004; Wray 2007). Another recent study showed an association of high levels of lactate in the amniotic fluid and an increased risk of instrumental delivery due to dystocia (Wiberg-Itzel, Pettersson et al. 2008). If the lowered pH is a cause or an effect of dysfunctional labour can be disputed.

How the widespread use of hormonal contraceptives and postponement of pregnancy influence the reproductive capability and uterine function of modern women is also an area to explore further.

All these biochemical processes are obvious targets for the research of parturition.

1.2.2 Oxytocin

Oxytocin is one of the most important hormones of parturition. It is secreted mainly from the posterior lobe of the pituitary gland but there is also a paracrine secretion in the uterus and decidua (Zeeman, Khan-Dawood et al. 1997; Blanks and Thornton 2003). In the uterus it binds to the membrane bound oxytocin receptor of the smooth muscle cells. The oxytocin receptor has G-protein coupling and through an ATP-dependent process cAMP and cGMP is produced and this leads to an increase of intracellular Ca2+-concentration (Shmygol, Gullam et al. 2006). This in turn leads to a coupling of actin and myosin and subsequently contraction of the muscle cells (Fig. 2). Estrogen stimulates the production of oxytocin receptors while progesterone inhibits it. There also seems to be a gradient with higher concentrations of the oxytocin receptor in the upper parts of the uterus compared to the isthmus and cervix. There is also some data suggesting lower concentrations of oxytocin receptors in women where induction of labour fails (Carbillon, Seince et al. 2001).

In Sweden oxytocin is used quite generously to augment labour and a previous study showed that 33.2% of all women received oxytocin during labour (Oscarsson, Amer-Wahlin et al. 2006). In local registry data from the south-eastern region of Sweden covering approximately 10 000 deliveries per year it has been recorded that as many as 50% of all women undergoing delivery might be given this medication (unpublished data). Widespread use of oxytocin in an uncontrolled manner might lead to an increased risk of adverse outcomes such as hyperstimulation of the uterus and subsequently asphyxia of the fetus. The hyperstimulation might also lead to uterine rupture, permanent neurological damage of the newborn and perhaps even death. Recent reviews of cases of malpractice in Sweden have shown that around 70% of the cases might be due to a injudicious use of oxytocin despite abnormal fetal heart rate

tracing (Jonsson, Norden et al. 2007; Berglund, Grunewald et al. 2008). In addition oxytocin might have negative effects on the mother as well such as changes in blood pressure, arrhythmias, and hyponatremia (Hayes and Weinstein 2008). It may be advisable to reduce the indiscriminate use of oxytocin and adhere more strictly to uniform indications and dosage regimens (Selin, Wallin et al. 2008).

1.2.3 Genetic regulation

The enormous development of genetics research technology, including the Human Genome Project, microarray technology, expression studies of mRNA and more has improved the understanding of the regulation of different bodily functions during the last decade. It has been estimated that humans have about 30 000 functional genes in our 23 chromosome pairs. Functional genomics studies of the uterus during the remodelling process throughout pregnancy show that this is a continuous process and the different metabolic pathways are modulated differently at diverse times of pregnancy (Chan, Fraser et al. 2002; Charpigny, Leroy et al. 2003). While the remodelling and maturation of the myometrium has been quite well characterized by these techniques the regulation of the onset of labour has not been possible to describe clearly by these methods (Breuiller-Fouche and Germain 2006). This might be due to the short time span in which these final events take place. In addition to functional genomics, several studies of proteomics and also systems biology have been published, promising a better understanding of some of these processes (Romero, Kuivaniemi et al. 2002; Romero and Tromp 2006). Even though the use of so called transcriptomics has been effective in identifying molecular signatures that are diagnostic and prognostic for diseases like cancer its value in the study of this functional condition has been questioned (Romero, Tarca et al. 2006).

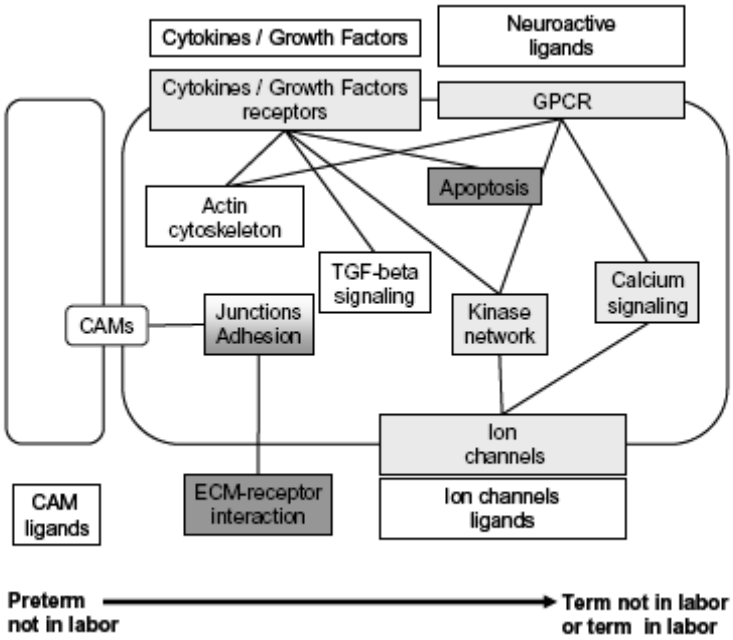

Figure 3. Differing genetic expression during pregnancy (Breuiller-Fouche, Charpigny et al. 2007). (CAM; cell adhesion molecules, ECM; extracellular matrix, GPCR; G-protein coupled receptor, TGF-beta; transforming growth factor beta.)

Many studies have been published regarding different expression patterns with both up- and down-regulation of genes in the uterus in labour compared to not in labour (Esplin, Fausett et al. 2005; Havelock, Keller et al. 2005; Hassan, Romero et al. 2006; O'Brien, Morrison et al. 2008). A recent review detailed 118 differentially expressed genes in non pregnant and preterm myometrium versus term not in labour and term in labour myometrium (Breuiller-Fouche, Charpigny et al. 2007). These could be grouped in 14 so called KEGG pathways (Kyoto Encyclopedia of Genes and Genomes) including pathways regulating cytoskeleton, calcium-signalling, cytokine production and junction formation (Fig 3.). KEGG is a bioinformatics resource to enable computational prediction of higher-level complexity of cellular processes from genomic and molecular information. It may be used to identify how different cellular processes interact. The mRNA expression of Connexin-43, a protein necessary for gap junction formation, is increased during the later part of pregnancy and the expression is also higher in the upper parts of the uterus. Conversely, the myometrial cyclooxygenase appear to be expressed at much greater levels in the lower compared to the upper uterine segment (Sparey, Robson et al. 1999). This kind of spatial difference at term as compared to earlier phases of pregnancy has also been shown in studies regarding mitogen-activated protein kinases (MAPK) with a higher expression in the upper parts of the uterus (Otun, MacDougall et al. 2005). The MAPK-pathway is one of the important intracellular signal transduction pathways.

1.2.4 Evolution

Throughout evolution a gene favouring difficult childbirth should hardly be propagated in the absence of the possibility of ending labour with a caesarean section. To the contrary there has instead been an increase of dystocia and from a Darwinian standpoint this might be explained by changes in the nutrition. The development from hunter-gatherers to our modern society with a highly agricultural-based diet might be one explanation of this increase (Roy 2003). During evolution the upright position probably has favoured narrower pelvises while the head of the fetus has increased in size due to a bigger brain, creating an evolutionary cephalo-pelvic conflict (Penn and Ghaem-Maghami 2001). The fact that dystocia increases over time further strengthens the notion that it is not dependant on a single gene mutation.

