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The association of beta-2 adrenoceptor genotype with short-cervix mediated preterm birth: a case-control study

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Abstract

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DISCLOSURE OF INTERESTS

The authors report no financial interests, commercial affiliations, or other possible conflicts of interest.

CONTRIBUTION TO AUTHORSHIP

Each author can take public responsibility for this manuscript.

Dr. Miller (R.S.M.) drafted the manuscript, assisted by Dr. Thom (E.A.T.), who provided substantial scientific input to the drafting and revision of this manuscript with regard to scientific content and form, conducted the statistical analysis, and approved the final manuscript as submitted.

All authors (R.S.M.; R.M.S.; E.A.T.; W.A.G.; J.D.I.; B.M.M.; G.S.; A.T.T.; U.M.R.; D.J.R.; Y.S.; S.C.B.; M.S.E.; J.E.T.; S.N.C.) participated in study design and protocol development, and contributed to critical aspects of the conduct of this research including but not limited to some or all of the following: assessment of patient recruitment; monitoring center performance; oversight of data quality; and evaluation and analysis of data. They provided significant intellectual contribution to the drafting and revision of this manuscript with regard to scientific content and form, and approved the final manuscript as submitted.

DETAILS OF ETHICS APPROVAL

The primary data collection was approved by the Institutional Review Boards of the Biostatistical Coordinating Center and the clinical sites at which patients were recruited, and all women provided written informed consent.

At Columbia University Medical Center, institution of the primary author, this study was designated as protocol IRB-AAAD5071 and first approved on August 7, 2008.

OBJECTIVE—To determine if β_2 -adrenoceptor (β_2 AR) genotype is associated with shortening of the cervix or with preterm birth (PTB) risk among subjects with a short cervix in the second trimester.

DESIGN—A case-control ancillary study to a multicenter randomized controlled trial.

SETTING—14 participating centers of the Maternal-Fetal Medicine Units Network of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

POPULATION—439 subjects, including 315 with short cervix and 124 with normal cervical length.

METHODS—Nulliparous women with cervical length <30 mm upon 16–22 week transvaginal sonogram and controls frequency-matched for race/ethnicity with cervical lengths ≥ 40 mm were studied. β_2 AR genotype was determined at positions encoding for amino acid residues 16 and 27.

MAIN OUTCOME MEASURES—Genotype distributions were compared between case and control groups. Within the short cervix group, pregnancy outcomes were compared by genotype, with a primary outcome of PTB <37 weeks.

RESULTS—Genotype data were available at position 16 for 433 subjects and position 27 for 437. Using a recessive model testing for association between short cervix and genotype, and adjusted for ethnicity, there was no statistical difference between cases and controls for Arg16 homozygosity (OR 0.7, 95% CI 0.4–1.3) or Gln27 homozygosity (OR 0.9, 95% CI 0.3–2.7). Among cases, Arg16 homozygosity was not associated with protection from PTB or spontaneous PTB. Gln27 homozygosity was not associated with PTB risk, although sample size was limited.

CONCLUSIONS— β_2 AR genotype does not seem to be associated with short cervical length or with PTB following the second-trimester identification of a short cervix. Influences on PTB associated with β_2 AR genotype do not appear to involve a short cervix pathway.

TWEETABLE ABSTRACT— β_2 AR gene variation was not associated with short cervix or with PTB risk following a short cervix diagnosis.

Keywords

Beta-2 adrenoceptor; Short cervix; Genotype

INTRODUCTION

Preterm birth, defined as delivery prior to 37 weeks gestational age, remains the leading cause of neonatal morbidity and mortality. It is well-established that risk of spontaneous preterm birth (SPTB) increases as sonographically-measured cervical length shortens in the second trimester of pregnancy.¹ However, scientific understanding of why some women develop a short cervix in the second trimester of pregnancy remains limited. While genetic influences have been implicated in short cervix pathophysiology,² understanding of the scope and magnitude of this association is incomplete.

