Genetic evidence of multiple loci in dystocia - difficult labour

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Abstract

Background: Dystocia, difficult labour, is a common but also complex problem during childbirth. It can be attributed to either weak contractions of the uterus, a large infant, reduced capacity of the pelvis or combinations of these. Previous studies have indicated that there is a genetic component in the susceptibility of experiencing dystocia. The purpose of this study was to identify susceptibility genes in dystocia.

Methods: A total of 104 women in 47 families were included where at least two sisters had undergone caesarean section at a gestational length of 286 days or more at their first delivery. Study of medical records and a telephone interview was performed to identify subjects with dystocia. Whole-genome scanning using Affymetrix genotyping arrays and non-parametric linkage (NPL) analysis was made in 39 women exhibiting the phenotype of dystocia from 19 families. In 68 women re-sequencing was performed of candidate genes showing suggestive linkage: oxytocin (OXT) on chromosome 20 and oxytocin-receptor (OXTR) on chromosome 3.

Results: We found a trend towards linkage with suggestive NPL-score (3.15) on chromosome 12p12. Suggestive linkage peaks were observed on chromosomes 3, 4, 6, 10, 20. Re-sequencing of OXT and OXTR did not reveal any causal variants.

Conclusions: Dystocia is likely to have a genetic component with variations in multiple genes affecting the patient outcome. We found 6 loci that could be re-evaluated in larger patient cohorts.

Background

Dystocia, defined as prolonged and difficult labour, is a common obstetric problem affecting 6-8% of all deliveries [1]. It is a major global health problem due to the increased risk of intrauterine asphyxia of the fetus, operative delivery and subsequently increased risk of both fetal and maternal morbidity such as haemorrhage, infections, pelvic floor trauma, subsequent placenta accreta and neurological disabilities [2-5]. Dystocia followed by instrumental delivery or caesarean section might also be major psychological trauma leading to increased levels of anxiety that by some authors even has been classified as post traumatic stress disorder [6,7].

Parturition is regulated by many factors and the mechanisms regulating labour and the expulsion of the child are incompletely understood [8]. Animal studies have shown that knock-out mice models of genes regulating parturition exhibit dystocia [9,10], but this has not yet been shown in humans [11]. A strong genetic influence has been shown in other obstetric conditions such as preeclampsia, low birth weight, and abnormal gestational length [12,13]. Studies have also detected an increased risk for dystocia in a woman with affected mother or sister [14,15]. We have previously estimated the heritability of dystocia to be 28% [16] and this finding has encouraged us to perform a genome-wide scan in a material consisting of affected sib pairs to further assess the genetic basis for dystocia.

Methods

Study population

We used the Swedish Medical Birth Registry [17] that covers almost all births in Sweden 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. The reason for this was to have a well defined end point and to increase the chance of finding subjects with...
dystocia since this condition is more frequent in primiparas and in prolonged gestation and a majority of operative deliveries after 41 weeks are emergency caesarean sections. Using the Swedish Multigeneration Registry [18] we managed to connect 75 sister pairs where both siblings fulfilled the above-mentioned criteria and subsequently contacted them for inclusion in the study. One of the investigators (M. Algovik) performed a structured telephone interview with all women to verify their eligibility to participate in the study, 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 the women’s subsequent obstetric history and their current health status. To be able to make a simple evaluation of the women’s overall experience of the delivery they were also asked to grade it on a five-step scale from very bad to very good. All participants gave their written consent to participate in the study and permission to review the medical records from their first delivery. The diagnosis was confirmed by study of the medical charts of the delivery including partogram. Women with breech presentation, contracted pelvis (sum of the pelvic diameters < 29.5 cm) or a child weighing more than 5000 g were not included in the genetic analysis (NPL). 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 we concluded that the phenotype is heterogeneous since not all of the women had dystocia as an indication for their caesarean section. Given that the primary focus of the study was uterine dysfunction rather than other reasons for dystocia and to enable us to pick the affected sib pairs with the most uniform phenotype for genetic analysis we divided the diagnosis of dystocia into four subgroups: 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 without other indications.

