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1 **Title**

2 Exploration of gene-environment interactions, maternal effects, and parent-of-origin effects in the  
3 etiology of hypospadias

4

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22

23 **Runninghead**

24 Gene-environment interactions in hypospadias

25

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49 **Disclosure summary**

50 The authors have nothing to disclose

51 **Abstract**

52 **Purpose** Hypospadias is a common congenital malformation of the male external genitalia.

53 Association studies for single nucleotide polymorphisms (SNPs) in genes encoding steroid-5-alpha-  
54 reductase (*SRD5A2*), estrogen receptors 1 (*ESR1*) and 2 (*ESR2*), and activating transcription factor 3  
55 (*ATF3*) have been equivocal. The aim of this study was to examine whether non-replication of  
56 findings for four SNPs in these genes could be due to interaction with environmental exposures.

57 **Materials and Methods** We genotyped 712 Dutch hypospadias case-parent triads for the four SNPs,  
58 used questionnaire information to determine exposures, and performed association tests using the log-  
59 linear approach. We studied gene-environment interactions for the four SNPs with exposure to  
60 estrogens, cytokines or cigarette smoke, multiple pregnancy, being born small for gestational age, and  
61 maternal hypertension or preeclampsia, high BMI, or primiparity. In addition, the presence of maternal  
62 genetic and parent-of-origin effects was tested.

63 **Results** Gene-environment interactions were identified for rs523349 in *SRD5A2* with estrogen  
64 exposure and maternal hypertension or preeclampsia, as well as for rs11119982 in *ATF3* with  
65 exposure to cytokines. Both SNPs only seemed to influence hypospadias risk in exposed cases. For  
66 rs6932902 in *ESR1*, only maternally derived alleles appeared to increase hypospadias risk in offspring.

67 **Conclusions** This study shows that interactions between genetic and environmental factors may help  
68 to explain non-replication in genetic studies of hypospadias.

69

## 70 **Introduction**

71 Hypospadias is a congenital hypoplasia of the penis, resulting from developmental arrest of urethral  
72 fusion. This leads to displacement of the urethral opening along the ventral side of the penis.

73 Hypospadias is one of the most common birth defects among boys, affecting 0.3-0.7% of newborn  
74 boys in Europe<sup>1</sup>. It shows familial clustering and segregation analyses suggest that the majority of  
75 cases have a multifactorial etiology<sup>2</sup>, involving both genes and environmental factors.

76 Some environmental factors have consistently been associated with hypospadias. Hypospadias  
77 occurs more often in children born small for gestational age (SGA), and in first, intracytoplasmic  
78 sperm injection (ICSI)-induced, or multiple pregnancies. In addition, maternal hypertension,  
79 preeclampsia, high body mass index (BMI), pre-existing diabetes, and use of anti-epileptic drugs  
80 increase hypospadias risk, as does maternal intra-uterine exposure to diethylstilbestrol (DES)<sup>1</sup>.

81 Genetic associations with hypospadias have also been reported, mainly for single nucleotide  
82 polymorphisms (SNPs) in endocrine-related genes, such as those encoding estrogen receptors 1  
83 (*ESR1*)<sup>3</sup> and 2 (*ESR2*)<sup>4</sup>, activating transcription factor 3 (*ATF3*)<sup>5</sup>, and steroid-5-alpha-reductase  
84 (*SRD5A2*)<sup>6,7</sup>. *ATF3* is an estrogen-responsive gene showing upregulation in hypospadias<sup>8</sup>, while  
85 *SRD5A2* encodes an enzyme that converts circulating testosterone in the genital tubercle to the more  
86 potent androgen dihydrotestosterone.

87 The numbers of samples analyzed in these genetic studies were relatively small, and most  
88 associations could not be replicated in a much larger study by our group<sup>9</sup>.

89 This lack of consistency might reflect differences in environmental exposures between  
90 populations<sup>9</sup>. Several reviews have called for studies simultaneously examining genes and  
91 environment in relation to hypospadias<sup>1,10</sup>, but so far, such studies have rarely been performed.  
92 Therefore, we set out to examine whether the lack of replication could be due to gene-environment  
93 interactions between the four SNPs described above and risk factors for hypospadias.

