Polymorphisms in Genes Coding for Cytokines, Mannose-Binding Lectin, Collagen Metabolism and Thrombophilia in Women with Cervical Insufficiency

Iben Sundtoft^a Niels Ulbjerg^a Rudi Steffensen^b Steffen Sommer^d
Ole Bjarne Christiansen^{c, e}

^aDepartment of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Departments of ^bClinical Immunology and ^cObstetrics and Gynecology, Aalborg University Hospital, Aalborg, ^dDepartment of Obstetrics and Gynecology, Horsens Hospital, Horsens, and ^eThe Fertility Clinic, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Key Words

Cervical insufficiency · Preterm birth · Cytokines · Mannose-binding lectin · Single nucleotide polymorphisms

Abstract

Objective: To study the association between cervical insufficiency and single nucleotide polymorphisms in seven genes coding for pro- and anti-inflammatory cytokine-related factors, mannose-binding lectin 2 (*MBL2*), collagen1 α 1 (*COL1A1*), *factor II* and *factor V Leiden* genes. **Methods:** In a case-control study, potential maternal biomarkers for cervical insufficiency were investigated in 30 women with a history of second-trimester miscarriage or preterm birth due to cervical insufficiency and in 70 control women. **Results:** Homozygous carriers of the interleukin 6 (*IL6*) –174 genotype GG had an odds ratio (OR) of 3.1 [95% confidence interval (95% CI) 1.3–7.4, $p = 0.01$] and *MBL2* genotypes coding for low or intermediate levels of plasma MBL had an OR of 3.3 (95% CI 1.2–9.0, $p = 0.01$) for cervical insufficiency compared with controls. Serum MBL levels were lower in women with cervical insufficiency than in controls (median 408 and 1,985 ng/ml, respectively, $p < 0.01$). **Conclusions:** Single nucleotide

polymorphisms in the *IL6* gene and the *MBL2* gene and low MBL levels related to the latter polymorphism may increase the risk of preterm birth due to cervical insufficiency.

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Introduction

Deliveries, whether term or preterm, are believed to share a common pathway of uterine contractility, cervical dilation and activation of the membranes. Term labor is a result of physiological activation of the components of the common pathway, whereas a pathological activation of the pathway may lead to preterm birth (PTB). The pathological processes implicated have been categorized as intrauterine infection/inflammation, uterine ischemia, uterine overdistension, abnormal allograft reaction, allergy, cervical insufficiency and hormonal disorders [1].

Cervical insufficiency is characterized by painless cervical dilation in the second trimester of pregnancy in the absence of uterine contractions causing second-trimester miscarriage or extreme PTB. The lack of pathognomonic findings and clear diagnostic criteria makes it difficult to

ascertain the actual incidence of cervical insufficiency, but estimates vary around 0.5–1% [2, 3].

Cervical insufficiency may be caused by a constitutional deficiency of the components of the cervical connective tissue. Thus the cervical collagen concentration has been shown to be lower in women with a history of cervical insufficiency [4, 5]. Congenital uterine malformation and surgery on the cervix including conization are other proposed causes of cervical insufficiency [6–9]. Cerclage treatment has been shown to be of benefit in selected cases of cervical insufficiency [10–12].

There is also some evidence that proinflammatory cytokines, such as interleukin 1 α (IL-1 α), IL-1 β , IL-6 and tumor necrosis factor α (TNF- α), play a role in cervical insufficiency as their levels in amniotic fluid are higher in women undergoing emergency cerclage surgery compared with normal pregnant women [13]. There may also be an increased risk of late miscarriage or PTB [14, 15] in women with thrombophilia caused by thrombosis in the placental vessels, but in theory this is expected to cause intrauterine fetal death rather than PTB due to cervical insufficiency.

The involvement of genetic factors in the pathophysiology of PTB and cervical insufficiency has been hypothesized based on studies of familial aggregation of PTB [16, 17] and case-control studies of presumed susceptibility genes [18–26].

