Isoflavone Intake in Early Pregnancy and Hypospadias in the Japan Environment and Children's Study

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OBJECTIVE To explore the association between isoflavone intake in early pregnancy (the critical window of masculinisation) and hypospadias. Since oestrogen is likely to contribute to the differentiation of male external genitalia, dietary intake of isoflavone, which has a similar structure to human oestrogen, may be associated with the occurrence of hypospadias. However, there has been little evidence of this association.

MATERIALS AND METHODS We used data of a nationwide birth cohort study, which recruited women as early in pregnancy as possible throughout Japan between 2011 and 2014. From the response to a self-administered food-frequency questionnaire, the daily intake of genistein (as a representative for isoflavone) was estimated. Information on hypospadias cases that were diagnosed until the first month after birth was obtained from medical records. Odds ratios (ORs) of hypospadias were estimated using a logistic regression model.

RESULTS Among 41,578 mothers, who delivered singleton live male births, the median genistein intake was 15.3 mg/day, and a total of 51 cases of hypospadias were identified. Compared with mothers in the reference group (genistein intake 11th-89th percentiles), those in the low intake group (≤10th percentile) had an elevated risk of their sons having hypospadias (multivariable-adjusted OR = 2.8, 95% confidence interval = 1.4-5.8). Adverse or beneficial effects of genistein on hypospadias were not observed in the high intake group (≥90th percentile) (OR = 0.9, 95% confidence interval = 0.4-2.4).

CONCLUSION Low maternal intake of isoflavone in early pregnancy was associated with an elevated risk of hypospadias. UROLOGY 124: 229–236, 2019. © 2018 The Author(s). Published by Elsevier Inc.

Hypospadias is a relatively frequent urogenital anomaly. The occurrence of hypospadias is related to difficulties in foetal masculinisation through sex hormone action, and the critical window is the 8th-14th week of gestation.[1] Although androgen is the main masculinisation-related sex hormone, it appears that oestrogen also contributes to the differentiation of male external genitalia,[2] and the importance of the balance between androgen and oestrogen has been noted.[3] In an experimental study, approximately half of male mice exposed to synthetic oestrogens in utero had hypospadias.[4] Therefore, hypospadias may be caused by a poor balance between androgen and oestrogen.

Isoflavones, such as genistein and daidzein, which are contained in soya and soya foods, and are able to cross the placenta,[5] have a similar structure to human oestrogen, and thus can bind to the oestrogen receptor.[6] Intake of isoflavone, has been reported to be associated with sex hormone-related cancer,[7] may be linked to the occurrence of hypospadias. In an experiment involving pregnant mice, hypospadias was observed in approximately 1 of 4 mice that were fed a diet comprising genistein, but was not observed in a control group with a soya-free diet.[8]

[1] *The study group members are listed in the Appendix.*

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The Avon Longitudinal Study of Pregnancy and Childhood revealed a positive association between a vegetarian diet, which included relatively more soya and soya foods than a nonvegetarian diet, and hypospadias.[9] However, a case-control study reported higher intake of isoflavones was associated with a decreased risk of hypospadias,[10] suggesting that isoflavone may not work in the direction of foetal masculinisation inhibition. Thus, further evidence of an association between isoflavone intake and hypospadias is desired, with the aim of preventing hypospadias and elucidating its aetiology.

Thus, we investigated the association between isoflavone intake and hypospadias in Japan, which was notable for its exploration of this association among Japanese individuals who consume much higher levels of isoflavone.

MATERIALS AND METHODS

Study Participants

The Japan Environment and Children's Study (JECS)[11,12] is an ongoing nationwide birth cohort study, and its concept and design have been previously detailed.[11] Briefly, we recruited women as early in pregnancy as possible, in 15 Regional Centres throughout Japan, and registered 103,099 pregnancies between 2011 and 2014.[12] After exclusion of 2321 with no subsequent delivery record, the remaining 100,778 pregnancies involved 101,779 foetuses, and resulted in 100,148 live births. The women's partners (fathers) were approached whenever possible, and roughly 50,000 fathers agreed to participate. The JECS protocol was approved by the Japanese Ministry of the Environment's Institutional Review Board on Epidemiological Studies, and the Ethics Committees of all participating institutions. All participating mothers and fathers had provided written informed consent.