The beta-2 adrenergic receptor (β_2 AR) is a well-studied G-protein-coupled receptor that is ubiquitously expressed in numerous human tissues and organs, including smooth muscle

cells in the uterine corpus and cervix.^{3, 4} β_2 AR stimulation by endogenous and exogenous agonists results in smooth muscle relaxation, potentially affecting cervical tone and resistance to mechanical distension. While human *in vitro* data suggest β_2 AR stimulation has an inhibitory effect on cervical contractile activity,⁵ animal studies indicate that stimulation increases cervical resistance to mechanical stretching.⁶

Single nucleotide polymorphisms in the β_2 AR gene (*ADRB2*) at codons encoding amino acids 16 and 27⁷⁻⁹ are believed to affect down-regulation and desensitization of the β_2 AR¹⁰⁻¹², resulting in differential responsiveness to circulating endogenous β -agonists. These polymorphisms have been implicated in a wide range of disease states,¹³⁻²² with polymorphic variation encoding for arginine homozygosity in *ADRB2* amino acid residue 16 (Arg16Arg) specifically associated with decreased risk of SPTB, slower rate of cervical dilatation in active labor, and altered responsiveness to β -agonist therapy.²³⁻²⁷

Given that β_2 AR genotype is associated with obstetrical phenotypes, as well as the fact that cervical β_2 AR expression appears to be functionally relevant, it is biologically plausible that β_2 AR genotype is related to pathophysiologic changes in cervical length. The objective of this study was to determine if Arg16Arg β_2 AR genotype is associated with decreased risk of developing short cervix or with protection from PTB risk following the second-trimester identification of a short cervix. Although genotype encoding for codon 16 was the focus of this investigation, we also analyzed genotype encoding for codon 27 given a known functional polymorphism with possible obstetrical associations at that location.^{28, 29} Furthermore, given an association between β_2 AR genotype and various disease states including hypertensive disorders and diabetes mellitus, a secondary aim of this study was to evaluate whether genotypes encoding for polymorphic variation at codons 16 or 27 are associated with the development of maternal medical complications of pregnancy.

METHODS

This was an ancillary study involving subjects participating in a multi-center randomized controlled trial investigating the effects of 17 alpha-hydroxyprogesterone caproate (17-OHPc) for the reduction of PTB following the sonographic identification of a short cervix in nulliparous women³⁰ conducted by 14 participating centers of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units (MFMU) Network. Approval for this investigation was obtained from the Institutional Review Board of each participating MFMU center.

Inclusion in the primary trial required a singleton nulliparous gestation complicated by a cervical length (CL) <30 mm identified by transvaginal ultrasound between 16 0/7 and 22 3/7 weeks gestational age. Subjects participating in the primary trial were approached to participate in this ancillary study, and those that agreed comprised the case group.

The first study aim, which was to determine if β_2 AR genotype is associated with shortening of the cervix, was addressed using a case-control study design. Normal cervical length control subjects who otherwise satisfied inclusion criteria for the primary trial but possessed a cervical length ≥ 40 mm were identified. ≥ 40 mm or greater was selected as the control group

inclusion cut-off because it is uncontroversial as a normal cervical length measurement. Short and normal cervical length subjects were frequency matched in a 2:1 fashion, to be similar in terms of race/ethnicity within each site. This was achieved by instituting a quota system, by site, of the number of 'normal cervix' women of given race/ethnicity that could be recruited on a monthly/quarterly basis. This ratio was chosen as a function of resources available to complete this investigation, while still yielding a large enough sample size to detect meaningful differences. Antenatal data were collected from all subjects at the time of study enrollment. Pregnancy outcomes were prospectively recorded for all short cervical length subjects.