1.3 DYSTOCIA

1.3.1 Clinical diagnosis and management

As stated previously the incidence of dystocia is between 8 and 10%. It is more common in primiparas and other risk factors include high maternal age, generally over 35 years (Treacy, Robson et al. 2006), high maternal BMI (Cedergren 2004; Roman, Goffinet et al. 2008), high fetal weight, longer gestational length, larger fetal head circumference, and shorter maternal height (Feinstein, Sheiner et al. 2002; Lowe 2007; Selin, Wallin et al. 2008). Longer interpregnancy interval has also been related to an increased risk of dystocia in subsequent deliveries (Zhu, Grigorescu et al. 2006). Dystocia is associated to several obstetric complications such as asphyxia of the infant, perineal lacerations, obstetric haemorrhage (Saunders, Paterson et al. 1992) and an increased risk for instrumental delivery and caesarean section. Dystocia is one of the

major indications for caesarean section (Macara and Murphy 1994; Penn and Ghaem-Maghami 2001; Lowe 2007). In order to avoid long and excruciating deliveries for both the mother and the child it is necessary to identify dystocia and act to stimulate labour (ACOG 2003). Prolonged labour also has negative psychological effects that might influence future reproduction (Waldenstrom, Hildingsson et al. 2004; Nystedt, Hogberg et al. 2006).

One regular way of classifying dystocia is problems with the four P's: *passenger, passage, power* and *psyche.* This classification points out that the size of the fetus, the anatomy of the passage, the force of contractions and even the psychological state of the mother all are important in a successful vaginal delivery.

To be able to follow the progress of labour a partogram, as originally described by Friedman (Friedman 1972), is of great importance. The use of a partogram or partograph is also recommended by WHO to monitor the progress of deliveries (WHO 1994). Even though Friedman's original work was based on relatively few observations in a limited material it has been widely accepted. In his way a graphic representation can be made of the dilatation of the cervix and the descent of the fetal head. The normal speed of dilatation is about 1 cm per hour during the active phase of labour. In the partogram it is easy to draw an "alert line" with the desired speed of opening and another line that is parallel but shifted in time two to four hours, the so called "action line" (Fig. 4). If the dilatation passes this line the midwife or obstetrician should be more active in stimulating labour. This could be made in several ways such as amniotomy, augmenting labour with oxytocin, shifting the position of the mother and more. If the action line should be two, three or four hours from the alert line is not definitely decided but much research supports a somewhat longer expectant period (Lavender, Alfirevic et al. 2006). Usage of the partogram is very widespread and all delivery wards in Sweden use them. Though much benefit has been shown regarding the use of partograms, a very recent Cochrane review has not been able to give evidence for any major improvements of its use with respect to operative deliveries or condition of the child (Lavender, Hart et al. 2008).

One way of defining dystocia is into *protraction* disorders, where the dilatation and descent slows down, and *arrest* disorders, where dilatation continues while the head stops at a station in the pelvis. The latter problem is often due to disproportion where the dimension of the birth canal is narrower than the presenting part of the fetus. Traditionally there has also been a division of dystocia into *primary* where the contractions are irregular and weak and never reach the desired force to dilate the cervix enough and *secondary* where the contractions initially seem to be of sufficient frequency and force but then the progress slows down or stops entirely. These two types of dystocia are often appearing in combination and are probably only different expressions of the same problem: a uterus where the intricate mechanisms of contraction have been disrupted for some reason. All these diverse ways of diagnosing dystocia may contribute to the confusion surrounding the diagnosis of this condition.

The treatment of dystocia is dependent on a correct way of defining the start of labour since different interventions before actual labour has started may be detrimental to the success of an uncomplicated vaginal delivery. The start of the active phase is usually described as a cervical dilatation of 3-4 centimetres and regular painful contractions every 2 to 3 minutes that last for around 40 seconds. There are no studies that really prove that early intervention prevents dystocia but when dystocia is diagnosed it should be treated promptly. Large prospective randomized controlled studies to evaluate

different ways of treating dystocia are unfortunately not very frequent. Several studies have shown, however, that amniotomy and administration of oxytocin can be of benefit to shorten the duration of labour and improve the well-being of the mother (Svardby, Nordstrom et al. 2007). No studies have so far been able to prove any reduction of the rates of instrumental or operative deliveries by these interventions (O'Driscoll, Foley et al. 1984; Blanch, Lavender et al. 1998). In the case of amniotomy there are evidence of a shortening of labour and a reduction of children with Apgar score less than 7 at 5 minutes after birth (Fraser, Turcot et al. 2000). As for oxytocin there is a general consensus that it has a favourable effect on the progress of labour if the contractions decrease in intensity and frequency. There is no agreement how the administration should be delivered, either in Sweden or worldwide. Several dosage regimens are used with different starting doses and steps of increase. When using oxytocin it is very important that the stimulation is made just to reach regular contractions at the most every two minutes to avoid overstimulation. One group that needs special consideration and extra attention are primiparas (Selin, Wallin et al. 2008).
The role of epidural anaesthesia in the development of dystocia is under debate. Several authors have found that the use of epidural anaesthesia is correlated to a higher frequency of dystocia and instrumental deliveries but there is no definite proof that epidurals lead to an increase in the caesarean section rate (Eltzschig, Lieberman et al. 2003; Wong, Scavone et al. 2005; Selin, Wallin et al. 2008). Some studies rather point to an increased need for local anaesthetics in labour with dystocia emphasising that dystocia in itself is more painful than normal labour (Panni and Segal 2003).

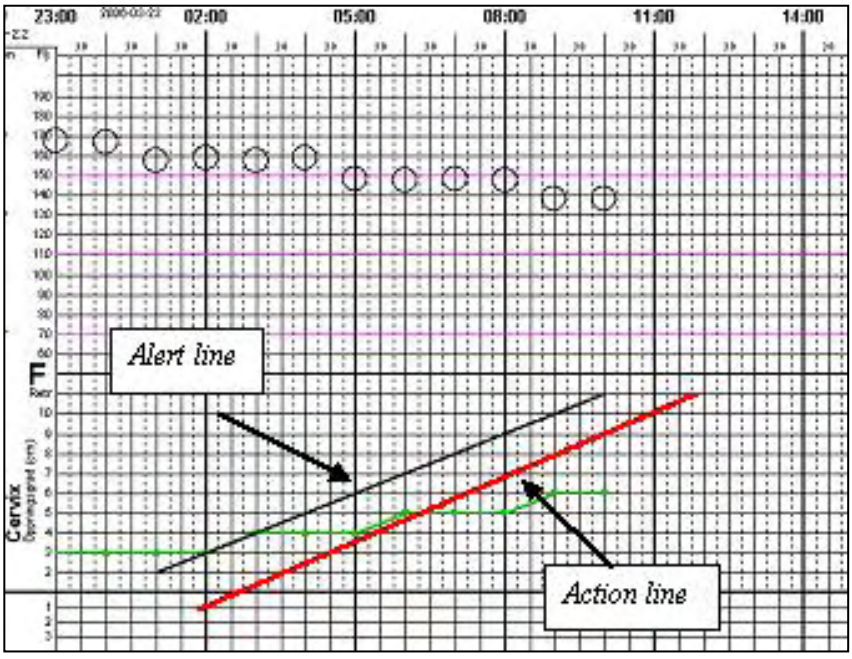

Figure 4. Partogram with representation of the descent of the fetal head, the dilatation of the cervix, Alert line and Action line.

1.3.2 "Active management of labour"

The concept of active management of labour was originally conceived at the National Maternity Hospital in Dublin in the 1960's. The concept is based on strict definitions of start of delivery and active labour, routine early amniotomy, administration of oxytocin

when labour progresses slowly with a dilatation of less than 1 cm per hour. Perhaps most important is one to one care with one attending midwife to each mother (O'Driscoll, Foley et al. 1984). This way of managing deliveries have been evaluated in several studies with somewhat divergent results. Although the shorter times of delivery have been appreciated by the mothers giving birth, no actual reduction of dystocia, instrumental or operative deliveries has been proven (Thornton 1997; Blanch, Lavender et al. 1998; Lavender, Alfirevic et al. 1998). A new Cochrane review did actually show a small but significant reduction of caesarean section rates but the authors conclude that further investigation is needed to clarify if there are parts of this package that are more effective than others (Brown, Paranjothy et al. 2008).