A single study on genes related to cervical connective tissue reported an association between polymorphisms in genes for collagen1 α 1 (*COL1A1*), transforming growth factor-beta (*TGF- β*) and cervical insufficiency [20]. Single nucleotide polymorphisms (SNPs) in genes associated with immune response and inflammation may be associated with recurrent miscarriage and second-trimester miscarriage. An association between polymorphisms in genes coding for proinflammatory cytokines and PTB has been demonstrated in several studies [19, 21–26]; the evidence for the *IL6* -174 genotype [27] and genetic polymorphisms predisposing to low plasma levels of mannose-binding lectin (MBL) [18, 19] being associated to PTB must a priori be considered strong. However, studies of the association between these SNPs and cervical insufficiency have not been published. In the present study we therefore aimed to study whether relevant DNA variants in the maternal genome were associated with second-trimester miscarriage and PTB due to cervical insufficiency. Sixteen genetic markers previously reported as possibly associated with second-trimester miscarriage and PTB were studied, thirteen SNPs from seven relevant cytokine and collagen genes in addition to functional, relevant

polymorphisms in the mannose-binding lectin 2 (*MBL2*) gene, and two thrombophilia-related SNPs. Finally serum MBL levels were measured in patients and controls.

Subjects and Methods

Study Population

At the Departments of Obstetrics and Gynecology, Aarhus University Hospital Skejby, Aarhus, Horsens Hospital, Horsens, and Rigshospitalet, Copenhagen University Hospital, Copenhagen, 100 Danish women were enrolled in this case-control study from September 2007 to August 2010. Cases and controls were recruited simultaneously. General exclusion criteria for both cases and controls were age <18 years, non-Caucasian ethnicity, a history of cervical dysplasia or conization, connective tissue disorder, menopause, pregnancy in the preceding 12 months, placental abruption, multiple pregnancy or fetal abnormality in the index pregnancies. Cases and controls were not further matched.

Cases

To identify women with cervical insufficiency, medical charts were studied for all patients with discharge ICD-10 codes for cervical insufficiency (DO343), late miscarriage (DO03, gestational age >15 weeks), threatening PTB (DO472) as well as external and abdominal cerclage (KMAB00, KLDD10). Approximately 850 women had a relevant discharge code, 262 of whom had clinical evidence of dilation of the cervix without labor in the second trimester. Women with vaginal bleeding or intrauterine infections during pregnancy or other symptoms uncharacteristic of cervical insufficiency, previous term birth, incomplete history or any of the aforementioned exclusion criteria were excluded after review of the medical charts. After critical review of the medical charts the inclusion criteria was met by 62 women, of whom 31 accepted to participate; 14 women did not respond, 5 were pregnant and 12 did not want to participate for other reasons. One woman was subsequently excluded because of failed blood sampling.

Controls

The control group consisted of 70 normal women hospitalized for sterilization or surgical procedures as part of the diagnosis and treatment of sterility, endometriosis, dysmenorrhea, premenopausal menometrorrhagia, leiomyoma, ovarian cysts or other benign conditions not involving the cervix. Exclusion criteria were any of the general criteria as well as previous PTB (birth before 37 weeks of gestation) or second-trimester miscarriage.

Blood Collection and Analysis

Blood samples were obtained from both cases and controls for analysis of activated partial thromboplastin time as a screening for presence of the lupus anticoagulant, IgG and IgM anticardiolipin antibodies (EliA anticardiolipin IgG and IgM, Phadia ApS, Alleroed, Denmark) and β 1-glycoprotein-1 antibodies (EliA anti- β 2-glycoprotein IgG and IgM, Phadia ApS). Serum MBL was determined by a monoclonal antibody-based time-resolved immunofluorometric assay described in a previous study [28].