As shown in Supplementary Figure 1, we analyzed the data of 41,578 mothers (41,578 male births) in the present study, after discounting multiple participation, restricting to mothers who delivered singleton male live births and for whom there was complete information on exposure and covariates (Table 1), and then excluding participants who reported daily energy intake outside the range of the 1st-99th percentile.

Isoflavone Intake

Self-administered questionnaires, including food-frequency questionnaires (FFQs), were distributed twice, first during the first trimester (median fill-in week of gestation = 15), and then again during the second/third trimester (27th week). In the first FFQ, we asked about usual dietary intake in the preceding year, and in the second, usual intake after awareness of pregnancy. In this study, data from the first FFQ were used as an exposure assessment, because we considered it to be a marker reflecting dietary intake in the critical window of external genitalia development (8-14 weeks).[1]

The JECS adopted the FFQ used in the Japan Public Health Centre-based Prospective Study for the Next Generation,[13] which investigated the intake frequency and usual serving size of various food items. This FFQ contained 10 items relating isoflavone intake: natto (fermented soyabeans), 6 tofu items [tofu for miso soup, tofu for other dishes, yushidofu (redrained tofu), koyadofu (freeze-dried tofu), namaage (thick deep-fried tofu), and aburaage (deep-fried tofu)], and 3 other soya foods [miso soup, soya milk, and *kinako* (roasted soya flour)]. We used 3 portion sizes [small (50% less than standard), medium (standard), and large (50% more than standard)], and 9 frequency categories (<1 time/month, 1-3 times/month, 1-2, 3-4, 5-6 times/week, 1 time/day, 2-3, 4-6, ≥7 times/day). For miso soup, there were 6 frequency categories (almost never, 1-3 times/month, 1-2, 3-4, 5-6 times/week, daily), and 9 choices of daily amount (<1, 1, 2, 3, 4, 5, 6, 7-9, ≥10 bowls). For soya milk, the FFQ contained 9 frequency categories (<1 time/week, 1-2, 3-4, 5-6 times/week, 1 glass/day, 2-3, 4-6, 7-9, ≥10 glasses/day).

The daily intakes of genistein and daidzein were estimated using a specifically developed food composition table for isoflavones in Japanese foods,[14] and the daily intakes of natto and tofu (sum of 6 items' intakes) were calculated by multiplying the frequency by the standard-equivalent portion size. After log-transformation of genistein, daidzein, natto, and tofu intake, we adjusted for total energy intake using the residual model.[15] As the estimate of genistein intake strongly correlated with that of daidzein intake ($\rho = 0.99$), we took genistein intake as a representative for isoflavone. In a validation study among 142 Japanese women aged 40-74 years, genistein intake estimated from the FFQ correlated with that estimated from the 12-day weighted food record ($\rho = 0.55$).[13]

The first FFQ included the following open question: "Do you take any health supplements more than once a week, continuously throughout the year?" The proportion of isoflavone supplement users was approximately 0.2%. Though we did not consider the genistein contained in such supplements when estimating genistein intake from the FFQ, we used this information in the sensitivity analysis (See statistical analysis.)

Hypospadias

According to the JECS in-house standard operating procedure, medical record transcripts are generated 3 times by physicians, midwives/nurses, and/or research co-ordinators: first during the first trimester, second after delivery, and finally at the first-month health check-up after delivery. We used the list of congenital anomalies in the transcription reports after delivery and at a month after delivery, and identified hypospadias diagnosed up to the first month after delivery without information on phenotypes. We also defined isolated cases of hypospadias, unaccompanied by other major or chromosomal anomalies, because different etiologies were attributed to such combined anomalies. In this study, major anomalies included anencephaly, spina bifida, encephalocele, microphthalmia, cleft palate, cleft lip (with or without cleft palate), congenital heart diseases (not including patent ductus arteriosus), oesophageal atresia, small intestinal atresia, anorectal malformation, gastroschisis, omphalocele, diaphragmatic hernia, and reduction defects of the upper and/or lower limbs.[16,17]

Statistical Analysis

The present study used the dataset jecs-ag-20160424, which was released in June 2016, and revised in October 2016, along with the supplementary dataset jecs-ag-20160424-sp1.