2. Likely dystocia: Induction of labour, long delivery time (> 20 h), all subsequent deliveries by caesarean section or instrumental delivery and no other indications.

3. Unlikely dystocia: No induction of labour, short delivery time (< 20 h), 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 with non-parametric linkage (NPL). See Table 2 for details regarding the study population. The study protocol was approved by the institutional review board at Karolinska University Hospital, Huddinge, Sweden.

**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 10 K 2.0 array containing approximately 10,000 SNP markers following the manufacturer’s instructions (Affymetrix, Santa Clara, CA, USA).

MERLIN [19] was used to calculate the Whittemore and Halpern non-parametric linkage (NPL) scores [20]. NPL is a way of measuring how often certain genetic areas are inherited together. Marker positions on DECODE genetic map were retrieved from Affymetrix using NetAffx™ [21], and allele frequencies were estimated using Caucasian allele frequencies provided by Affymetrix. We had low power to detect genotyping errors due to study design, but MERLIN flagged 124 genotypes as unlikely and these were removed from linkage analysis. All individuals were analyzed 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-

### Table 1: Diagnose codes

| 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-     |
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.

Re-sequencing
Genomic DNA from 68 affected individuals with dystocia and a historic reference material consisting of 107 healthy women without adverse obstetric history who had given a written consent to participate in studies on complications of pregnancy were used to sequence oxytocin (OXT) and oxytocin receptor (OXTR) genes. Cases were not matched with the reference group, but were used to establish an estimate of the population frequency of potential mutations. Exons including 100 bp flanking sequence on both sides and 1 kb 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 [22].

Results
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. Clinical characteristics have been collected in Table 3 for all interviewed women, Table 4 for the affected sib pairs included in the genetic analysis (NPL) and Table 5 for the 68 women that were used for the re-sequencing of OXT and OXTR. Since additional female relatives were included after the initial selection to create complete pedigrees some of these values differ from the original selection criteria.

Genetic analysis, non-parametric linkage (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

| Characteristic                  | Mean | Range     | SD   |
|--------------------------------|------|-----------|------|
| Age                            | 30.2 | 19.2-43.1 | 5.0  |
| BMI                            | 27.4 | 19.1-39.5 | 3.7  |
| Gestational length             | 41 + 6 | 38 + 0 - 43 + 4 |      |
| Time from start of delivery to parturition (h) | 26:33 | 4:25 - 96:00 |      |
| Birth weight (g)               | 3824 | 2360 - 5260 | 568  |
corresponded to and NPL-score of 1.98. Our 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. Linkage results are sum-
marized in Table 6. 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, cal-
cium-calmodulin dependent kinases, and phospholipase
c-like genes, but we were most interested in oxytocin on
chromosome 20, oxytocin receptor on 3, endothelin con-
verting enzyme on 3 and endothelin receptor type A on 4.
We did not succeed in identifying any dystocia candidate
genes in the linkage peak regions on chromosomes 6, 10
and 12.

Re-sequencing of OXT and OXTR
Initially, we sequenced all OXT and OXTR exons includ-
ing splice sites and putative promoter regions in five indi-
viduals with dystocia and one control to detect common
variations. One known polymorphism was detected
within the OXT locus, but did not differ in allele fre-
quency from that expected from dbSNP data. We were
unable to sequence exon 2 due to high (> 70%) GC con-
tent. Sixteen variations were identified within the OXTR
locus, of which four were novel and the remaining 12
were included in dbSNP with rs numbers. Eleven OXTR
polymorphisms were selected for sequencing in 68 dysto-
cia cases (Table 5) to assess their allele frequencies in
comparison with a historic reference group consisting of
107 women with characteristics described in Table 7. OXTR polymorphisms are summarized in Table 8. All
detected polymorphisms, including the three successfully
genotyped newly detected ones (rsOXTR_01, 02 and 04),
were observed to have population frequencies above 1%,
and without even suggestive evidence for association,
making it unlikely that they would be involved in risk of
dystocia.