A 5 mL venous blood sample was obtained from each study participant. DNA was extracted and purified with a Puregene extraction kit (Gentra, Minneapolis, MN) and tested for quantity, purity and quality by optical densitometry (ratio, 260/280 nm). All samples underwent genotyping at Prevention Genetics (Marshfield, WI). Genotyping assays were run as submicroliter PCR-based assays on Array Tape (Douglas Scientific, Alexandria, MN) to determine the β_2 AR single-nucleotide polymorphisms (SNPs) within codons encoding amino acids 16 (rs1042713) and 27 (rs1042714)^{31–33} Of note, in an effort to remain consistent with convention established in prior *ADRB2* publications, β_2 AR polymorphisms in this manuscript are described by the amino acid residues they encode for and not the polymorphism designation. Quality control (QC) was internally performed at Prevention using a gender marker (rs25601). External QC was performed by re-genotyping approximately 10% of the samples at our laboratories at the University of Geneva, Switzerland. As described in previous work, DNA was amplified by polymerase chain reaction using specific and validated primers for single-copy DNA regions encoding for amino acid changes Arg16Gly and Gln27Glu located in the single exon of *ADRB2*.³⁴ β_2 AR SNPs for rs1042713 and rs1042714 were determined by Sanger sequencing reaction and electrophoresis on a fluorescent DNA fragment analyzer apparatus (ABI3100; Applied Biosystems, Foster City, CA). There was greater than 99% agreement between the genotyping at Prevention and in Geneva.

Power calculations were based upon variation at amino acid 16, the protein site of primary interest, given previously-demonstrated biologic association with obstetric outcomes. The first specific aim was to determine whether the odds of *ADRB2* polymorphic variation encoding for Arg16 homozygosity (Arg16Arg) is significantly different between those with and without short cervix. Based upon an anticipated 80% recruitment rate from the planned cohort of 1000 subjects in the primary trial, we expected a total of 1200 women would be enrolled in this ancillary study (800 with short cervical length and 400 with normal cervical length). This sample size was sufficient to yield at least 80% power, with an alpha of 5%, two-sided, to detect an odds ratio of 0.6 for Arg16Arg presence in the short cervix group compared with the normal length group, if 16% or more of the women in the normal cervical length group have the Arg16Arg genotype.

The second aim, which was to determine whether Arg16Arg is protective against PTB among cases with a short cervix, was addressed by examining outcomes among subjects in the case group stratified by β_2 AR genotype. The primary outcome was PTB, defined as delivery <37 0/7 completed weeks gestational age. Assuming a sample size of 400 subjects

with short cervical length in the placebo arm, a PTB rate in the whole group of 20% (as assumed for the parent trial) a PTB rate in the Arg-16 homozygous group of 10%, and a 20% rate of Arg16Arg homozygosity (80 subjects), we calculated that there was 71% power to detect a difference. If the PTB rate in the Arg-16 homozygous group were as low as 5%, there would be more than 99% power to detect a difference.

Secondary outcomes included PTB <35 weeks, PTB <32 weeks, spontaneous PTB, indicated PTB, preterm premature rupture of membranes (PPROM), and maternal health complications.

Chi-square or Fisher's exact test were used to compare categorical variables between groups and the Wilcoxon Rank Sum test was used to compare continuous variables. Chi-square tests for Hardy-Weinberg equilibrium were conducted for the control group. Logistic regression models were used to test differences between cases and controls after correcting for ethnicity. Odds ratios and 95% confidence intervals are reported. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. All tests were two-tailed and $p < 0.05$ was used to define statistical significance. No adjustments were made for multiple comparisons. A recessive model was used upon consideration of the published *ADRB2* literature. Analyses were performed using SAS Version 9.2 (SAS Institute, Inc, Cary, NC).

RESULTS

Between May, 2007 and May, 2011, 657 subjects were randomized in the primary trial before the DSMB halted the study for futility since the conditional power, given the results accumulated by that time, to show a benefit for progesterone was extremely low.²⁸ Of these, 380 subjects were approached to participate in the ancillary study; 315 (82.9%) agreed and supplied a blood sample. Among 198 candidate control subjects approached for participation, 124 (62.6%) consented and supplied a sample.

Among demographic and baseline antenatal characteristics, significant differences were observed between case and control groups with regard to maternal age, pre-pregnancy BMI, and gestational age at screening (Table 1). Consistent with study design, mean cervical length in the short cervix (case) group (2.4 cm, range 0.5–3.0 cm) was significantly shorter than in the control group (4.6 cm, range 4.0–7.1 cm, $p < .0001$). There was significantly more cervical funneling (21.9% vs. 0.8%, $p < 0.001$) and debris (11.1% vs. 4.0%, $p = 0.02$) in these women when compared with controls.