In line with an earlier study,[10] we focused on the distribution at the lower and upper ends of genistein intake, and categorized participants into 3 groups: ≤10th percentile, 11th-89th percentile (reference), and ≥90th percentile. To examine the association between genistein intake in early pregnancy and hypospadias, a logistic regression model was applied to estimate maternal age-adjusted odds ratios (ORs) and 95% confidence

Table 1. Baseline characteristics of 41,578 pregnant women with respect to genistein intake in early pregnancy.

	Number of Women	≤10th Percentile (n = 4158) (%)	11th-89th Percentile (n = 33,262) (%)	≥90th Percentile (n = 4158) (%)
Dietary intake in early pregnancy				
Genistein (mg/day), median	41,578	3.3	15.3	45.3
Natto (g/day), median	41,578	0	10.3	32.0
Tofu (g/day), median	41,578	5.1	20.5	40.7
Fish (g/day), median	41,578	21.4	33.3	34.1
Vegetables (g/day), median	41,578	107.9	159.7	209.0
Fruit (g/day), median	41,578	82.9	118.8	132.3
Energy (kcal/day), median	41,578	1606	1705	1698
Age at delivery (years)				
<25	3598	14.4	8.2	6.2
25-29	11,366	32.3	27.2	23.5
30-34	14,903	31.1	36.4	36.6
≥35	11,711	22.2	28.2	33.7
Educational background (years)				
<13	14,306	41.4	34.0	31.1
≥13	27,272	58.6	66.1	68.9
Household income (million Japanese-yen/year)				
<6	30,379	76.7	73.0	70.3
≥6	11,199	23.3	27.0	29.8
Occupation in early pregnancy				
Administrative, managerial, professional, or engineering	9996	21.9	24.4	23.3
Clerical	7307	17.7	17.5	18.3
Sales or service	8975	27.1	21.2	18.9
Others	3847	10.4	9.2	9.0
Homemaker	11,453	22.9	27.7	30.6
Smoking habits				
Never smoked	24,506	54.5	59.4	59.6
Ex-smokers who quit before pregnancy	9798	20.0	23.5	27.6
Smokers during early pregnancy	7274	25.5	17.1	12.8
Alcohol consumption				
Never drank	14,232	36.4	34.2	32.0
Ex-drinkers who quit before pregnancy	7548	18.4	17.9	20.2
Drinkers during early pregnancy	19,798	45.2	47.9	47.8
Body mass index before pregnancy (kg/m^2)				
<18.5	6676	16.4	16.0	16.6
18.5-24.9	30,489	72.1	73.4	74.0
≥25.0	4413	11.5	10.7	9.4
Current history of diabetes or gestational diabetes				
No	40,212	96.5	96.8	96.3
Yes	1366	3.5	3.2	3.7
Current history of hypertensive disorders in pregnancy				
No	40,260	96.4	96.8	97.3
Yes	1318	3.6	3.2	2.7
Parity				
0	18,107	49.9	42.5	45.7
≥1	23,471	50.1	57.5	54.3
Infertility treatment				
No	38,715	94.3	93.4	89.8
Ovulation stimulation/artificial insemination by sperm from husband	1545	3.5	3.5	5.4
Assisted reproductive technology	1318	2.2	3.1	4.7

Continued

Table 1. Continued

	Number of Women	≤10th Percentile (n = 4158) (%)	11th-89th Percentile (n = 33,262) (%)	≥90th Percentile (n = 4158) (%)
Routine use of folic acid supplements				
No (<4 times/week)	30,066	76.4	72.7	65.2
Yes (≥4 times/week)	11,512	23.6	27.3	34.9
Gestational age (week), median	41,578	39	39	39
Preterm (<37 weeks)	2128	4.9	5.1	5.3
Paternal age at delivery (21,773 mothers with participating fathers)				
<25	1275	5.7	9.9	3.3
25-29	4875	22.2	27.3	19.3
30-34	7304	33.8	31.6	33.3
≥35	8319	38.3	31.3	44.1