Discussion
According to our knowledge, this is the first paper assess-
ing the genetic origin of dystocia through non-parametric
linkage analysis. There is strong suggestive evidence of
linkage at chromosome 12p12 and we found several pos-
sible 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 allow us to identify any potential causal mutations.
However, it is possible that we have overseen regulatory
variants affecting gene expression, mRNA stability or
localization of protein product. It is also possible that we
have overlooked true candidate genes on regions showing
suggestive linkage on chromosomes 4, 6, 10 and 12. We
chose comparison with a healthy historic reference mate-
rial for the re-sequencing of OXT and OXTR. Evidently
this group differs from our cases regarding age, BMI, ges-
tational length and birth weight but we did not manage to
find any mutations. If we actually had found any muta-
tions a comparison with matched controls would of
course have been preferable.

The results of the present study may be explained in
different ways. One obvious weakness is that the pheno-
type of dystocia is not strictly defined due to clinical het-
erogeneity and its diagnosis depends both on patient
characteristics and the obstetric experience of the local
hospital staff. Thus, the use of the clinical entity dystocia
varies according to local preferences and traditions, and
institutions are likely to diagnose dystocia in a non-stan-
dardized manner. This is well illustrated in Sweden where
dystocia prevalence in different delivery wards differs
widely between 4 and 33 percent [23]. Since the study
design is retrospective and partly based on telephone

| Table 4: Clinical characteristics for affected sib pairs included in the genetic analysis (NPL) (n = 39) |
|-------------------------------------------------|---|---|---|
| Characteristic                  | Mean  | Range       | SD |
| Age                            | 32.3  | 21.9-43.1   | 4.8|
| BMI                            | 27.1  | 20.3-39.5   | 3.8|
| Gestational length             | 41+6  | 40+6-43+1  |     |
| Time from start of delivery to parturition (h) | 28:01 | 6:00-96:00 |     |
| Birth weight (g)               | 3928  | 2750-4850   | 454|

| Table 5: Clinical characteristics of the women included in the re-sequencing of OXT and OXTR (n = 68) |
|-------------------------------------------------|---|---|---|
| Characteristic                  | Mean  | Range       | SD |
| Age                            | 31.0  | 20.7-43.1   | 5.0|
| BMI                            | 27.5  | 19.1-39.5   | 3.9|
| Gestational length             | 41+6  | 38-5-43+3  |     |
| Birth weight (g)               | 3886  | 2750-4850   | 477|
interviews there is a possibility of recall bias, but the interviews were combined with a thorough review of the medical charts. Traditionally dystocia is attributed to three general causes, the three P’s in obstetrics; power, passenger and pelvis. With the present approach excluding fetal-maternal disproportion and maternal overweight we attempted to predominantly study the power (uterine muscle activity) within this phenotype.

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. We cannot exclude the possibility that the linkage is in fact to some other condition such as high birth weight or post term pregnancy but since these conditions often are related, the genetics of each might be quite complicated to elucidate.

The present study is an example of a project where data from several medical registers has been combined and where we have been granted permission 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.

This study indicates that dystocia is a complex disease that is probably not 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 [24]. The study population included only a limited sample size making it underpowered for association analysis. Our results thus merely established that the newly detected variants were polymorphisms (population frequency > 1%) and adding more subjects would be necessary to increase the opportunity to perform a well-powered genetic association analysis.

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 we have so far not studied any possible male phenotype.

Since the occurrence of dystocia is likely to involve allelic variations in several different loci we think that 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. An alternative and complimentary approach would be to focus on extensive re-sequencing of candidate genes in affected individuals and controls to assess genetic variability within candidate loci and identify possible causal variants.

### Table 6: Linkage results

| Chr | Marker range | Physical range | NPL score | Genomic P |
|-----|--------------|----------------|-----------|-----------|
| 20  | rs2013961 - rs674110 | 2 - 4 Mbp | 2.23 (3.7 Mbp) | 0.97 |
| 12  | rs1405608 - rs722918  | 20 - 62 Mbp | 3.15 (51 Mbp) | 0.21 |
| 10  | rs726176 - rs1409317  | 97 - 121 Mbp | 2.92 (115 Mbp) | 0.37 |
| 6   | rs1979541 - rs979515  | 159 - 165 Mbp | 2.84 (164 Mbp) | 0.43 |
| 4   | rs723794 - rs1464452  | 147 - 157 Mbp | 3.03 (154 Mbp) | 0.28 |
| 3   | rs1508722 - rs2358693, rs725318 - rs763342 | 7 - 22 Mbp, 179 - 189 Mbp | 2.43 (13 Mbp), 2.55 (185 Mbp) | 0.87, 0.74 |