Genotyping was carried out in six SNPs in the *MBL2* gene, three located in the promoter region: *MBL2* -619 G > C (called H/L, respectively; rs11003125), *MBL2* -290 G > C (called Y/X;

rs7096206), *MBL2* -66 C > T (called P/Q; rs7095891) and three located in the first exon of the structural *MBL2* gene: *MBL2* +154 C > T (called A/D; rs5030737), *MBL2* +161 G > A (called A/B; rs1800450), and *MBL2* +170 G > A (called A/C; rs1800451). Relevant SNPs in the *COL1A1* gene coding for collagen 1 α 1 (rs1800012) [20], in the *factor II* gene (rs1799963) and the *factor V Leiden* gene (rs6025) were also investigated. Finally, 12 SNPs located in seven cytokine or cytokine-related genes were investigated: *IL1A* +4845 G > T (rs17561), *IL1A* +889 C > T (rs1800587), *IL1B* +31 T > C (rs1143627), *IL1B* -511 C > T (rs16944), *IL6* -174 G > C (rs1800795), *IL6R* G > A (rs8192282), *IL10* -1082 A > G (rs1800896), *LTA* -82 G > C (rs746868), *LTA* +252 A > G (rs909253), *TNFA* -308 G > A (rs1800629), *TNFA* -857 C > T (rs1799724), and *TGFB* +174 G > C (rs1800471). Information about SNP IDs and nucleotide changes is given in online supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000381620). The SNPs were selected based on the available literature and hypothesized proinflammatory pathways and are mainly those that have been previously reported to be associated with recurrent or late miscarriages or PTB.

Genotyping Technique

DNA was extracted from EDTA-stabilized blood samples using a Maxwell 16 blood DNA purification kit (Promega, Madison, Wis., USA). All SNPs were tested using a real-time polymerase chain reaction (PCR) technique using minor groove binder TaqMan probes (Applied Biosystems, Foster City, Calif., USA). Information about primers, probes and array references is presented in online supplementary table 1. For 10 SNPs in cytokine genes pre-designed SNP Genotyping Assays (Applied Biosystems) were used, for four SNPs (*LTA* -82 G > C, *TGFB* 74 G > C, *factor II* G20210 G > A, and *factor V Leiden* 1691 G > A) primers and probes were designed for this study, and finally the remaining six SNPs (in the *MBL2* gene) were investigated as previously described [29, 30]. DNA amplification was carried out in 5 μ l containing 20 ng DNA, 0.9 μ M primers and 0.2 μ M probes (final concentrations). The product was amplified using TaqMan Universal PCR Master Mix (Applied Biosystems). Reactions were performed in 384-well plates with the following protocol on a GeneAmp PCR 9700 or a 7900 HT Sequence Detection System: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. To determine genotypes, end-point fluorescence was read on the 7900 HT Sequence Detection Systems using SDS version 2.3 software.

Statistical Analysis

Differences in demographic characteristics between the cervical insufficiency and the control group were compared using the Wilcoxon rank-sum test. Frequencies of SNPs were determined and recorded as allele frequencies (proportion of positive chromosomes) and genotypes (proportion of women possessing homozygous or heterozygous combinations of SNPs). With regard to the SNPs in the *MBL2* gene, recognized haplotypes and genotypes encompassing SNPs in the promoter region and exon 1 were established. The Hardy-Weinberg equilibrium was estimated separately for each polymorphism in the case and the control group, respectively. Comparisons of frequencies of individual SNPs and MBL genotypes associated with high, intermediate or low MBL concentrations [31] in patients with cervical insufficiency and controls were assessed by the χ^2 test. A two-sided p value <0.05 was

considered statistically significant. Odds ratios (ORs) were reported with 95% confidence interval (95% CI). Statistical analysis was performed with Stata (statistical software package version 10.1 for Windows, StataCorp LP).

Results

The baseline characteristics of the included participants are shown in table 1. Detailed clinical data on the women with cervical insufficiency are shown in online supplementary table 2. We found no difference in the characteristics of the women with cervical insufficiency who did not participate (n = 31) in the study compared to those participating (n = 31) (results not shown).

All SNPs were in Hardy-Weinberg equilibrium in both the patient and the control group, except for the SNPs in *IL1B* -31 (both patient and control group) and *IL1B* -511 (control group).

Two genotypes were significantly associated with cervical insufficiency compared with controls: homozygous carriers of the *IL6* -174 G allele (genotype GG) (OR 3.1, 95% CI 1.3–7.4, p = 0.01; table 2) and carriers of *MBL2* genotypes associated with low and intermediate MBL levels (OR 3.3, 95% CI 1.2–9.0, p = 0.01; table 3).