intervals (CIs) of hypospadias, using Stata 14 (StataCorp LP, College Station, TX). In addition to maternal age at delivery (<25, 25-29, 30-34, ≥35 years), a multivariable model included the following variables reported to be associated with congenital anomalies including hypospadias: educational background (<13, ≥13 years); household income (<6, ≥6 million Japanese-yen/year); occupation in early pregnancy (administrative, managerial, professional, or engineering; clerical; sales or service; other; homemaker); smoking habits (never smoked, ex-smokers who quit before pregnancy, smokers during early pregnancy); alcohol consumption (never drank, ex-drinkers who quit before pregnancy, drinkers during early pregnancy); body mass index before pregnancy (<18.5, 18.5-24.9, ≥25.0 kg/m^2); current history of diabetes or gestational diabetes (no, yes); current history of hypertensive disorders in pregnancy (no, yes); parity (0, ≥1); infertility treatment (no, ovulation stimulation or artificial insemination by sperm from husband, assisted reproductive technology (ART)); use of folic acid supplements [routine use (defined as ≥4 times/week), nonroutine use]; and fish, vegetable, or fruit consumptions in early pregnancy, estimated from the first FFQ, as markers of healthy dietary habit. The association between soya foods, including natto and tofu, and hypospadias was also assessed. Expecting to find no measurable association, we used intakes of genistein and soya foods in mid-late pregnancy (outside the critical window of masculinisation), as estimated from the second FFQ, and investigated the association with hypospadias. For this analysis, we included 41,486 mothers who had valid data from the second FFQ and delivered their sons after 28 weeks of gestation.

To confirm the robustness of the association between genistein intake in early pregnancy and hypospadias, we added several subgroup and sensitivity analyses. First, we excluded mothers aged 35 years or older, and mothers who had utilized ART for this specific pregnancy, because these factors are likely to increase the risk of hypospadias.[18] Second, we excluded mothers with severe morning sickness, to prevent the misclassification of isoflavone intake in early pregnancy. Third, we excluded users of isoflavone supplements in early pregnancy. Fourth, we excluded mothers exposed to insecticides and/or herbicides at work, because some of these may have endocrine disrupting action and have been reported to have an association with hypospadias.[19] Fifth, we additionally adjusted for genistein intake in mid-late pregnancy. Finally, we used the subpopulation with participating fathers, and adjusted for paternal age at delivery (<25, 25-29, 30-34, ≥35 years),[20] after excluding fathers exposed to insecticides and/or herbicides at work.[19]

RESULTS

In this population, the median genistein, natto, and tofu intakes were 15.3 mg/day, 10.0 g/day, 19.5 g/day, respectively, and a total of 51 cases of hypospadias were identified (12.3/10,000 male live births). Table 1 shows the characteristics of mothers based on genistein intake groups. Increasing maternal intakes of fish, vegetables, or fruit is accompanied by increasing genistein intake. The distributions of maternal age, educational background, household income, occupation, smoking habits, parity, use of folic acid supplements, and paternal age showed the different patterns among the 3 groups.

Table 2 shows the association of genistein and soya food intake in early pregnancy with hypospadias. Compared with mothers in the 11th-89th percentiles genistein intake group, those in the ≤10th percentile group had an elevated risk of their sons having hypospadias (multivariable-adjusted OR = 2.8, 95% CI = 1.4-5.8). This association was also observed when restricting to isolated cases of hypospadias (OR = 2.5, 95% CI = 1.1-5.6). In contrast, there was no difference in the risk of hypospadias between the reference and the ≥90th percentile groups (OR = 0.9, 95% CI = 0.4-2.4). With respect to soya foods, low natto intake was clearly associated with hypospadias; the multivariable-adjusted OR for the no natto intake group (≤15th percentile) vs the reference group (16th-89th percentiles) was 2.1 (95% CI = 1.1-4.1). As shown in Supplementary Table 1, there was no measurable association of genistein and soya food intakes in mid-late pregnancy with hypospadias. The Spearman's correlation coefficients between early and mid-late pregnancy intake were 0.60 for genistein, 0.64 for natto, and 0.50 for tofu.