Genotype data were available at *ADRB2* position 16 (rs1042713) for 433 subjects and position 27 (rs1042714) for 437 subjects. The minor allele frequencies (MAF) were 0.47 and 0.25 for rs1042713 and rs1042714, respectively, which are close to the global MAFs of 0.47 and 0.24 as reported in the National Center for Biotechnology Information (NCBI) dbSNP database. Expected Hardy-Weinberg equilibrium was demonstrated at both positions ($p = 0.856$ for Arg16 homozygosity and $p = 0.279$ for Gln27 homozygosity). Overall, Arg16Arg prevalence was 23.1% and Gln27Gln prevalence was 7.8%. There was no evidence for lack of fit for any of the logistic models.

Table 2 presents *ADRB2* genotype by maternal race for SNPs rs1042713 and rs1042714. There was no difference in β_2 AR genotype between cases and controls after correcting for population stratification. Arg16Arg was present in 70 (22.4%) of cases and 30 (25.0%) of controls ($p=0.56$). Similarly, Gln27Gln was present in 25 (8.0%) cases and 9 (7.3%) controls ($p=0.72$). Using an ethnicity-adjusted recessive model testing for association between short cervix and genotype, there was no statistical difference between cases and controls for Arg16 homozygosity (OR 0.7, 95% CI 0.4–1.3) or Gln27 homozygosity (OR 0.9, 95% CI 0.3–2.7).

As there was no difference in PTB rates between 17-OHPc and placebo arms in the primary trial, subjects with short cervical length were considered as a single group without regard to trial randomization when evaluating associations between β_2 AR genotype and obstetrical outcomes (Tables 3 and 4). There was similarly no difference in PTB based upon treatment type among subjects with short cervix participating in this ancillary study (data not shown).

The overall rate of PTB among cases was 21.3%. Arg16Arg was not associated with PTB among subjects with a short cervix (Table 3). Gln27Gln was not associated with overall PTB or spontaneous PTB, but was associated with indicated PTB (Table 3). Secondary delivery outcomes were also not influenced by β_2 AR genotype encoding for amino acid positions 16 or 27. Genotype at positions 16 (Table 3) and 27 (Table 4) was not associated with antenatal complications including gestational diabetes, gestational hypertension, or preeclampsia. However, Arg16Arg was associated with an increased rate of placental abruption (10.0% versus 1.2%, $p = 0.002$).

DISCUSSION

MAIN FINDINGS

This study failed to demonstrate any relationship between β_2 AR genotype and short cervix or its consequences. Our results do not add to previous literature associating β_2 AR genotype with labor-related obstetrical phenotypes, as well as data suggesting that genotype at codon 16 affects β_2 AR function, resulting in altered myometrial responsiveness to endogenous and exogenous β -agonists, that could affect uterine tone and the risk of contractions and labor. Independent case-control studies by Landau and Doh each identified a strong relationship between Arg16Arg and SPTB suggestive of a genotype-specific protective effect.^{23, 25} A follow-up study by Landau demonstrated that an intravenous hexoprenaline tocolysis regimen significantly prolonged pregnancy in the Arg16Arg subset of subjects undergoing treatment for preterm labor.²⁶ Most recently, Miller demonstrated that Arg16Arg was associated with a significantly slower rate of dilatation in active labor among an unselected cohort of late preterm and term parturients.²⁷

It is possible that the inability to demonstrate a genotype effect in this study is due to its focus on cervical function, whereas prior β_2 AR studies have evaluated effects largely attributed to myometrial activity. While it is clear that there is a β_2 AR component to the maintenance of tone within the fibromuscular cervix, this effect is not as well understood, nor perhaps as important as it is within the uterus. Using biopsies taken from the human internal cervical os at term, Bryman demonstrated that although α -adrenoceptor-mediated

excitatory effects predominated, β -adrenoceptor effects could also be demonstrated.⁵ In contrast, while physiologic correlates within the human cervix are unclear, Gaspar demonstrated in rat models that β_2 AR stimulation with terbutaline *increased* cervical resistance against mechanical stretching.⁶ The above findings notwithstanding, current results may be interpreted to support at best a limited role of β_2 AR stimulation upon the maintenance of cervical tone, and no evidence for an effect of genotype variation.