94 In addition to gene-environment interactions, other (epi)genetic mechanisms may be involved  
95 in the etiology of hypospadias. Maternal genotype may affect the intra-uterine environment, thus  
96 modulating hypospadias risk, and gene imprinting may cause the copy derived from one parent to be

97 more fully expressed than the copy derived from the other parent<sup>11</sup>. Therefore, we also examined the  
98 maternal genotype and imprinting effects.

99

## 100 **Materials and methods**

### 101 *Cases and parents*

102 AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for  
103 Anomalies in children) is a large data- and biobank at the Radboud University Nijmegen Medical  
104 Centre in the Netherlands, in which questionnaire data and DNA samples are collected from patients  
105 with congenital malformations or childhood cancer and their parents. For the current study, DNA was  
106 available from 796 hypospadias cases born between 1980 and 2008 and 1,422 parents. Medical  
107 records of all cases were reviewed to identify syndromic hypospadias cases, collect clinical  
108 characteristics, and obtain information about anatomical location of the urethral opening. The regional  
109 Committee on Research Involving Human Subjects approved the study protocol and all parents and  
110 children over 11 years of age gave written informed consent.

111

### 112 *Environmental risk factor data*

113 Questionnaires were sent to the parents of all patients, containing a variety of questions on health and  
114 lifestyle just before and during pregnancy, which were used to define environmental risk factors.  
115 Although exogenous exposure to estrogens is not a known risk factor for hypospadias<sup>1</sup>, we included it  
116 in the gene-environment interaction analyses, because *SRD5A2*, *ESR1*, and *ESR2* are involved in  
117 endocrine processes and *ATF3* is an estrogen-responsive gene. Exogenous exposure to estrogens was  
118 defined as continued use of oral contraceptives during early pregnancy or consumption of soy or  
119 linseed products, which contain high amounts of phytoestrogens<sup>12</sup>, at least once a week in the first 14  
120 weeks after conception. Women with a hormonal coil implanted who became pregnant were excluded  
121 because of weak estrogen exposure. Women exposed to pesticides at work were also excluded because  
122 pesticides can have either estrogenic or anti-estrogenic effects.

123 In addition, we studied interactions with factors associated with hypospadias occurrence: SGA  
124 (defined as birth weight < 10<sup>th</sup> percentile for that gestational age, using Dutch reference curves<sup>13</sup>),  
125 mothers with hypertension or preeclampsia, high BMI (defined as BMI > 25 kg/m<sup>2</sup>), primiparity, and  
126 multiple pregnancy.

127 In most tissues, *ATF3* mRNA can be induced by various stress signals, such as cytokines and  
128 chemicals from cigarette smoke<sup>14,15</sup>. Therefore, we also included these exposures in the gene-  
129 environment interaction analyses for *ATF3*. Because the placental barrier may be permeable to  
130 cytokines<sup>16,17</sup> and chemicals from cigarette smoke<sup>18</sup>, we categorized cases whose mothers smoked at  
131 least one cigarette per day during some time in the first 14 weeks after conception as exposed to  
132 cigarette smoke, and cases whose mothers reported the presence of an infection and/or inflammation in  
133 this period as exposed to cytokines.

134

### 135 *Genotyping*

136 Blood was collected in EDTA containing tubes (n=1,405) or saliva using Oragene containers (n=687;  
137 DNA Genotek Inc., Ottawa, Canada). DNA extraction and genotyping was performed as described  
138 previously<sup>9</sup>.