With respect to the subgroup and sensitivity analyses of the association between genistein intake in early pregnancy and hypospadias (Table 3), the elevated risk in the low intake group was also observed when excluding mothers aged 35 years or older, those who had utilized ART, those with severe morning sickness, isoflavone supplement users, and those exposed to insecticides and/or herbicides at work. Even after mutual adjustment for genistein intakes in early and mid-late pregnancy, low genistein intake in early pregnancy was associated with hypospadias (OR = 2.4, 95% CI = 1.0-

Table 2. Association of genistein and soya food intake in early pregnancy with hypospadias.

	≤10th Percentile		11th-89th Percentile	≥90th Percentile	
	OR	95% CI		OR	95% CI
Genistein					
Intake (mg/day)		≤5.0	5.1-36.1		≥36.2
No. of participants		4158	33,262		4158
No. of cases		10	36		5
Maternal age-adjusted model	2.3	(1.1-4.7)	Reference	1.1	(0.4-2.8)
Multivariable model*	2.8	(1.4-5.8)	Reference	0.9	(0.4-2.4)
No. of isolated cases†		8	32		5
Multivariable model*	2.5	(1.1-5.6)	Reference	1.0	(0.4-2.7)
Natto					
Intake (g/day)		0‡	0.1-29.5		≥29.6
No. of participants		6359	31,061		4158
No. of cases		13	32		6
Maternal age-adjusted model	2.0	(1.1-3.9)	Reference	1.4	(0.6-3.3)
Multivariable model*	2.1	(1.1-4.1)	Reference	1.3	(0.5-3.1)
No. of isolated cases†		11	28		6
Multivariable model*	2.0	(1.0-4.1)	Reference	1.5	(0.6-3.6)
Tofu (tofu for miso soup, tofu for other dishes, yushidofu, koyadofu, namaage, and aburaage)					
Intake (g/day)		≤4.5	4.6-52.4		≥52.5
No. of participants		4158	33,262		4158
No. of cases		8	40		3
Maternal age-adjusted model	1.7	(0.8-3.6)	Reference	0.6	(0.2-1.9)
Multivariable model*	1.9	(0.9-4.2)	Reference	0.5	(0.2-1.7)
No. of isolated cases†		6	36		3
Multivariable model*	1.6	(0.6-3.8)	Reference	0.6	(0.2-2.0)

CI, confidence interval; OR, odds ratio.
* Adjusted for maternal age at delivery, educational background, household income, occupation in early pregnancy, smoking habits, alcohol consumption, body mass index before pregnancy, current history of diabetes or gestational diabetes and hypertensive disorders in pregnancy, parity, infertility treatment, routine use of folic acid supplements, and fish, vegetable, or fruit consumptions in early pregnancy.
† Defined as unaccompanied by other major (anencephaly, spina bifida, encephalocele, microphthalmia, cleft palate, cleft lip (with or without cleft palate), congenital heart diseases (not including patent ductus arteriosus), oesophageal atresia, small intestinal atresia, anorectal malformation, gastroschisis, omphalocele, diaphragmatic hernia, and reduction defects of the upper and/or lower limbs), and chromosomal anomalies (Down syndrome, 18 trisomy, and 13 trisomy).
‡ Participants did not consume natto (≤15th percentile).

5.4), but intake in mid-late pregnancy was not (OR = 1.3, 95% CI = 0.5-3.2). The elevated risk was also confirmed among the subpopulation with participating fathers.

DISCUSSION

To the authors' knowledge, this is the first prospective study to reveal an association between maternal intake of isoflavone in early pregnancy (estimated as genistein intake) and hypospadias. We observed an increase in the risk of delivering sons with hypospadias in the low isoflavone intake group (genistein intake ≤10th percentile), and this association did not change after excluding users of isoflavone supplements, implying that dietary intake of isoflavone contributes to the occurrence of hypospadias. It is understandable that natto intake showed a clear association with hypospadias rather than tofu, because the former is rich in the aglycone form of isoflavone,[21] which has superior absorptivity compared to the glucoside form.[22]