### Table 7: Clinical characteristics of the reference group (n = 107)

| Characteristic  | Mean  | Range         | SD  |
|-----------------|-------|---------------|-----|
| Age             | 28.4  | 17.0-43.0     | 4.7 |
| BMI             | 22.7  | 17.2-34.5     | 3.7 |
| Gestational length | 39 ± 4 | 37 + 0-42 + 0 |     |
| Birth weight (g) | 3540  | 2560-4540     | 385 |
have focused on the maternal genotypes but during pregnancy it is probably of interest to take the genetics of the child into account as well. For example, our study does not assess whether dystocia risk is affected by the properties of the fetus, but this might be worthwhile to pursue further.

Conclusions

In the overall assessment of a woman giving birth the obstetrician might find the knowledge that epidemiological studies have shown an increased risk of dystocia in a primiparous woman if her mother or sister has experienced the same problem to be useful. Our study indicates that there actually might be a genetic background for dystocia through a strong suggestive evidence of linkage at chromosome 12p12 and also at 5 other loci that might be of interest. We believe that larger studies including patients with a well defined phenotype of dystocia might lead to new insights into the aetiology and physiology of this important condition.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

MA participated in planning the study, collected the material, participated in the genetic and statistical analysis and wrote the main part of the manuscript. HP and KK participated in designing the study, and made the main part of the NPL-analysis and re-sequencing as well as the statistical analysis. JK and MW originated the study, participated in its design and helped in writing the manuscript. All authors read and approved the final manuscript.

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Table 8: Sequencing results for oxytocin receptor gene (OXTR). rsOXTR_01-04 are novel polymorphisms detected in this study.

| Marker name | Location         | Alleles | MAF cases | MAF controls | Yates corrected Chi square | P value |
|-------------|------------------|---------|-----------|--------------|---------------------------|---------|
| rs2268498   | Upstream of exon 1 | T/C     | 0.48 (63) | 0.40 (99)    | 1.94                      | 0.16    |
| rs2268497   | Upstream of exon 1 | A/G     | 0.47 (65) | 0.39 (103)   | 1.59                      | 0.21    |
| rsOXTR_01   | Upstream of exon 1 | C/T     | 0.023 (66) | 0.014 (104)  | 0                         | 1       |
| rs1465386   | Upstream of exon 1 | G/T     | 0.095 (68) | 0.025 (20)   | 1.25                      | 0.26    |
| rs3806675   | Upstream of exon 1 | G/A     | 0.40 (67) | 0.40 (107)   | 0                         | 1       |
| rsOXTR_02   | Upstream of exon 1 | G/C     | 0.05 (62) | 0.06 (17)    | 0                         | 1       |
| rs2301260   | Exon 1            | G/A     | 0.11 (66) | 0.065 (78)   | 1.55                      | 0.21    |
| rs968389    | Exon 1            | A/G     | 0.40 (63) | 0.39 (82)    | 0.02                      | 0.88    |
| rsOXTR_03   | Intron 1          | C/T     | NA        | NA           | NA                        | NA      |
| rs237915    | Intron 1          | T/C     | 0.27 (62) | 0.37 (20)    | 1.03                      | 0.31    |
| rs237913    | Intron 2          | C/A     | 0.27 (62) | 0.37 (20)    | 1.03                      | 0.31    |
| rsOXTR_04   | Exon 3            | G/A     | 0.15 (63) | 0.15 (20)    | 0                         | 1       |
| rs2228485   | Exon 3            | G/A     | NA        | NA           | NA                        | NA      |
| rs4686302   | Exon 3            | C/T     | NA        | NA           | NA                        | NA      |
| rs237902    | Exon 3            | G/A     | NA        | NA           | NA                        | NA      |
| rs1042778   | 3’UTR             | G/T     | NA        | NA           | NA                        | NA      |

*Yates corrected Chi square
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