1.3.3 Increase of caesarean section rate

In the entire western world including Sweden there has been a continuous increase of the caesarean section rate as well as the frequency of instrumental deliveries during the past decades (Fig. 5). The indications for caesarean section fall into several categories where 30% are repeat caesarean sections due to a previous caesarean, 10% are due to fetal distress, 11% due to breech presentation and 30-38% is because of dystocia (Macara and Murphy 1994; Penn and Ghaem-Maghami 2001).

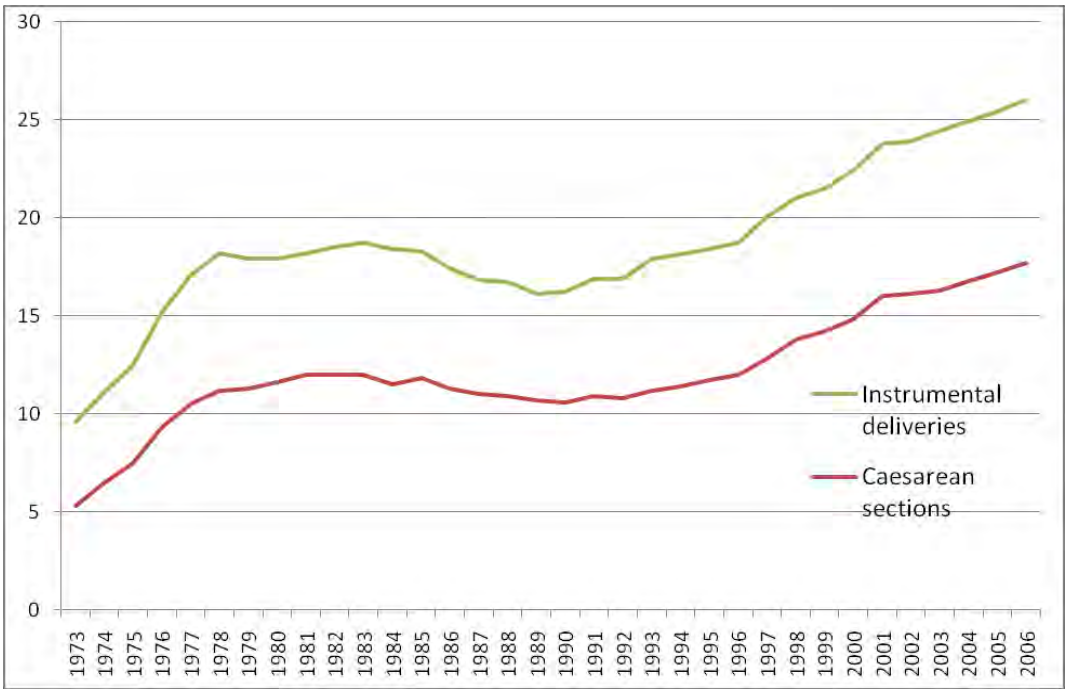

Figure 5. Frequency of instrumental deliveries and caesarean sections in Sweden (data from the Swedish Medical Birth Registry).

This increase is of great concern since operative deliveries are associated with an increase in complications both for the infant and the mother. Even though the safety of surgery and analgesia has improved during recent years a review of caesarean sections by the Swedish Board of Health and Welfare showed an increase in the risks of major haemorrhage, serious thromboembolic events, endometritis, and uterine rupture during the next pregnancy (Källén 2005). More caesarean sections do not seem to reduce the

use of instrumental deliveries since both modes of delivery are becoming more common in a parallel fashion. To explain this increase there might be several factors that contribute, for example older mothers at the first delivery, an increase in maternal BMI (Barau, Robillard et al. 2006), more mothers with a uterine scar after a previous caesarean, and perhaps a higher anxiety level among the mothers as well as the health care personnel (Florica, Stephansson et al. 2006).
Many authors have been alarmed about this increase and the WHO has set a desired goal of a maximum caesarean rate of 15% (WHO 1985). Several authors have proposed different ways of dealing with this problem and knowing which level is the ideal is of course dependant on population statistics and the status of the local health care system. A method that has gained popularity in Sweden is categorizing the women giving birth in *ten groups* where each woman only can be placed in one category depending on parity, spontaneous start of labour or induction, etc. The rates of caesarean sections in each group can then be analyzed to make it easier to focus on which kind of efforts are needed in each group (Robson 2001). The concern that trial of labour after a prior caesarean might lead to an increase in the morbidity of the mother has been disputed (Cahill and Macones 2007; Rossi and D'Addario 2008). It is generally accepted, however, that it is important to keep the frequency of caesarean sections in primiparas as low as possible, since an operative first delivery will lead to an increased risk of repeat caesarean sections.

1.4 IDENTYFYING GENETIC FACTORS IN OBSTETRICS

So far no certain relationships with single gene mutations and pregnancy complications has been shown, even though one study has presented an association with preterm labour and the Factor V gene (*F5*) in women (Hao, Wang et al. 2004). It seems that these disorders do have a more complex background. Association studies have shown promising results in many fields of medicine and are probably very useful in obstetrics as well if they are designed in a suitable way (Romero, Kuivaniemi et al. 2002).

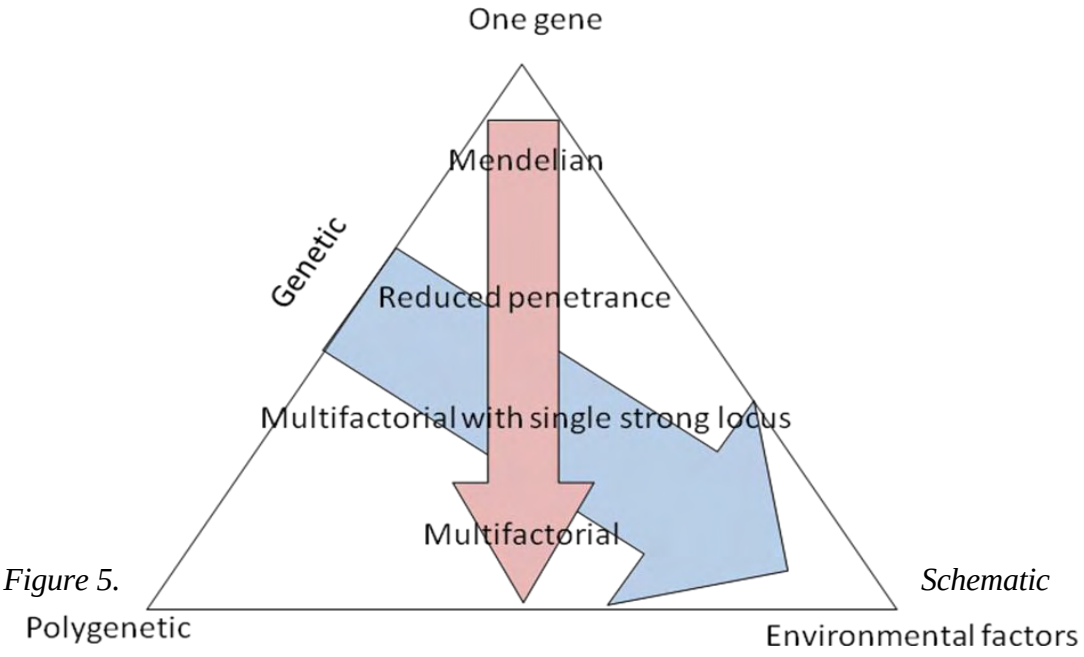

Figure 5. Schematic representation of the genetic and environmental influence.

Complex diseases are defined as an interaction between the genetic material and environmental factors (Dempfle, Scherag et al. 2008; Ward 2008). The genetic background will make any individual less or more susceptible to a certain medical condition and this will influence the impact of different environmental factors. There is a continuum of the genetic influence from the monogenic diseases with a single disease causing gene locus with a strict Mendelian inheritance to the majority of diseases that are dependent on several genes. In between are the diseases caused by genes with reduced penetrance (Reich, Cargill et al. 2001; Reich and Lander 2001) (Fig. 5).
The study of complex diseases must take both the genetic material and the environment into account and the emphasis of this thesis has been to uncover the genetic background of dystocia. These polygenic conditions, where several factors must collaborate for the disease to develop, result in so called "threshold effects". (Fig. 6) The genotype of a disease-associated gene subdivides the population into two groups of individuals that are homozygotic and either has a decreased (p^2) or increased (q^2) liability of disease. In-between is the heterozygotic group (2pq) with an intermediate risk. The environment will then influence the liability of individuals in an either positive or negative direction. In pregnancy the situation is even more complicated due to the influence of both fetal and paternal genes and also the possibility of different epigenetic effects. Epigenetics are the variations of phenotype depending on modification of the DNA other than sequence changes, for example methylation. Once established in a differentiated cell type, epigenetic signals are stably inherited through mitosis and are essential to maintain the correct genetic expression profile within cells of a certain type (Tremblay and Hamet 2008).