Although β_2 AR genotype has been associated with hypertensive disorders and diabetes mellitus in the non-obstetrical literature,^{12, 18, 35, 36} genotypes at positions 16 and 27 were not associated with gestational diabetes, gestational hypertension, or preeclampsia in this study. Arg16Arg appeared to be associated with increased abruption risk when compared with other genotypes, however small numbers limit the strength of this finding. As with the association between Gln27Gln and indicated PTB, it is difficult to determine if these associations reflect true underlying genetic predispositions or instead are the unintended consequences of performing multiple statistical comparisons. Although this study was not specifically designed to analyze potential associations between *ADRB2* and maternal health complications, findings do not strongly suggest a genotype effect on maternal or obstetrical complications.

STRENGTHS AND LIMITATIONS

A strength of this investigation is that it correlated β_2 AR genotype with obstetrical outcomes in a moderately sized cohort of women with pregnancies complicated by short cervix. Limitations of our analyses include that the final subject recruitment fell short of initial sample size estimates, largely due to premature discontinuation of the primary trial. This premature study completion limited the number of subjects with short cervical lengths and those with normal cervical lengths that were ultimately enrolled in this investigation. However, for the analysis of genotype and PTB, this was mitigated by incorporating subjects enrolled in the active treatment group rather than just the placebo group, since there was no difference found between active and placebo. In addition, there was no appreciable data trend to suggest a reasonable probability that with full recruitment a statistically significant difference between groups would have been identified.

INTERPRETATION

These data do not demonstrate an association between β_2 AR genotype and short cervix nor PTB risk following the identification of a short cervix. Putative protection from PTB associated with polymorphic variation encoding for Arg16Arg within the β_2 AR does not appear to involve a short cervix pathway.

CONCLUSION

Study limitations notwithstanding, based upon these results β_2 AR genotype is a less compelling candidate for further research efforts regarding genetic contributions to a short cervix or its consequences. Future investigations into the relationship between β_2 AR genotype and PTB should target other pathways.

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Appendix

In addition to the authors, other members of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network are as follows:

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Table 1

Study Demographics

	Cervical length <30 mm N = 315	Cervical length 40 mm N = 124	P value
Maternal age at screening, years	21 (19, 25)	23 (20, 28)	0.003
Race			0.77
Black, non-Hispanic	170 (54.0)	64 (51.6)	
Hispanic	47 (14.9)	18 (14.5)	
White, non-Hispanic	86 (27.3)	39 (31.5)	
Other	12 (3.8)	3 (2.4)	
Pre-pregnancy BMI, kg/m ²	24.1 (20.9, 29.0)	26.0 (23.0, 30.0)	0.006
Prior pregnancy loss between 13–19 weeks	11 (3.5)	8 (6.5)	0.16
Cigarette smoker during this pregnancy	48 (15.2)	18 (14.6)	0.87
Beta-agonist exposure this pregnancy	8 (2.5)	0 (0.0)	1.00
Gestational age at screening, weeks	20 (18, 20)	19 (18, 20)	0.02
Cervical length at screening, cm	2.6 (2.2, 2.8)	4.4 (4.2, 4.7)	<.0001
Debris	35 (11.1)	5 (4.0)	0.03
Funneling	69 (21.9)	1 (0.8)	<.0001

Data presented as N (%) or median (25th, 75th percentile)

Table 2

ADRB2 genotype distribution by maternal race for SNPs rs1042713 and rs1042714

	rs1042713			rs1042714		
	Codon 16 (n=433)*			Codon 27 (n=437)*		
	AA, n(%)	AG, n(%)	GG, n(%)	CC, n(%)	CG, n(%)	GG, n(%)
Total	100 (23.1)	203 (46.9)	130 (30.0)	249 (57.0)	154 (35.2)	34 (7.8)
Black, non-Hispanic	62 (27.0)	103 (44.8)	65 (28.3)	148 (63.5)	68 (29.2)	17 (7.3)
Hispanic	17 (26.2)	32 (49.0)	16 (24.6)	42 (64.6)	21 (23.3)	2 (3.1)
White, non-Hispanic	18 (14.6)	61 (49.6)	44 (35.8)	49 (39.2)	61 (48.8)	15 (12.0)
Other	3 (20.0)	7 (46.7)	5 (33.3)	10 (71.4)	4 (28.6)	0 (0.0)

* Polymorphisms are described by the amino acid residues for which they encode. For codon 16, A=Arginine and G=Glycine. For codon 27, C=Glutamine and G=Glutamic acid.