We found no significant difference between the distributions of other genotypes in the two groups (table 2). There was a non-significant (p = 0.20) difference in the frequency of carriers of the T polymorphism in the in *COL1A1* gene (table 4).

Serum MBL levels were significantly lower in women with cervical insufficiency (median 408 ng/ml, 95% CI 118–1,463) than in controls (1,985 ng/ml, 95% CI 1,265–2,735, p < 0.01; table 3). 40% of women with cervical insufficiency had MBL levels <200 ng/ml compared with 18.8% of controls (OR 2.9, 95% CI 1.1–7.4).

There were no significant differences in the prevalence of increased activated partial thromboplastin time or positivity for IgM and IgG anticardiolipin and IgM and IgG β 2-glycoprotein-1 antibodies between the patients and controls. The rate of biomarkers related to the antiphospholipid syndrome in patients was <3.3%. None of the patients or controls carried the *factor II* 20210 G > A SNP, whereas 7.1% of patients and 10.1% of controls carried the *factor V* 1691 G/A SNP (p = 0.64).

A significant difference in parity was found between cases and controls, with more multiparous and no nulliparous subjects in the group of women with cervical insufficiency. The higher frequency of multiparity in the case group was due to a higher rate of stillbirths and ex-

Table 1. Baseline characteristics of patients with cervical insufficiency (n = 30) and controls (n = 70)

	Cervical insufficiency		Controls		p value
	n (%)	median (range)	n (%)	median (range)	
Age, years		35 (22–45)		31 (18–49)	<0.01
Birth		2 (0–4)		1 (0–6)	<0.01
Nullipara	3 (10)		30 (43)		<0.01
Primipara	4 (13)		16 (23)		0.28
Multipara	23 (77)		24 (34)		<0.01
Neonatal death ^a	20 (67)	1 (0–3)	0		<0.01
Ectopic pregnancy	2 (7)	0 (0–1)	0		0.03
Early miscarriage	12 (40)	0 (0–3)	13 (19)	0 (0–2)	0.02
Late miscarriage	14 (47)	0 (0–3)	0		<0.01
Induced abortion	10 (33)	0 (0–4)	11 (16)	0 (0–2)	0.03
Cerclage treatment			0		
Transcervical	17 (57)	1 (0–3)	0		<0.01
Transabdominal	16 (53)	1 (0–1)	0		<0.01
Uterine malformations ^b	4 (13)	0 (0–1)	0		<0.01

Birth: gestational age ≥ 22 weeks; early miscarriage: gestational age < 15 weeks; late miscarriage: gestational age 15–22 weeks; induced abortion: gestational age < 12 weeks.

^a Defined as death within the first 28 days of life. ^b Defined as uterine septum, uni- or bicornuate uterus.

treme PTBs (online suppl. table 2). Excluding all nulliparous controls (n = 30) did not change the results; homozygous carriers of the *IL6* –174 GG genotype had an OR of 3.5 (95% CI 1.2–10.7, p = 0.01) and carriers of *MBL2* genotypes associated with low and intermediate MBL levels had an OR of 2.6 (95% CI 0.9–7.7, p = 0.05) for cervical insufficiency when compared with controls.

Discussion

This is the first study of a possible association between several maternal DNA variants associated with predisposition to inflammation, immune dysregulation or collagen metabolism and cervical insufficiency. We found that the *IL6* –174 GG genotype and polymorphisms in the *MBL2* gene associated with low or intermediate levels of plasma MBL were significantly associated with cervical insufficiency. In addition, by measuring the MBL protein directly in serum, the median levels were significantly lower and the frequency of low (< 200 ng/ml) MBL levels was significantly higher in women with cervical insufficiency than controls.