139

### 140 *Statistical analyses*

141 We used the case-parent triad design. The most frequent homozygous genotypes in parents served as  
142 reference genotypes in the log-linear approach<sup>19</sup> that was applied to assess genetic associations. Log-  
143 linear models were fitted without assumption of Hardy-Weinberg equilibrium (HWE). Information on  
144 families with one missing parental genotype was included in the analyses using the expectation-  
145 maximization algorithm<sup>20</sup>. Likelihood ratio tests (LRT), comparing full models including both mater-  
146 nal and offspring genotypes to reduced models including either maternal or offspring genotype only,  
147 were computed to determine the relevance of maternal and offspring genotypes for hypospadias risk.  
148 We also conducted these analyses separately for the groups of anterior, middle, and posterior  
149 hypospadias cases, because different risk factors may be responsible for the different phenotypes<sup>21,22</sup>.  
150 Although the case-parent triad design is robust to population-stratification when testing genetic  
151 effects, effects of environmental exposures cannot be estimated.

152 Parent-of-origin analyses were conducted in two steps. As an initial screening, we used the  
153 transmission asymmetry test (TAT)<sup>19</sup>. This approach provides insights into the data, but is invalid



154 when maternal effects exist. Therefore, the parent-of-origin LRT (PO-LRT), was used to confirm the  
155 results<sup>23</sup>.

156 Interactions between environmental exposures and offspring genotypes were tested using log-  
157 linear models with the LRT comparing a full model including gene-environment interactions to a  
158 reduced model including only the offspring genotypic effect<sup>24</sup>. We used a dominant interaction  
159 parameter, assuming that the environmental factor affects carriers with one or two copies of the variant  
160 allele similarly. We did not correct the critical *P*-value for multiple testing, as we only tested a limited  
161 number of well-founded hypotheses. If the LRT indicated the presence of an interaction ( $P_{LRT} < 0.05$ ),  
162 relative risks (RR) and 95% confidence intervals (95% CI) were calculated separately for the different  
163 strata of the exposure variable using the variance calculated with the LEM program, which takes into  
164 account missing genotypes<sup>25</sup>. All other analyses were performed using the SAS System for Windows,  
165 release 8.02 (SAS Institute, Cary, North Carolina).

166

## 167 **Results**

168 Of the 796 available hypospadias cases, 38 patients were excluded due to lack of parental DNA. To  
169 ensure independence, we excluded the youngest brother from 22 sib-pairs, while from three twin-pairs,  
170 one brother was excluded at random. We excluded 19 patients because of syndromic hypospadias,  
171 chromosome abnormalities, or a known cause of hypospadias. Finally, two triads were excluded  
172 because of Mendelian errors. The final data set consisted of 712 cases. For 668 cases, DNA of both  
173 parents was available, while for 44 cases, we only had DNA from one parent. Environmental data  
174 were missing for 70 families. The majority of cases were of European Caucasian descent (91%), and  
175 the remaining were of non-European (5%) or unknown descent (4%). Almost 60% of cases had an  
176 anterior hypospadias, while 20% and 13% had middle and posterior urethral openings, respectively.  
177 Table 1 shows the distribution of the environmental risk factors studied. Exogenous exposure to  
178 estrogens, multiple pregnancies and fetal exposure to cytokines were relatively rare (<10%), whereas  
179 the other factors were more common.

180 Genotyping of the SNPs was completed with a success rate of more than 98.5%. All genotype  
181 frequencies in parents were in HWE ( $P \geq 0.28$ ). Genetic association results showed that offspring  
182 genotype of the variant in *ESR1* was associated with hypospadias, as reported earlier in a partly  
183 overlapping sample<sup>9</sup>, whereas results for the variant in *ATF3* were suggestive of an association.  
184 Maternal genotypes were not associated with hypospadias in offspring (Table 2). Repeating the  
185 analyses separately for subgroups of anterior, middle, and posterior hypospadias cases showed  
186 comparable results.

187 The results of the gene-environment interaction analyses pointed towards interactions between  
188 offspring genotype of rs523349 in *SRD5A2* and exogenous estrogen exposure and maternal  
189 hypertension or preeclampsia (Table 3). Offspring carrying the variant allele seemed to be at increased  
190 risk of hypospadias when estrogen exposure occurred and at decreased risk when the mother had  
191 hypertension or preeclampsia. Furthermore, an interaction was observed between rs11119982 in *ATF3*  
192 and exposure to cytokines, with an increased risk of hypospadias for offspring carrying the variant  
193 allele only when the mother reported an infection and/or inflammation (Figure 1). Due to small  
194 numbers of cases with certain exposures, we also considered a reduced model assuming HWE, which

195 handles small sample size situations better. The risk estimates from this model showed the same  
196 direction of gene by exposure interaction for the SNP in *SRD5A2* and exogenous estrogen exposure,  
197 albeit less strongly (Figure 2). For the other interactions, similar results were obtained as in the full  
198 model.