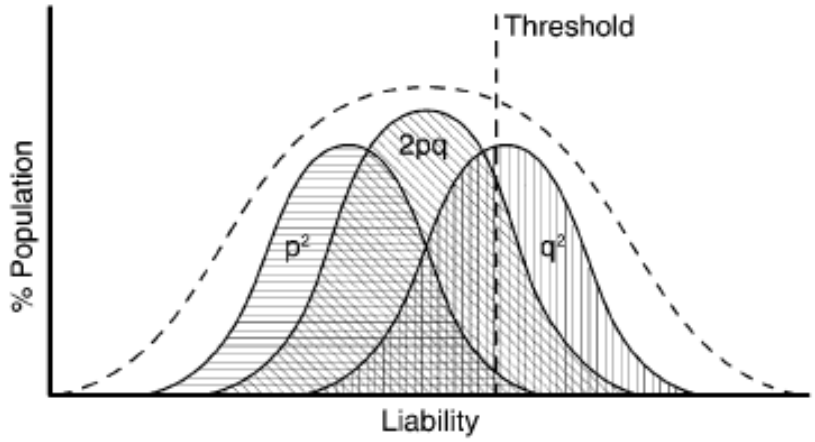

Figure 6. Schematic representation of the liability of disease for different allele combinations.

1.5 EPIDEMIOLOGY & SWEDISH MEDICAL BIRTH REGISTRY

All studies except study I are based on original data that was obtained from the Swedish Medical Birth Registry (MBR), that prospectively has collected information on all births in Sweden since 1973, and it has also been validated (Cnattingius, Ericson et al. 1990; Rosen and Ericson 1999). It covers almost 99% of all births in Sweden since all delivery wards register their data in a systematic fashion with standardized forms including details of the pregnancy, the delivery and the health of the newborn. Together with these detailed data the diagnoses of the mother and the newborn are also

registered making it possible to perform extensive and comparative studies in a population based material. This is systematically performed by The Centre for Epidemiology at The National Board of Health and Welfare.
While epidemiological studies are of great interest for identifying and quantifying diseases, pathological conditions and different risk factors in the population they have to be interpreted cautiously. One obvious problem regarding the MBR is that the diagnose coding is made retrospectively and can often be erroneous and incomplete. Another predicament is that epidemiological studies can be of limited value in ascertaining causal relationships. Supplementary studies to prove actual causation with biochemical and pathophysiological focus are often necessary.

2 AIMS OF THE STUDY

The main aim of the study was to further investigate the problem of dystocia, its genetics and its impact on the health of the pregnant woman and her child. This was broken down into the following areas.

- To elucidate if there is any common genetic factor that influences the risk of a woman to experience dystocia.

- To investigate how genetic and environmental interaction can augment the risk of certain diseases with a special focus on dystocia.

- To get a better understanding of the process of human parturition and the influence of difficult labour on the health of the mother and child.

- To find ways to identify pregnant women at risk of experiencing dystocia to improve the care of these women.

3 MATERIAL AND METHODS

3.1 ETHICS

All studies have been reviewed and approved by the medical review board at Karolinska Institutet, Karolinska University Hospital, Huddinge with the approval protocols numbered 184/97 and 116/03.

3.2 STUDY I

3.2.1 Cases

Charts for patients that gave birth at Huddinge University Hospital and Västervik County Hospital from 1st of March 1996 and 30th of September 1997 were examined. Women that had completed 286 days of gestation and who were subject to a caesarean section were asked to participate. A total of 23 individuals were included.

3.2.2 Mutation screening with Single Stranded Conformation Polymorphism

Constitutional DNA was prepared from peripheral blood according to standard protocols with phenol chloroform-extraction and ethanol precipitation. PCR products were generated by exon flanking primers according to published sequences of the genes of steroid-5-α-reductase (*SRD5A1*) (Jenkins, Hsieh et al. 1991), prostaglandin F 2α-receptor (*PTGFR*) (Abramovitz, Boie et al. 1994), and endothelin 1 (*EDN1*) (Inoue, Yanagisawa et al. 1989). In order to analyse the prostaglandin F 2α-receptor gene, exon flanking intronic sequences were characterised (Betz, Lagercrantz et al. 1999), and exon flanking primers were then designed for the gene.
PCR rections were performed in 50 µl, containing 200 ng of template DNA, 0.2 mM dNTP´s, 10-50 pmol of each primer, 1.25 U Taq DNA polymerase with the appropriate buffer and overlaid by 20 µl mineral oil. Successful amplification of the PCR products was checked on 1.5% agarose gels and the products were then subject to cold SSCP after denaturation for 5 min at 96 ^{o}C before. The 20% polyacrylamide gels was run at 4^{o}C and 24^{o}C on a Novex Thermo Flow ^{TM} electrophoresis unit (Novex, San Diego, CA) and the gels were silver stained (Bio-Rad Laboratories, CA).

3.2.3 DNA sequencing

Aberrant fragments were subject to a new round of PCR after purifying the fragment with WizardPrep (Promega), direct sequencing was performed according to standard procedures using cycle sequencing (Thermo Sequenase Radiolabeled Terminator Cycle Sequencing kit, Amersham LIFE SCIENCE). The products were run on a 6% denaturing polyacrylamide gel. The gels were dried and exposed to films (Hyperfilm TM-MP from Amersham) for 12-24 hours at room temperature.

3.3 STUDY II

3.3.1 Material

The data was extracted through record linkage between the Swedish Medical Birth Registry, the Multi-Generation Register (Statistics-Sweden 2001) and the Twin Registry (Karolinska Institutet). The Multi-Generation Register provides information on all first-degree relatives for residents born in Sweden 1941 and later. An individual had to be alive in 1960 or born thereafter to be included. Adoptions and other non-biological relations are noted. The unique national registration number assigned to each Swedish resident permits cross-linking of information about individuals between these registers (Lunde, Lundeborg et al. 1980). The diagnoses at delivery are noted at discharge and reported to the Medical Birth Registry. Diagnoses are classified according to the International Classification of Diseases (ICD), see table 1.

Table 1

Diagnose code	**Text**	**ICD revision**	**Year**
657.0, 657.1	Prolonged labour, primary and secondary	ICD-8	1973-1986
661A-C, 662A-C	Primary and secondary dystocia	ICD-9	1987-1996
O62.0-1, O63.0-1	Primary and secondary dystocia	ICD-10	1996-

All pregnancies during the study period were used in order to define whether the women had had dystocia in any pregnancy. In all, we included information from 2 539 534 registered births during the years 1973-97. Sisters and mothers to women with dystocia could be identified and pairs of healthy, disease discordant and disease concordant women were made. Individuals lacking information about mother were not included in the analysis. Additional information on the sisters included the degree of biological relationship (full- or half-sisters). Unique sister and mother-daughter pairs were then created: a sib-ship that contain one affected and one unaffected woman counts as a discordant pair; a sib-ship with three affected women (sister A, sister B, and sister C) will be counted as three concordant pairs (AB, AC and BC); whereas a sib-ship with two affected and one unaffected woman will be counted as one concordant and two discordant pairs.

3.3.2 Statistical analysis

The relative risk of dystocia for women whose mother or sister had been diagnosed with dystocia, compared with women whose mother or sister had not been diagnosed with dystocia, was estimated as an odds ratio (OR), using 95 percent confidence intervals (CI) (Kuritz, Landis et al. 1988).