The mechanism linking immunoregulatory genetic polymorphisms to late adverse pregnancy outcomes has been proposed as a predisposition to an abnormal inflam-

matory response following an otherwise harmless microbial colonization of the cervix or amniotic membranes or microbial invasion of the amniotic cavity. Increased levels of inflammatory cytokines have been found in the amniotic fluid of women in preterm labor ending in PTB. Pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6, are believed to induce synthesis and release of prostaglandins and metalloproteinases, stimulating uterine contractions, softening of the cervix and weakening of the chorioamniotic membranes [32], whereas anti-inflammatory cytokines such as IL-10 and TGF- $\beta 1$ modulate the effect of the proinflammatory cytokines. These findings make it reasonable to study associations between cervical insufficiency and polymorphisms in genes coding for pro- and anti-inflammatory cytokines.

It has been reported that carriers of the *IL6* –174 CC genotype have significantly lower plasma concentrations of IL-6 than individuals with the GG and GC genotypes [33], and it is reasonable to suggest that the latter genotypes are also associated with higher amniotic fluid concentrations of IL-6 and therefore predisposition to PTB. Our study of a highly selected population consisting of women with classic cervical insufficiency, excluding those with signs of intrauterine infections, labor or vaginal bleeding at time of delivery, confirms the association between the maternal *IL6* –174 GG genotype and

Table 2. Allele and genotype frequencies of SNPs in cytokine or cytokine receptor genes in patients with cervical insufficiency (n = 30) and controls (n = 70)

Gene polymorphisms		Cervical insufficiency		Controls		OR	95% CI	p value
		n	%	n	%			
<i>IL1A</i> 889 (C > T)	CC	13	43.3	32	45.7	0.9	0.3–2.3	0.83
	CT	15	50.0	31	44.3	1.3	0.5–3.2	0.60
	TT	2	6.7	7	10.0	0.6	0.1–3.7	0.59
	allele C	41	68.3	95	67.9	1.0	–	0.95
	allele T	19	31.7	45	32.1			
<i>IL1A</i> 4845 (G > T)	GG	13	43.3	32	45.7	0.9	0.3–2.3	0.83
	GT	16	53.3	31	44.3	1.4	0.6–3.7	0.41
	TT	1	3.3	7	10.0	0.3	0.0–2.6	0.26
	allele G	42	70.0	95	67.9	1.1	0.5–2.3	0.77
	allele T	18	30.0	45	32.1			
<i>IL1B</i> –31 (T > C)	TT	11	36.7	33	47.1	0.6	0.2–1.7	0.33
	TC	13	43.3	23	32.9	1.6	0.6–4.1	0.32
	CC	6	20.0	14	20.0	1.0	–	1.0
	CC + CT	19	63.3	37	52.9	1.5	0.6–4.1	0.30
	allele T	25	41.7	51	36.4	0.8	0.4–1.6	0.48
	allele C	35	58.3	89	63.6			
<i>IL1B</i> –511 (C > T)	CC	11	36.7	33	47.1	0.6	0.2–1.7	0.33
	CT	13	43.3	24	34.3	1.5	0.6–3.8	0.39
	TT	6	20.0	13	18.6	1.1	0.3–3.6	0.87
	TT + CT	19	63.3	37	52.9	1.5	0.6–4.1	0.30
	allele C	35	58.3	90	64.3	0.8	0.4–1.5	0.43
	allele T	25	41.7	50	35.7			
<i>IL6</i> –174 (G > C)	GG	16	53.3	19	27.1	3.1	1.3–7.4	0.01
	GC	11	36.7	33	47.1	0.7	0.3–1.6	0.33
	CC	3	10.0	18	25.7	0.3	0.1–1.2	0.08
	GG + GC	27	90.0	52	74.3	3.1	0.8–11.5	0.08
	allele G	43	71.7	71	50.7	2.4	1.3–4.7	0.01
	allele C	17	28.3	69	49.3			
<i>IL6R</i> (G > A)	GG	21	70.0	42	60.0	1.6	0.6–4.4	0.34
	GA	9	30.0	26	37.1	0.7	0.3–2.0	0.49
	AA	0	0.0	2	2.9			
	allele G	51	85.0	110	78.6	1.5	0.7–4.0	0.29
	allele A	9	15.0	30	21.4	1.5	0.7–4.0	0.29
<i>IL10</i> –1082 (A > C)	AA	11	36.7	16	22.9	1.9	0.7–5.4	0.15
	AC	12	40.0	34	48.6	0.7	0.3–1.8	0.43
	CC	7	23.3	20	28.6	0.8	0.2–2.2	0.59
	allele A	34	56.7	66	47.1	1.5	0.8–2.8	0.22
	allele C	26	43.3	74	52.9			
<i>LTA</i> –82 (C > G)	CC	9	30.0	28	40.0	0.6	0.2–1.7	0.34
	CG	15	50.0	34	48.6	1.1	0.4–2.7	0.90
	GG	6	20.0	8	11.4	1.9	0.5–7.1	0.26
	allele G	33	55.0	90	65.3	0.7	0.4–1.3	0.22
	allele C	27	45.0	50	35.7			
<i>LTA</i> 252 (A > G)	AA	14	46.7	27	38.6	1.4	0.5–3.6	0.45
	AG	12	40.0	31	44.3	0.8	0.3–2.2	0.69
	GG	4	13.3	12	17.1	0.7	0.2–2.8	0.63
	allele A	40	66.7	85	60.7	1.3	0.7–2.6	0.43
	allele G	20	33.3	55	39.3			