199           The results of the parent-of-origin effects analyses are shown in Table 4. For rs6932902 in  
200 *ESR1*, the estimated PO-LRT relative risk for an imprinting effect was 1.61 (95% CI=1.02-2.53),  
201 indicating that a maternally derived copy seemed to be associated with a greater risk of hypospadias  
202 than a paternally derived copy. The TAT showed that only the maternally derived copy increased the  
203 risk of hypospadias (RR=1.8, 95% CI=1.3-2.7).

204

## 205 Discussion

206 This study is a follow-up to our earlier association study of genetic variants in *SRD5A2*, *ESR1*, *ESR2*,  
207 and *ATF3* and hypospadias risk in which 620 cases were included. For the current study, we excluded  
208 37 cases because DNA of both parents was not available or a brother was present in the dataset, and  
209 included 129 cases not included in the earlier study because of non-Caucasian or unknown ethnicity or  
210 because they were collected after 2007. We included gene-environment interactions as well as  
211 maternal and parent-of-origin effects in an attempt to reconcile our findings with those of others.

212 The estimated interaction between offspring genotype of the SNP in *SRD5A2* and exogenous  
213 estrogen exposure during early pregnancy suggests that offspring carrying the variant have a more  
214 than eight fold increased risk of hypospadias only in case of exogenous estrogen exposure in the full  
215 log-linear model, and an almost three times increased risk in the reduced model. The interaction  
216 between this variant in *SRD5A2* and exogenous estrogen exposure seems biologically plausible, as it  
217 causes a valine to leucine substitution (V89L) resulting in an approximately 30% decrease in enzyme  
218 activity<sup>26</sup> and thus in less dihydrotestosterone. Additional estrogen exposure might cause an androgen-  
219 estrogen imbalance in carriers of this variant, resulting in hypospadias. The gene-environment  
220 interaction observed could help to explain differences in findings for this SNP between our study and  
221 studies from Sweden and China<sup>6,7,9</sup>. The latter two observed associations with the malformation, not  
222 taking environmental parameters into account, while we did not. However, phytoestrogen exposure is  
223 known to be higher in Chinese and Swedish populations compared to the Dutch. Chinese people  
224 consume more soy products, while in Nordic countries more rye bread and berries are consumed.  
225 These food products contain large amounts of isoflavonoids and lignans, respectively, whereas in a  
226 typical Western diet, both lignans and isoflavonoids are almost completely lacking<sup>27</sup>.

227 The SNP in *SRD5A2* seemed to decrease hypospadias risk in case of maternal hypertension or  
228 preeclampsia, which may result from placental insufficiency. The latter may also lead to decreased  
229 provision of human chorionic gonadotropin (hCG) to the foetus. As hCG stimulates foetal testicular  
230 steroidogenesis before the foetus's own pituitary-gonadal axis is established, and the SNP in *SRD5A2*  
231 may result in even less DHT being formed, we would expect the SNP to increase hypospadias risk in  
232 case of maternal hypertension or preeclampsia.

233           The SNP in *ATF3* seemed to be associated with an increased hypospadias risk only when the  
234 mother reported an infection and/or inflammation during pregnancy. *ATF3* shows strong upregulation  
235 in hypospadias patients<sup>8</sup> and is upregulated in response to cytokines<sup>14</sup>. While the rs11119982 variant  
236 has not been functionally characterized, a working hypothesis could be that the variant underlying the  
237 association with hypospadias causes an increased expression of *ATF3* in response to cytokines.  
238 However, this finding does not reconcile our results with those reported earlier, describing a decreased  
239 hypospadias risk in the presence of the variant<sup>5</sup>.