In the model-fitting analysis dystocia was treated as a binary trait with the assumption of an underlying normal distribution of liability, with multiple factors contributing additively. This liability is the sum of genetic and environmental effects and the liability distribution has a threshold value that discriminates between dystocia and not dystocia (Falconer 1967). Women affected by dystocia are thus assumed to be above the threshold value of the liability distribution. The similarity between relatives with respect to dystocia was calculated as the correlation of liability (tetrachoric correlation) (Neale, Walters et al. 1994). The importance of genetic effects is indicated by higher

relative risks, and correlation among monozygotic twins compared to dizygotic and higher correlation among first degree compared to second-degree relatives. The importance of shared environmental effects is indicated by excess in similarity among family members not accounted for by genetic effects.
Contingency tables over disease status in the different pairs of relatives were entered into the structural model-fitting program (Mx) (Neale and Miller 1997), where separate disease thresholds were calculated for each table. A structural equation for any woman is: D=A+B+C+E, where D is the liability to disease, A is the genotype, B is the shared familial environment specific for mother-daughter relations, C is the shared familial environment between sisters, and E the non-shared environment. In the model fitting analysis the assumptions are that the correlation between;

- Monozygotic twins is 100 % (making the equation D=A+C).
- Dizygotic twins, biological full-sisters depend on common genes and common familial environment (D=0.5A+C).
- Mother/daughter pairs depend on common genes and common familial environment (D=0.5A+B).
- Maternal biological half-sisters depend on common genes (average 25%) and common familial environment (D=0.25A+C), since we assume that most children continue to live with their mother. Paternal biological half-sisters thus depend on common genes only (D=0.25A).

Five models were tested. In the full model parameters were estimated for heritability (A), shared environment among mother/daughter relations (B), shared familial environment among sisters (C), and non-shared environment (E), separately. The significance of each factor (A, B, C, E) was tested by excluding that parameter from the model. In Model 2 the genetic factor (A) is excluded, so the above equation becomes D=B+C+E. In subsequent models each factor is excluded to test which factor has the most importance on the liability for disease. The significance of parameters that differ between two models can then be examined by using the difference in chi-square value between the two models compared. This difference is in itself distributed as chi-square statistic, with the same degrees of freedom as the difference in the degrees of freedom between the two models. For example, if we want to test if the genetic heritability parameter (A) is significant, the difference in chi-square value between Model 2 (in which A is excluded) and Model 1 (the full model) can be calculated, compared with the chi-square distribution, with one degree of freedom, to obtain a p-value.

3.4 STUDY III

3.4.1 Study population

The Swedish Medical Birth Register was used to identify women who had given their first birth with caesarean section between 1982 and 1997 at a gestational length of more than 286 days. Using the Multi-Generation Register (Statistics-Sweden 2001) 75 sister pairs were connected where both siblings fulfilled the above-mentioned criteria and subsequently contacted them for inclusion in the study. A structured telephone interview was performed with all women to verify the diagnosis of dystocia, and to investigate whether their mothers had experienced problems giving birth or if there were any other close relatives with such problems. The telephone interview also included questions about how the women had experienced their first delivery, their subsequent obstetric history, and their current health status. All participants gave their

written consent for this study and permission to review the medical records from their first delivery. Women with breech presentation, contracted pelvis (sum of the pelvic diameters <29,5 cm) or a child weighing more than 4999 g were excluded from the genetic analysis. Similarly, multiple pregnancies and families where one or more members refused to participate were excluded.
Following the telephone interviews and the study of the medical records it was clear that the phenotype was heterogenic since not all of the women had dystocia as an indication for the caesarean section. The diagnosis of dystocia was divided into four subgroups to enable the selection of affected sib pairs with the most uniform phenotype for genetic analysis: certain dystocia, likely dystocia, unlikely dystocia and no dystocia. These subgroups are described below:

1. Certain dystocia: Definite diagnosis (see table 1), all subsequent deliveries by caesarean section
2. Likely dystocia: Induction of labour, long delivery time (>20 h), all subsequent deliveries by caesarean section or instrumental delivery
3. Unlikely dystocia: No induction of labour, short delivery time (<20), other indication for caesarean i.e. relative disproportion, child > 5000 g , subsequent deliveries without caesarean section or instrumental delivery
4. No dystocia: Absolute disproportion, breech presentation, caesarean section before start of labour, delivery misclassified as caesarean but was actually vaginal.

Additional sisters and cousins were invited to participate in families where several women were identified with delivery-related problems. Of the initial 150 women, 56 were excluded and 10 new cases added making the study population a total of 104. Of these 104 women, 83 provided a blood sample for the genetic analysis. Only families with women in group 1 and 2 were used in the genetic analysis.

3.4.2 Genotyping and genetic analysis

Single nucleotide polymorphism (SNP) genotyping was performed for 18 affected sib-pairs and one family with three affected siblings using the Affymetrix GeneChip® Mapping 10K 2.0 array containing approximately 10 000 SNP markers following the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA).
MERLIN (Abecasis, Cherny et al. 2002) was used to calculate the Whittemore and Halpern non-parametric linkage (NPL) scores (Whittemore and Halpern 1994). Marker positions on DECODE genetic map were retrieved from Affymetrix using NetAffx™ (Liu, Loraine et al. 2003), and allele frequencies were estimated using Caucasian allele frequencies provided by Affymetrix. Clear genotyping errors (but not unlikely genotypes) were removed using MERLIN. All individuals were analysed as affected. NPL scores were calculated for all families, and graphs were created using GNUPLOT. In the graphs, x-axis represents the DECODE genetic map locus, and y-axis represents the NPL score. Genome-wide and chromosome-wide significance of NPL-scores was estimated by simulating data 1 000 times with Merlin and extracting the highest NPL-score from each simulation. Physical positions of peak regions were verified manually against the NCBI dbSNP build 126, which gives chromosomal coordinates for human genome build 36.

3.4.3 Re-sequencing

Genomic DNA from 68 affected individuals with dystocia and 107 healthy controls were used to sequence oxytocin (*OXT)* and oxytocin receptor (*OXTR)* genes. Exons including 100bp flanking sequence on both sides and 1kb upstream of the first exon were amplified using polymerase chain reaction (PCR). Purified PCR products were sequenced using DYEnamic ET dye terminator kit following manufacturer's instructions (Amersham Biosciences, Buckinghamshire, UK) and electrophoresed using a MegaBACE 1000 instrument (Amersham Biosciences, Uppsala, Sweden). Sequence analysis was performed using the MegaBACE Sequence Analyser 3.0 software (Amersham Biosciences) and Staden package computer programs (Bonfield, Smith et al. 1995).

3.5 STUDY IV

Data was obtained from the Swedish Medical Birth Registry and the entire cohort of women who gave birth to their first child in Sweden from 1 January 1973 to 31 December 1993 was extracted. All their subsequent births up to 31 December 2004 were also noted. Data was collected on parity, gestational length, age, mode of delivery, maternal height, weight at admission to maternity care, maternal diagnoses, birth weight, birth length, Apgar score, diagnoses of the child, and head circumference. Women with multiple pregnancies, prematurity (< 259 days or 37 weeks) and/or non-cephalic presentations were excluded from further study. Women who had at least one of the diagnoses of dystocia according to ICD (see table 1) during the first delivery were grouped in the dystocia group (D). Women who had not received any of these diagnoses were grouped in a non-dystocia (ND) group. The number of subsequent births was noted for each woman and the interval to the second delivery was recorded. In all analyses of the second delivery, except from the total number of children, women with an interdelivery interval shorter than 9 months and/or had a gestational length shorter than 259 days were excluded. This was made to make the analysis of gestational length, fetal weight and other outcomes of the children between the first and second delivery comparable. Finally the same outcome variables of the second delivery as the first delivery were studied.

The diagnoses of the children were grouped as follows:

1. Impaired general condition and/or depressed cerebral function
2. Impaired respiratory/circulatory function
3. Haemorrhage/neonatal jaundice
4. Miscellaneous
5. Ante- or intrapartal death

The fluctuation of the diagnosis of dystocia over time during the study period was also studied as well as the variation between different hospitals in Sweden.