Table 2 (continued)

Gene polymorphisms		Cervical insufficiency		Controls		OR	95% CI	p value
		n	%	n	%			
<i>TGFB1</i> 74 (G > C)	GG	27	90.0	55	78.6	2.5	0.6–14.2	0.17
	GC	3	10.0	14	20.0	0.4	0.1–1.8	0.22
	CC	0	0.0	1	1.4	–	–	0.51
	allele G	57	95.0	124	88.6	2.5	0.7–13.6	0.16
	allele C	3	5.0	16	11.4			
<i>TNFA</i> –308 (G > A)	GG	22	79.3	47	67.1	1.3	0.5–4.0	0.54
	GA	6	20.0	22	31.4	0.5	0.2–1.6	0.24
	AA	2	6.7	1	1.4	4.9	0.2–294.8	0.16
	allele G	50	83.3	116	82.9	1.0	–	0.93
	allele A	10	16.7	24	17.1			
<i>TNFA</i> 857 (C > T)	CC	25	83.3	63	90.0	0.6	0.1–2.5	0.35
	CT	5	16.7	6	8.6	2.1	0.5–9.2	0.24
	TT	0	0.0	1	1.4			0.51
	allele C	55	91.7	132	94.3	1.5	0.4–5.5	0.49
	allele T	5	8.3	8	5.7			

Table 3. *MBL2* genotypes associated with high, intermediate and low MBL levels and plasma MBL levels measured by ELISA in women with cervical insufficiency (n = 30) and controls (n = 70)

	Cervical insufficiency		Controls		OR	95% CI	p value
	n	%	n	%			
<i>MBL2</i> genotype							
YA/YA ^a	4	13.3	33	47.1	0.17	0.04–0.6	0.01
YA/XA ^a	7	23.3	13	18.6	1.32	0.4–4.2	0.59
XA/XA ^b	2	6.7	1	1.4	4.93	0.2–294.8	0.16
YA/YO ^b	9	30.0	13	18.6	1.88	0.6–5.6	0.21
XA/YO ^c	5	16.7	7	10.0	1.8	0.4–7.3	0.35
YO/YO ^c	3	10.0	3	4.3	2.48	0.3–19.5	0.27
Genotype-deduced plasma MBL level							
High	11	36.7	46	65.7	0.30	0.1–0.8	0.01
Intermediate	11	36.7	14	20.0	2.32	0.8–6.6	0.08
Low	8	26.7	10	14.3	2.18	0.7–7.0	0.14
Low + intermediate	19	63.3	24	34.3	3.31	1.2–9.0	0.01
Measured plasma MBL level, ng/ml (n, median)	30	408	69	1,985			0.008

The structural mutant alleles (B, C and D) are collectively designated 'O'. The haplotypes HYPA, LYQA and LYPA associated with high MBL plasma levels are commonly evaluated as a joined diplotype group 'YA'. The LXPA haplotype is analyzed as 'XA' and the HYPD, LYPB, LYQC and LYPD haplotypes as 'YO'.