240           The parent-of-origin analyses indicated that only a maternally derived copy of the variant in  
241 *ESRI* seems to be associated with an increased hypospadias risk. This suggests that the maternally  
242 derived allele of *ESRI* is more fully expressed than the paternally derived allele. Although *ESRI* is not  
243 one of the currently known imprinted genes ([www.geneimprint.com](http://www.geneimprint.com)), the experimental identification  
244 of imprinted genes is challenging, because monoallelic expression of imprinted genes may occur only  
245 in particular tissues, at particular stages of development, or in one of the isoforms<sup>28</sup>. Therefore, it is  
246 unlikely that all human imprinted genes are already known. However, the observed parent-of-origin  
247 effect could also have arisen from an effect of maternal-fetal genotype interaction. Unfortunately, we  
248 do not have enough statistical power to disentangle these two possible effects.

249           We have to acknowledge some limitations. For all factors studied, we relied on information  
250 from questionnaires which may result in misclassification due to recall problems, especially since the  
251 average time between birth and filling out the questionnaires was 10.2 years, ranging from 0 to 27  
252 years. However, most factors studied are relatively easy to remember. Also, this misclassification  
253 probably does not depend on genotype and would have resulted in attenuation of the results only,  
254 which may have obscured some effects. Misclassification due to measurement error could also account  
255 for not finding gene-environment interactions for well-known factors such as SGA or primiparity,  
256 which may be proxies for underlying causes that are difficult to measure, such as placental  
257 insufficiency. To our knowledge, we investigated the largest sample of hypospadias cases thus far  
258 reported in genetic studies, while the power of our study was further increased by including  
259 information on families with one missing parental genotype using the expectation-maximization

260 algorithm. Nevertheless, numbers of cases having a specific genotype and being exposed to a  
261 particular environmental risk factor were still small, resulting in large confidence intervals for the  
262 effect estimates. Our definition of exogenous estrogen exposure, for example, assured a selective  
263 group of women experiencing high levels of exposure, but resulted in low numbers of exposed  
264 women. This indicates that very large samples are needed to study gene-estrogen-exposure  
265 interactions. In addition, the gene-environment interaction test assumes that the SNP under study is a  
266 disease-causing mutation. If a marker in linkage disequilibrium with the causative mutation is studied  
267 instead, the test is susceptible to exposure-related population-stratification<sup>29</sup>.  
268

269 **Conclusions**

270 We showed that parent-of-origin effects and gene-environment interactions contribute to the etiology  
271 of hypospadias, and that environmental factors can explain genetic non-replication between studies.  
272 Our results warrant further research directed at elucidating combined effects of genetic and  
273 environmental factors for this frequently occurring urological birth defect.

274

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## Tables

**Table 1.** Distribution of environmental risk factors for hypospadias patients.

Exposure	yes		no		unknown <sup>a</sup>	
	n	(%)	n	(%)	n	(%)
Exogenous exposure to estrogens	29	(4%)	580	(81%)	103	(14%)
<i>Use of oral contraceptives during pregnancy</i>	8	(1%)	626	(88%)	78	(11%)
<i>Consumption of soy products during pregnancy</i>	16	(2%)	620	(87%)	76	(11%)
<i>Consumption of linseed products during pregnancy</i>	7	(1%)	626	(88%)	79	(11%)
Known hypospadias risk factors						
Small for gestational age	125	(18%)	505	(71%)	82	(12%)
Hypertension or preeclampsia	107	(15%)	533	(75%)	72	(10%)
BMI > 25 kg/m <sup>2</sup>	140	(20%)	468	(66%)	104	(15%)
First pregnancy	361	(51%)	277	(39%)	74	(10%)
Multiple pregnancy	49	(7%)	590	(83%)	73	(10%)
Fetal exposure to cytokines	67	(9%)	499	(70%)	146	(21%)
<i>From a severe cold</i>	15	(2%)	574	(81%)	123	(17%)
<i>From other viral, bacterial or fungal infections</i>	28	(4%)	563	(79%)	121	(17%)
<i>From chronic inflammatory diseases</i>	29	(4%)	605	(85%)	78	(11%)
Fetal exposure to cigarette smoke	117	(16%)	505	(71%)	90	(13%)