The statistical data was computed using Statistica (data analysis software system), version 7.1, StatSoft, Inc. (2005) with standard methods of counting frequencies, computing means and standard *t*-test comparing differences in continuous variables between the D and ND group. Significance in differences of categorical variables was measured with χ-square test. A *p*-value of <0.001 was considered significant and since the material is so large almost all differences were significant. To test significance of different risk factors both univariate and multivariate logistic regression was performed.

4 RESULTS

4.1 STUDY I

Of the 23 women 12 cases had a familial history with a sister and/or mother with dystocia. In the group with a gestational length of more than 42 weeks 6 out of 7 had another family member with dystocia and in the group with a pregnancy length of between 41 weeks and 4 days - 6 days 3 /8 had a family history of dystocia.
No mutations were detected in this highly selected group of 23 women. A number of polymorphisms were found. The results are presented separately for each gene. Polymorphisms, mutations and base pair position relates to the sequences deposited in Genebank (Homo sapiens endothelin-1 accession J05008, Human steroid 5-α-reductase accession M32313 and M68882, Homo sapiens prostanoid FP receptor accession L24470).
Two polymorphisms in exon 2 of the steroid-5-α-reductase gene were detected, comparing with the normal sequence. Pro103Pro (CCG->CCA) were found in homozygous form in 14 cases. Nine additional cases were heterozygous for this polymorphism but were also heterozygous for another polymorphism, Ala116Ala (GCA->GCG). This gives a frequency of 80 and 20%, respectively of the two polymorphisms.
Two aberrant bands on SSCP were detected in the first part of exon 1 of the endothelin 1 gene. Sequencing failed to show any mutations in the exon and over 60 intronic flanking base pairs.
A mutation, in homozygous form, was detected in the first part of exon 5 in one case and in heterozygous form in 4 cases. This mutation is substituting Lys198 to Asn (AAG -> AAT). It is present in 6 of 46 chromosomes analysed, giving a frequency of 15%. In the normal population (tested on 100 chromosomes) the corresponding frequency was 20%.
In the prostaglandin F2α-receptor gene a total of three different polymorphisms were found. In the non coding part of exon 1 a polymorphism, in heterozygous form, was detected in one case (2%). This polymorphism is substituting 104C->T. In exon 2 a polymorphism, in homozygous form, was detected in two cases (9%), altering the last base of codon Thr 21 (ACC->ACT). In the non coding part of exon 3 a polymorphism was detected, at position 1490, in four cases. It was present in heterozygous form in three cases and in homozygous form in one case (11%).

4.2 STUDY II

In all, 190 747 women with 204 204 births (8.0 %) diagnosed with dystocia in any form was identified. Compared to a monozygotic twin whose co-twin did not have dystocia, a monozygotic twin whose co-twin had dystocia faced an almost fourfold increase in the risk of dystocia in general. When all diagnoses of dystocia were added together we found the highest odds ratio (OR) for monozygotic twins (3.82). For dizygotic twins, biological full-sisters, and half-sisters with the same mother, and mother-daughter pairs corresponding risks were lower, while the odds ratio was 1.66, 1.85 and 1.60, respectively. Half-sisters sharing the same father had a somewhat lower risk. The

correlation of liability confirms the assumption of a genetic component in the development of dystocia.
The results of model-fitting, which takes all relatives for all diagnoses into consideration, show a heritability estimate (A) for dystocia in general is 28 %. The influence of shared environment between sisters (C) was smaller, while the shared environment between mother and daughter (B) had a negligible influence. This model shows that the largest influence on dystocia originate from the non-shared environment (E) at 67 %. When which of the factors that contributes to the model was tested, only exclusion of shared environment between mother and daughter (B) could be done without significant decrease in fit.
The data was also subdivided into primary or secondary dystocia. The pattern is the same for primary and secondary dystocia as for dystocia in general: compared to women who did not have a sister or mother with dystocia women who had a sister with (primary or secondary) dystocia generally had an increased risk of (primary or secondary) dystocia themselves. The results of model-fitting for primary dystocia alone showed approximately the same parameter estimates and a larger influence of non-shared environment. Secondary dystocia showed a reduced influence from the genotype, and a more pronounced effect of non-shared environment.
The material was subdivided further to find out if the relative risk was higher in an extreme subgroup of women with primary dystocia who had undergone Caesarean section. In this group the risk was increased in all groups of pairs, but the risk was only statistically significant in monozygotic twins and mother-daughter pairs. Dizygotic twins were not included due to a lack of concordant affected pairs. Also in this subgroup the correlation of liability decreases from monozygotic twins to sisters to mother –daughter pairs.
Furthermore, analyses were made with subgroups of secondary dystocia: secondary dystocia combined with Caesarean section; secondary dystocia combined with a gestational length of 280 days or more (with or without Caesarean section); and secondary dystocia combined with a gestational age of 294 days or more. Finally, the same analyses with primiparas only were made. All these analyses resulted in smaller groups and made calculations less certain, without altering the general results (data available at request).

4.3 STUDY III

4.3.1 Characteristics of the study population

We identified 76 women (73.1%) exhibiting a phenotype of certain or likely dystocia. Since many of the subjects were post-term, 53 (51.0%) of them had undergone induction of labour. In 16 of the 47 families (34.0%), the mother had had dystocia or some other obstetric problem such as instrumental or breech delivery. Thirty-eight (36.5%) women said that the delivery had been a bad or very bad experience and 22 (21.2%) said that their first delivery had negatively influenced the number of children they had born.

4.3.2 Genetic analysis (NPL)

According to simulations performed with Merlin, significant P-value (identified by an NPL-score reached in 5% of simulations) would have corresponded to an NPL-score of

3.64, while suggestive P-value (identified by an NPL-score reached at least once per simulation) would have corresponded to and NPL-score of 1.98. The best linkage peak was located on chromosome 12p12 (NPL score 3.15), and other peaks with suggestive linkage were found on chromosomes 3, 4, 6 and 20. The division into the subgroups with certain, likely, unlikely and no dystocia did not increase the significance of genetic analysis. Similarly, division of data into subgroups according to geographical region did not produce significant change to analysis results. Peak regions contained several apoptosis-related factors, calcium-calmodulin dependent kinases, and phospholipase c-like genes, but the interest was focused on oxytocin on chromosome 20, oxytocin receptor on 3, endothelin converting enzyme on 3 and endothelin receptor type A on 4. We did not manage to identify any dystocia candidate genes in the linkage peak regions on chromosomes 6, 10 and 12.

4.3.3 Re-sequencing of *OXT* and *OXTR*

Initially, we sequenced all oxytocin (*OXT*) and oxytocin receptor (*OXTR*) exons including splice sites and putative promoter regions in five individuals with dystocia and one control to detect common variations. One known polymorphism was detected within the *OXT* locus, but did not differ in allele frequency from that expected from dbSNP data. We were unable to sequence exon 2 due to high (>70%) GC content. Sixteen variations were identified within the *OXTR* locus, of which four were novel. Eleven *OXTR* polymorphisms were selected for sequencing in up to 68 dystocia cases and 107 controls to assess their allele frequencies in a bigger sample set.

4.4 STUDY IV

4.4.1 Variation over time and between hospitals

There was a variation of the diagnosis of dystocia with a continuing increase during the study period which also corresponded to an increase in the frequency of caesarean sections and instrumental deliveries. There was also a great variation of the reported frequency of the diagnosis of dystocia between hospitals in Sweden where it varied between 6.0% and 37.8%! There was a tendency that hospitals with a larger number of deliveries had a higher frequency of dystocia but there was no uniform pattern. There was however also a tendency that hospitals with a high frequency of dystocia diagnosis had a higher proportion of instrumental deliveries, but not caesarean sections.