^a Associated with high plasma MBL levels (500–5,000 ng/ml). ^b Associated with intermediate plasma MBL levels (50–1,200 ng/ml).

^c Associated with low plasma MBL levels (0–200 ng/ml).

cervical insufficiency. In accordance, Wu et al. [27] in a meta-analysis found the SNP *IL6* –174 genotype CC to be protective against PTB in women of European descent.

Inability to prevent microbial invasion of the amniotic cavity can also, in theory, be caused by a genetic immunodeficiency such as a deficiency of MBL. Our study found significant associations between low serum levels

Table 4. Association between the *COL1A1* 1023 polymorphisms related to connective tissue metabolism and cervical insufficiency (cervical insufficiency: n = 30; controls: n = 70)

<i>COL1A1</i> 1023 G > T	Cervical insufficiency		Controls		OR	95% CI	p value
	n	%	n	%			
GG	17	56.7	49	70.0	0.6	0.2–1.5	0.20
GT	12	40.0	20	28.6	1.7	0.6–4.4	0.26
TT	1	3.3	1	1.4	2.4	0.3–189.4	0.53
TT + GT	13	43.3	21	30.0	1.8	0.7–4.7	0.20
Allele T	46	76.7	118	84.3	0.6	0.3–1.4	0.20
Allele G	14	23.3	22	15.7	1.6	0.7–3.7	0.20

n = Number of participants.

of the MBL protein, genotypes associated with low or intermediate MBL levels and cervical insufficiency. MBL binds to carbohydrates on the surfaces of microorganisms, which results in complement activation and thereby elimination of the microorganisms, but MBL can probably also modulate secretion of cytokines such as TNF- α from the lymphocytes. The mechanism through which low MBL may contribute to PTB (and cervical insufficiency) is unknown, and studies on the association between MBL deficiency and PTB are conflicting [18, 34–38].

We found a trend for an association between cervical insufficiency and a SNP in the *COL1A1* gene that may predispose to an increased connective tissue metabolism, which support findings by Warren et al. [20].

There are several limitations to this study. The small sample size can cause a type 2 error, as the study population will be underpowered to detect weak genetic effects that may be of importance in a complex condition as cervical insufficiency. We tested 12 SNPs in cytokine-related genes, and regarding some of these we had no prior hypothesis about which may be associated with cervical insufficiency; therefore, it could be argued that some of the p values in table 2 should be corrected by the Bonferroni method. However, based on the meta-analysis by Wu et al. [27], the *IL6* –174 GG genotype was a priori expected to confer susceptibility to cervical insufficiency, and therefore in our view the p value for this genotype needs no correction. Similarly, the *MBL2* genotypes associated with low and intermediate MBL levels and the *COL1A1* TT + GT genotypes have previously been reported to be associated with late miscarriage and cervical insufficiency and were therefore a priori candidate susceptibility genetic variants not needing Bonferroni correction.

The strength of this study is the highly selected group of cases meeting the criteria of classic cervical insufficiency: a painless progressive dilation and effacement of the uterine cervix during the second trimester of pregnancy without vaginal bleeding or signs of intrauterine infection. It adds to the strength that cases and controls all originated from the same ethnic population (Caucasians), although our findings may not allow generalization to other ethnic populations.

In conclusion, our results confirm that both polymorphisms in the *MBL2* gene associated with low or intermediate levels of serum MBL and low plasma MBL levels increase the risk of PTB due to cervical insufficiency. Furthermore, the *IL6* –174 GG genotype also seems to confer susceptibility to PTB due to cervical insufficiency, which confirms findings in more heterogeneous populations. Overall our results support the theory of cervical insufficiency being, in part, a genetic disorder.

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Statement of Ethics

This study was approved by the local Ethics Committee (project identification number M-20070185) and the Danish Data Protection Agency (project identification number 2007-41-0834). Informed consent was obtained from all participants.

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