*Percentages do not add up to 100% due to rounding and overlapping categories; n, number; <sup>a</sup>for 70 patients, environmental data were completely missing because parents did not fill out the questionnaires.*

**Table 2.** Genetic association results for offspring and maternal genotypes of single nucleotide polymorphisms in *SRD5A2*, *ESR1*, *ESR2* and *ATF3* with hypospadias.

Single nucleotide polymorphism	Geno-type	Offspring genotype					Maternal genotype				
		Cases n (%)	RR	95% CI	P-value	$P_{LRT}^a$	Mothers n (%)	RR	95% CI	P-value	$P_{LRT}^b$
rs523349 in <i>SRD5A2</i>	CC	333 (48%)	ref			0.87	326 (47%)	ref			0.64
	CG	298 (43%)	1.1	0.9, 1.3	0.60		313 (45%)	1.0	0.8, 1.3	0.69	
	GG	70 (10%)	1.0	0.7, 1.5	0.83		57 (8%)	0.9	0.6, 1.3	0.46	
rs6932902 in <i>ESR1</i>	GG	513 (72%)	ref			$3 \times 10^{-3}$	523 (75%)	ref			0.42
	AG	177 (25%)	1.5	1.2, 2.0	$1 \times 10^{-3}$		159 (23%)	1.1	0.8, 1.4	0.61	
	AA	18 (3%)	2.0	1.1, 3.8	0.03		16 (2%)	1.7	0.7, 3.7	0.21	
rs2987983 in <i>ESR2</i>	AA	345 (49%)	ref			0.13	328 (47%)	ref			0.98
	AG	293 (42%)	0.8	0.7, 1.0	0.06		297 (43%)	1.0	0.8, 1.3	0.97	
	GG	67 (10%)	0.8	0.5, 1.1	0.12		72 (10%)	1.0	0.7, 1.4	0.86	
rs11119982 in <i>ATF3</i>	CC	163 (23%)	ref			0.07	180 (26%)	ref			0.09
	CT	354 (50%)	1.2	1.0, 1.6	0.07		332 (48%)	0.9	0.7, 1.1	0.23	
	TT	188 (27%)	1.4	1.0, 1.9	0.02		187 (27%)	1.1	0.8, 1.6	0.37	

Percentages do not add up to 100% due to rounding; CI, confidence interval; LRT, likelihood ratio test; n, number; RR, relative risk; <sup>a</sup>P-value of the likelihood ratio test comparing a full model including maternal and offspring genotypes to a reduced model including only maternal genotypes; <sup>b</sup>P-value of the likelihood ratio test comparing a full model including maternal and offspring genotypes to a reduced model including only offspring genotypes.

**Table 3.** Results of the tests for gene-environment interactions for single nucleotide polymorphisms in *SRD5A2*, *ESR1*, *ESR2* and *ATF3*.

Single nucleotide polymorphism	Environmental risk factor	$P_{LRT}^a$
rs523349 in <i>SRD5A2</i>	Exogenous exposure to estrogens	$7 \times 10^{-3*}$
	Small for gestational age	0.92
	Hypertension or preeclampsia	0.04*
	BMI > 25 kg/m <sup>2</sup>	0.17
	First pregnancy	0.42
	Multiple pregnancy	0.44
rs6932902 in <i>ESR1</i>	Exogenous exposure to estrogens	0.45
	Small for gestational age	0.11
	Hypertension or preeclampsia	0.06
	BMI > 25 kg/m <sup>2</sup>	0.89
	First pregnancy	0.10
	Multiple pregnancy	0.72
rs2987983 in <i>ESR2</i>	Exogenous exposure to estrogens	0.42
	Small for gestational age	0.92
	Hypertension or preeclampsia	0.86
	BMI > 25 kg/m <sup>2</sup>	0.28
	First pregnancy	0.27
	Multiple pregnancy	0.11
rs11119982 in <i>ATF3</i>	Exogenous exposure to estrogens	0.78
	Small for gestational age	0.11
	Hypertension or preeclampsia	0.21
	BMI > 25 kg/m <sup>2</sup>	0.82
	First pregnancy	0.40
	Multiple pregnancy	0.17
	Fetal exposure to cytokines	0.02*
Fetal exposure to cigarette smoke	0.60	