4.4.2 First delivery

Mothers: The total material included 901 370 women who had 2 049 115 deliveries and of these 792 044 (87.9%) fulfilled the criteria of inclusion with singleton pregnancy, cephalic presentation and a gestational length of more than or equal to 259 days. Of the included women 106 192 had a diagnosis of dystocia at the first delivery, which is 11.8% of all women and 13.4% of the included. All studied descriptive variables differed significantly between the two studied groups, except Apgar score below 4 at 5 minutes.
The total number of deliveries in the dystocia group (D) was 2.18 compared to 2.29 in the non-dystocia (ND) group ($p<0.001$). The interval between the first and second delivery was not longer in the D group as we had anticipated, however, and was 41.7 months in the D group and 42.6 months in the ND group.

To explore further if a traumatic first delivery influences number of children and interdelivery interval we examined the outcomes at the second delivery in women who in addition of being diagnosed with dystocia also had their first delivery by caesarean section or by ventouse. Interestingly these subgroups had fewer children but shorter interdelivery intervals. There was an increase in the proportion of women who had only one child in the dystocia group, 20.4% compared to 17.5% in the ND group. Among those women who had dystocia and a delivery by caesarean section the first pregnancy the proportion that had only one child was even more increased to 25.0%.
Neonatal outcome: The children had an increased morbidity in the D group with a significant increase in the number of children with an Apgar score of less than 7 at 5 minutes ($p<0.001$). The proportion of children with an Apgar score of less than 4 at 5 minutes did not differ between groups. Regarding the neonatal diagnoses at the first delivery the D group had 25.2% children with any diagnosis but only 14.7% in the ND group ($p<0.001$).
Since the frequency of deaths was higher in the ND group the time of death was also analyzed and the majority of these cases were deaths before labour. The deaths during labour were slightly more frequent in the D group with 0.027% compared to 0.026% in the ND group (NS).

4.4.3 Second delivery

Mothers: At the second delivery, women who were diagnosed with dystocia at the first delivery had an increased risk of being diagnosed with dystocia and the frequencies were 9.9% in the D group and 2.8% in the ND group. Even during the second delivery there was a significant increase in the frequency of operative delivery in the D group with 5.4% ventouse/forceps and 13.4% caesarean section compared to 1.7% and 7.0% in the ND group.
Neonatal outcome: The children had an adverse outcome even after the second delivery in the dystocia group. Interestingly the frequency of diagnoses of complications was significantly increased even though the women did not receive a diagnosis of dystocia at the second delivery.

4.4.4 Logistic regression

A univariate and multivariate logistic regression was performed using the parameters maternal age, maternal height, maternal BMI, gestational length, fetal gender, fetal weight, and fetal head circumference. All of these parameters were shown to independently influence the risk of being diagnosed with dystocia. In this analysis only women with known BMI were included (n=219 926). The univariate analysis for each parameter with all women was also performed, but that did not change the results in any substantial way.

5 DISCUSSION

When asking a skilled obstetrician or midwife how they would describe dystocia there are probably several ways of defining the condition. Local traditions and personal experience may explain these large differences. The heterogeneity of the phenotype of dystocia is also probably the major problem of this thesis, which is clearly shown in study IV where the differences between diagnose coding in different Swedish hospitals is so obvious. Using ordinary diagnose coding in a retrospective manner is probably too imprecise to be very useful in making this kind of genetic survey.

In study I the focal point was to screen a selected group of 23 women, with a history of dystocia, for the presence of mutations in three candidate genes. All of these genes are expressed in the uterus and many studies have presented evidence of a strong genetic component in the risk of developing dystocia. A certain number of genes can therefore be expected to be altered. Animal data have strongly suggested two of them, the genes for testosterone 5-α reductase type 1 and for the prostaglandin F2α receptor, as candidates for dystocia. The product of the third gene, Endothelin-1, has been shown to act directly on the myometrium causing muscle contractions. All these three analysed genes should represent good candidates for dystocia.

There could be several possible explanations to the lack of finding any mutations in study I. The material might have been too small and not selected enough towards a familial aggregation of dystocia. The SSCP screening method probably detects between 70 and 80% of mutations and therefore some mutations could have been left unfound. The first part of steroid 5-α reductase type 1 gene was not analysed due to failure in amplifying this segment. However the results of study I strongly suggest that the three genes analysed are not commonly causing dystocia in humans.

One might also question whether animal models in all cases are a good tool to find genes involved in disease, or determining traits in humans. It might very well be that the mechanisms of parturition differ between humans and other species.

According to the population-based study II, heritability was estimated to contribute with 28 % in the liability of developing dystocia. The relative risk and correlation of liability for dystocia decreased with decreasing genetic similarity. As the influence of the shared environment was found to be very small, the results from study II is in accordance with the previously reported familial aggregation studies indicating an increased risk of dystocia if the mother and/or sister have been affected (Varner, Fraser et al. 1996; Berg-Lekas, Hogberg et al. 1998). Study II shows that this familial similarity is primarily due to shared genes. The similarity of the correlations for the mother-daughter pairs and the sister pairs suggests additive, rather than dominant or epistatic modifying, genetic effects. The finding of slightly lesser genetic influence in secondary dystocia when compared to primary might indicate differing mechanisms in originating labour and the continuing work of the uterus. Secondary dystocia might also to a higher degree be influenced by local traditions and different protocols of augmenting labour and treating labour abnormalities, as indicated above.

The overall incidence of dystocia was found to be 8.0 %, which is in the agreement with results from previous prospective and retrospective studies on incidence (Macara and Murphy 1994). The separation of dystocia into primary and secondary is often quite arbitrary and are probably only different aspects of the same problem.

Furthermore, the diagnose coding at the time of delivery is often problematic and imprecise. It is also possible that the time span between the births of the mother and daughters may have influenced the coding, but that will probably not affect the conclusion for dystocia in general. It may have some impact on the accuracy of the analyses for primary and secondary dystocia. Since the study is population based, the risk of selection bias is negligible. Furthermore, information of dystocia was collected independent of zygosity, and known relationships between sisters, or mother and daughter, and recall bias is therefore inconceivable.

The most likely mechanism of dystocia is through a defect ripening of the cervix, a malfunction of the myometrium or both, and perhaps also in the timing of these events. Considering the clinical impact of dystocia a single gene mutation should probably have been erased by evolutionary selection. The design of study II was to estimate maternal genetic effects and if fetal genetic effects are important for dystocia, the total genetic influence will be higher because paternal inheritance is not accounted for. Other possibilities could be maternal pelvic anomalies - even though frequency of major anomalies of the female pelvis in Sweden is very low (Ohlsen 1980) - or genetically related psychological causes, e.g. level of general anxiety, which might be a part in the occurrence of dystocia. Since the process of giving birth also depends on the psychological state of the woman, women with relatives which have experienced dystocia may also be influenced by a "psychological inheritance" and therefore have an increased risk of dystocia themselves.

Even though study II showed that the non-shared environment had the strongest influence on the liability for dystocia the heritability was encouraging enough in the pursuit of finding genes related to this condition.

Study III was the first paper assessing the genetic origin of dystocia through non-parametric linkage analysis. There was strong suggestive evidence of linkage at chromosome 12p12 and several possible genes in the areas of interest but none that struck as being solely responsible for this condition. The re-sequencing of oxytocin (*OXT*) and oxytocin receptor (*OXTR*), both of which are obvious candidate genes, did not identify any potential causal mutation. It is possible that regulatory variants affecting gene expression, mRNA stability, epigenetic effects or localization of protein product have been overseen. It is also possible that true candidate genes on regions showing suggestive linkage on chromosomes 4, 6, 10 and 12 have been overlooked. Also study III is influenced by the fact that the phenotype of dystocia is not strictly defined due to clinical heterogeneity and its diagnosis depends both on patient characteristics and the obstetric experience of the local hospital staff. Since the study design is retrospective and partly based on telephone interviews there is a possibility of recall bias, but the interviews were combined with a thorough review of the medical charts. To overcome these uncertainties cases demonstrating other well known causes for difficult labour such as fetal-maternal disproportion and maternal overweight were excluded. Although this may have led to the identification of a more consistent group in regards to poor labour it might also have introduced a certain degree of selection bias. The possibility that the linkage is in fact due to some other condition such as high birth weight or post term pregnancy cannot be excluded but since these conditions often are related, the genetics of each might be quite complicated to elucidate.