Table 3

Obstetrical and maternal outcomes and *ADRB2* genotype encoding for position 16 (rs1042713) among subjects with short cervix

Outcome	Arg16Arg* N = 70	Other* N = 243	Odds Ratio (95% CI)**	P value
Preterm Birth				
<37 weeks	15 (21.4%)	51 (21.0%)	1.0 (0.5–1.9)	0.996
<35 weeks	12 (17.1%)	23 (9.5%)	2.0 (0.9–4.2)	0.079
<32 weeks	6 (8.6%)	8 (3.3%)	2.7 (0.9–8.0)	0.079
Spontaneous <37 weeks	9 (12.9%)	37 (15.2%)	0.8 (0.4–1.8)	0.601
Indicated <37 weeks	6 (8.6%)	14 (5.8%)	1.5 (0.5–4.0)	0.455
Cerclage	2 (2.9%)	3 (1.2%)	2.4 (0.2–21.6)***	0.627
Preterm premature rupture of membranes	2 (3.1%)	9 (3.9%)	0.7 (0.2–3.5)	0.699
Chorioamnionitis	2 (2.9%)	20 (8.2%)	0.3 (0.1–1.5)	0.145
Abruption	7 (10.0%)	3 (1.2%)	8.3 (2.1–33.4)	0.003
Cesarean delivery	11 (15.7%)	51 (21.0%)	0.7 (0.4–1.5)	0.382
Gestational hypertension or preeclampsia	8 (11.4%)	31 (12.8%)	0.8 (0.4–1.9)	0.629
Preeclampsia alone	5 (7.1%)	16 (6.6%)	0.95 (0.3–2.9)***	1.00
Gestational diabetes mellitus	6 (8.6%)	9 (3.7%)	2.4 (0.8–7.2)	0.110

* Data presented as N (%)

** Models are adjusted for ethnicity

*** Exact logistic regression used

Table 4

Obstetrical and maternal outcomes and *ADRB2* genotype encoding for position 27 (rs1042714) among subjects with short cervix

Outcome	Gln27Gln* N = 25	Other* N = 288	Odds Ratio (95% CI) **	P value
Preterm Birth				
<37 weeks	7 (28.0%)	60 (20.8%)	1.8 (0.7–4.7)	0.213
<35 weeks	4 (16.0%)	32 (11.1%)	1.6 (0.5–5.1)	0.424
<32 weeks	2 (8.0%)	13 (4.5%)	1.9 (0.4–9.4)	0.410
Spontaneous <37 weeks	3 (12.0%)	44 (15.2%)	1.0 (0.3–3.4)	0.954
Indicated <37 weeks	4 (16.0%)	16 (5.5%)	3.4 (1.0–11.6)	0.048
Cerclage	0 (0.0%)	5 (1.7%)	--	--
Preterm premature rupture of membranes	0 (0.0%)	11 (4.0%)	--	--
Chorioamnionitis	2 (8.0%)	20 (6.9%)	1.3 (0.3–6.2)	0.727
Abruption	0 (0.0%)	10 (3.5%)	--	--
Cesarean delivery	7 (28.0%)	55 (19.0%)	1.5 (0.6–3.8)	0.401
Gestational hypertension or preeclampsia	4 (16.0%)	35 (12.1%)	1.5 (0.5–4.7)	0.499
Preeclampsia/eclampsia	2 (8.0%)	19 (6.6%)	1.4 (0.1–6.9) ***	0.958
Gestational diabetes mellitus	2 (8.0%)	13 (4.5%)	2.8 (0.5–814.0)	0.218

* Data presented as N (%)

** Models are adjusted for ethnicity

*** Exact logistic regression used