*LRT*, likelihood ratio test; <sup>a</sup>*P*-value of the likelihood ratio test comparing a full model including gene-environment interactions to a reduced model including only offspring genotypes; \* indication of gene-environment interaction.

**Table 4.** Results of the parent-of-origin analyses.

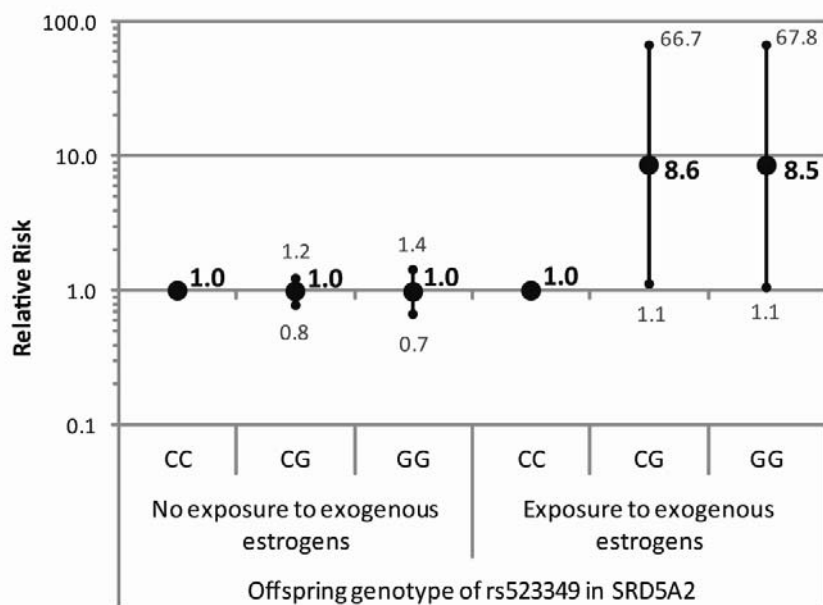
Single nucleotide polymorphism	Minor allele <sup>a</sup>	Transmission asymmetry test (TAT)										PO-LRT		
		Mothers					Fathers					<i>P</i> <sub>TAT</sub>	RR	<i>P</i> -value
		T	NT	RR	95% CI	<i>P</i> -value	T	NT	RR	95% CI	<i>P</i> -value			
rs523349 in <i>SRD5A2</i>	G	81	80	1.0	0.7, 1.4	0.94	66	81	0.8	0.6, 1.1	0.22	0.34	1.18	0.27
rs6932902 in <i>ESR1</i>	A	79	43	1.8	1.3, 2.7	1×10 <sup>-3</sup>	58	53	1.1	0.8, 1.6	0.64	0.05	1.61	0.07
rs2987983 in <i>ESR2</i>	G	59	95	0.6	0.5, 0.9	4×10 <sup>-3</sup>	64	82	0.8	0.6, 1.1	0.14	0.33	0.76	0.27
rs11119982 in <i>ATF3</i>	T	83	71	1.2	0.9, 1.6	0.33	111	70	1.6	1.2, 2.1	3×10 <sup>-3</sup>	0.17	0.79	0.25

*CI*, confidence interval; *NT*, minor allele not transmitted; *PO-LRT*, parent-of-origin likelihood ratio test; *RR*, relative risk; *T*, minor allele transmitted; *TAT*, transmission asymmetry test; <sup>a</sup>the least frequent allele in the parents.