Study III is an example of a project where data from several medical registers has been combined and where permission was granted by the Institutional Review Board to contact the patients directly for obtaining biological samples. This is a quite unique

approach and it is notable that this approach was well received by the patients. Only 15 women (10%) declined to participate directly at the telephone interview and no case of offending a woman by this direct approach was encountered. On the contrary, the majority of women were willing to donate blood samples without any compensation. Thus, the direct approach appears feasible and has great potential to increase the scientific value of medical registers.

Study III indicates that dystocia is a complex disease that probably not is caused by a single locus disease allele. Knowledge of the genetic architecture of complex diseases is still incomplete and it is possible that the risk for any common disease is dependent on a large number of loci, each with a number of low frequency disease-predisposing alleles (Reich and Lander 2001). The study population included a limited sample size and adding more subjects might increase the power.

During evolution there is balance between mutation and selection and since dystocia-causing mutations would not be brought on to the next generation in the absence of the possibility of caesarean section, there is most likely genetic heterogeneity responsible for the phenotype. The disease-causing alleles can of course also be propagated by male offspring, but any possible male phenotype has not been studied. Study III focused on possible maternal genotypes but during pregnancy it is probably of interest to take the genetics of the child into account as well. Since the occurrence of dystocia is likely to involve allelic variations in several different loci it might be very cumbersome and costly to collect a large enough sample set to reach significance in any single locus with genome-wide linkage analysis.

In study IV the large population based material confirmed earlier studies (Feinstein, Sheiner et al. 2002; Cedergren 2004; Zhu, Grigorescu et al. 2006; Lowe 2007; Roman, Goffinet et al. 2008; Selin, Wallin et al. 2008) of the incidence of dystocia and also some of the factors that have been associated with an increased risk of dystocia. Higher maternal age, higher maternal BMI, shorter maternal height, increasing gestational length, increasing fetal weight and larger fetal head circumference were all independently associated with an increased risk of dystocia. A male fetus was also independently associated with an increased risk of dystocia. Furthermore, study IV confirmed a previous study that the diagnosis of dystocia is significantly related to an increased rate of instrumental deliveries and caesarean section and impaired neonatal outcome (Olesen, Westergaard et al. 2003).

A novel finding in study IV was the higher proportion of boys in the dystocia group at the first delivery. The reason for this is unknown but male fetuses are associated with several risk factors for dystocia such as larger fetal size and longer gestational length (Magnus, Bakketeig et al. 1993; Magnus, Bakketeig et al. 1997; Divon, Ferber et al. 2002; Gidlof, Wedell et al. 2007). One could speculate that the endocrine influence of the feto-placental unit influence the quality of the uterine activity. In accordance with such a speculation is a recent clinical study on mothers carrying a female homozygotic fetus affected by adrenogenital syndrome. Also these women have longer gestational length and more instrumental deliveries (Gidlof, Wedell et al. 2007). An endocrine influence of pregnancy and delivery outcome is interesting in many aspects not least since it might be influenced by epigenetic and environmental factors in our societies. The biology of and interaction between the male fetus and the mother is a very interesting path of research that might lead to new insights regarding the endocrine regulation of pregnancy and parturition.

It was obvious in study IV that dystocia influences the reproductive outcome, at least in this western European setting and reduces the number of children. However, the study did not show that women affected by dystocia who decide to have another child have a longer interval between the first and second child. Thus, it seems that these women are not affected by reduced fertility but rather are not willing to undergo pregnancy and delivery again. It seems highly likely that an excruciating experience of a long period of labour followed by an instrumental or operative delivery might influence the couples's willingness for reproduction in the future (Gottvall and Waldenstrom 2002).
Interestingly, women who had a diagnosis of dystocia during the first delivery also had an increased risk of getting this diagnosis at the second delivery and also an increased risk of having an operative or instrumental delivery. This can be a sign of an underlying dysfunction of the uterus in some women but also demonstrates that dystocia probably has many causes that can differ from one time to another.
Furthermore it was obvious from study IV that the diagnosis of dystocia increased over the study period and that hospitals with a high incidence of dystocia also had higher frequencies of instrumental deliveries and caesarean sections. The increase of caesarean sections (Robson 2001) is a well known problem all over the world and to some extent this can perhaps be explained by dystocia since maternal age at first delivery, maternal BMI and fetal weight all are risk factors that are rising (Barau, Robillard et al. 2006; Treacy, Robson et al. 2006).
Study IV was based solely on registry material and showed a great variation in the diagnosis of dystocia between hospitals making it likely that local traditions influence both the management of deliveries and the diagnose coding. This is an indication of the difficulties of correctly diagnosing this condition and, to some extent at least, the diagnosis is probably made to justify an intervention during delivery, which also has been shown (Oppenheimer, Holmes et al. 2007). Because of this study IV can be influenced by selection bias but the results are in concordance regarding the overall incidence of dystocia as compared to previous studies. The increase in complication diagnoses of the children is hardly influenced by this bias, however.
In these studies on dystocia the identification of certain genotypes that are associated with an increased risk has been tried. It might be that the limited success so far in these efforts is partly explained by a poorly defined phenotype. Thus, registry data with obvious inherent limitations in regard to homogeneous composition might not be suitable for this purpose. Rather it would be of value for the future to create an improved classification of this condition.
In agreement with previous studies, study IV clearly demonstrates that children to women affected by dystocia are at a higher risk for complications at birth. However, that the risk is also increased for the subsequent child although not affected by dystocia has not previously been reported. The reason for this is unknown but one hypothesis is that this might be due to some unknown underlying mechanism and if so dystocia might only be a symptom rather than the primary cause for poor perinatal outcome. This observation might be significant and emphasize the importance of elucidating the pathophysiology of this condition.
Other reflections when discussing this condition are that in Sweden obstetricians do not generally take part in the normal vaginal delivery and this might lead to a lack of experience and a risk for misinterpretation of the progress of labour. In addition the high turnaround in modern delivery wards might lead to a failure of letting normal labour take its time and instead ending the delivery with caesarean or vacuum

extraction. In many of today's delivery wards in Sweden the focus of young interns is to learn to perform caesarean sections since this is a requirement for being able to be on call by themselves. It might be easier to finish a prolonged delivery with a caesarean instead of continuing to monitor slow progress and balance it with the risk of ending up with a child with asphyxia. There is also the ever present risk of processing by the Medical Responsibility Board and/or litigation.

To conclude it is very useful for the obstetrician to know that several epidemiological studies have shown an increased risk of dystocia in a primiparous woman if her mother and/or sister experienced the same problem. The adherence to strict protocols might be a possible way of reducing the interventions due to dystocia (Oppenheimer, Holmes et al. 2007; Selin, Wallin et al. 2008). Obstetricians are also accustomed to using different scoring systems such as the Bishop score for evaluating the cervix and Apgar score for evaluating the newborn. Perhaps a "Dystocia score" could be of assistance where for example genetics, maternal BMI and height, estimated weight of the child and other relevant factors could be put together to make a more accurate estimation for the risk of developing dystocia?

6 CONCLUSIONS

6.1 GENERAL CONCLUSIONS

- Dystocia is an increasing problem in obstetric care that has a major impact on the reproductive health.
- The diagnosis of dystocia is imprecise and should be made in a more structured manner. This will be of benefit both for the mothers giving birth and for future research.
- Dystocia is a complex condition that has a genetic background but it is hardly the result of a mutation in a single gene.
- The direct approach of contacting research subjects after extraction from medical registers has been well accepted without offending the subjects.

6.2 FOCUS OF FUTURE RESEARCH

Larger studies including patients with a well defined phenotype of dystocia might lead to new insights into both the physiology of uterus as well as the aetiology of dystocia. Focus on extensive re-sequencing of candidate genes in the genetic areas of interest in affected individuals and controls to assess genetic variability within candidate loci and identify possible causal variants.
Development and validation of some kind of scoring system for an evaluation of the risk of dystocia will lead to improvement both of the clinical diagnosis and management as well as future obstetric research.
Research during pregnancy is very complex and must take maternal and paternal genetic factors into consideration in addition to epigenetic and environmental effects.

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