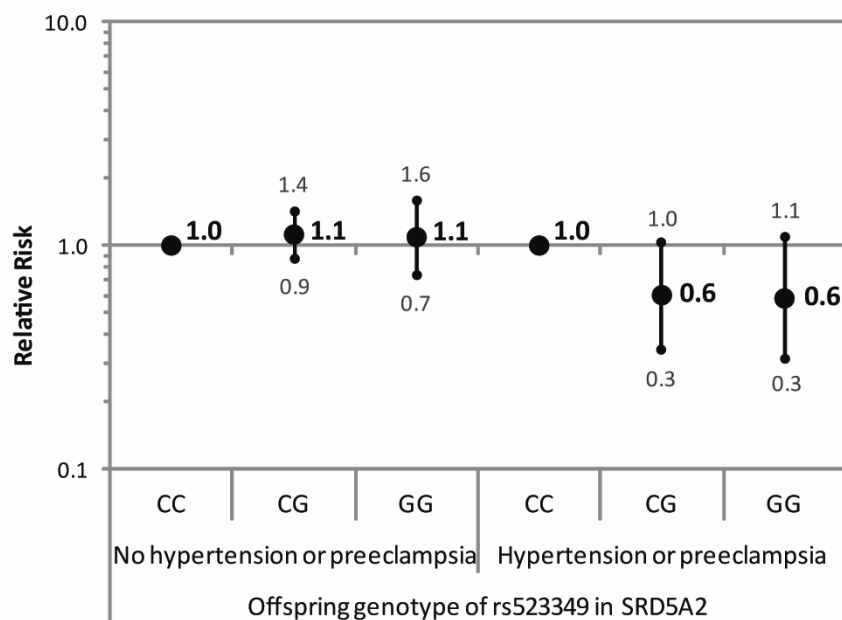
## Figures

Figure 1. Relative risks of hypospadias with 95% confidence intervals for genotypes of (a) rs523349 in *SRD5A2* within strata of exogenous estrogen exposure (b) rs523349 in *SRD5A2* within strata of maternal hypertension or preeclampsia and (c) rs11119982 in *ATF3* within strata of fetal exposure to cytokines.

**A**



**B**





C

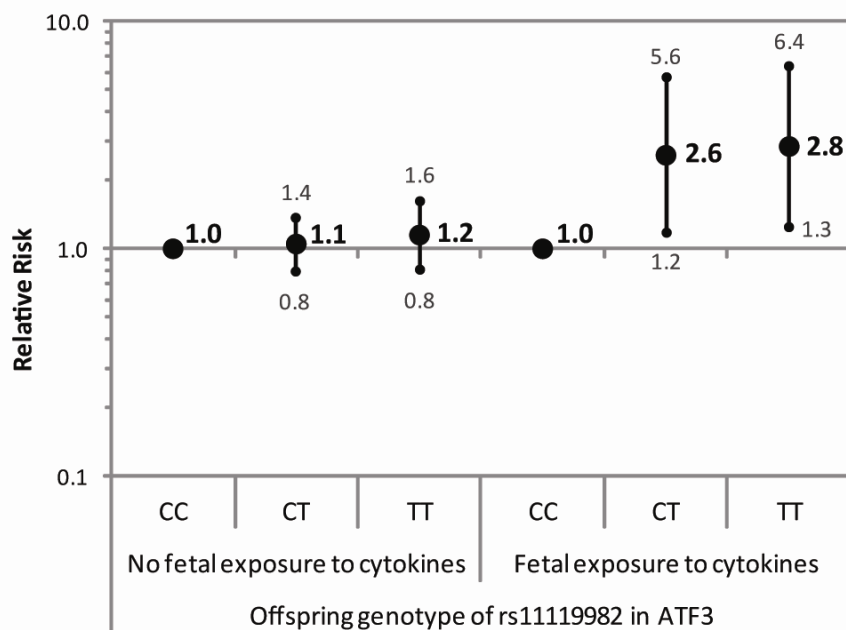


Figure 2. Relative risks of hypospadias with 95% confidence intervals for genotypes of rs523349 in *SRD5A2* within strata of exogenous estrogen exposure using a reduced model assuming Hardy Weinberg equilibrium.