The present finding, that the low isoflavone intake group had a higher risk of hypospadias, was not inconsistent with the report from the National Birth Defects Prevention Study.[10] This case-control study analyzed the data on mothers of 1250 sons with second- or third-degree hypospadias, along with 3118 controls, between 1997 and 2005, and showed that a high intake of genistein [≥0.0418 mg/day (≥90th percentile)] was associated with a decreased risk of hypospadias when compared with the reference [0.0108-0.0417 mg/day (11th-89th percentile)]. In the present study, in those participants whose dietary isoflavone intake was much higher than those in that previous study, we observed that a certain amount of isoflavone intake was associated with a relatively low risk of hypospadias, and the risk did not increase among the participants with high intake. Previously, an association between hypospadias and a vegetarian diet, which typically involves increased consumption of soya, was reported,[9] but this was not replicated in other studies.[23-25] We thus consider that isoflavone appears to be a risk-decreasing rather than risk-increasing factor for hypospadias. Isoflavone produces oestrogenic or antioestrogenic effects as the situation demands,[7] and may be related to the maintenance of the balance between androgen and oestrogen. Anti-inflammatory and antioxidant effects of isoflavone have also been inferred,[26] because inflammation and oxidative stress have been proposed as etiologic contributors to congenital anomalies in general.[27]

A methodologic advantage of this study is its prospective cohort design, which is less likely to be affected by

Table 3. Subgroup and sensitivity analyses of the association between genistein intake in early pregnancy and hypospadias.

	≤10th Percentile		11th-89th Percentile	≥90th Percentile	
	OR	95% CI		OR	95% CI
Main result in Table 2					
No. of participants		4158	33,262		4158
No. of cases		10	36		5
Multivariable model*	2.8	(1.4-5.8)	Reference	0.9	(0.4-2.4)
Excluding mothers aged 35 years or older					
No. of participants		3234	23,877		2756
No. of cases		9	27		3
Multivariable model*	3.4	(1.5-7.4)	Reference	0.8	(0.2-2.7)
Excluding mothers who utilized assisted reproductive technology					
No. of participants		4067	32,232		3961
No. of cases		9	34		5
Multivariable model*	2.7	(1.3-5.7)	Reference	1.0	(0.4-2.6)
Excluding mothers with severe morning sickness					
No. of participants		3758	30,029		3753
No. of cases		10	34		5
Multivariable model*	2.8	(1.4-5.9)	Reference	1.0	(0.4-2.6)
Excluding users of isoflavone supplements in early pregnancy					
No. of participants		4155	33,213		4144
No. of cases		10	36		5
Multivariable model*	2.8	(1.4-5.8)	Reference	0.9	(0.4-2.4)
Excluding mothers exposed to insecticides and/or herbicides at work					
No. of participants		3959	31,365		3953
No. of cases		10	35		5
Multivariable model*	2.9	(1.4-6.0)	Reference	0.9	(0.4-2.3)
Adjusted for genistein intake in mid-late pregnancy†					
No. of participants		4147	33,192		4147
No. of cases		9	36		5
Multivariable model*	2.4	(1.0-5.4)	Reference	0.8	(0.3-2.4)
Adjusted for paternal age at delivery after excluding fathers exposed to insecticides and/or herbicides at work (n = 19,326)					
No. of participants		1858	15,433		2035
No. of cases		3	10		1
Multivariable model*	4.4	(1.1-16.9)	Reference	0.5	(0.1-4.1)

* Adjusted for maternal age at delivery, educational background, household income, occupation in early pregnancy, smoking habits, alcohol consumption, body mass index before pregnancy, current history of diabetes or gestational diabetes and hypertensive disorders in pregnancy, parity, infertility treatment, routine use of folic acid supplements, and fish, vegetable, or fruit consumptions in early pregnancy.
† We included 41,486 women who had valid data from a food-frequency questionnaire during the second/third trimester and delivered their sons after 28 weeks of gestation.

recall bias than the case-control studies, as may become apparent in the temporality of the observed association. Unlike the case-control studies, however, cohort designs are not appropriate for investigating cases of rare disease. Among the approximately 40,000 mother-son pairs of this study, there were only 51 cases of hypospadias (12.3/10,000 male live births); and a Japanese nationwide hospital-based monitoring survey for congenital anomalies reported only 61 cases of hypospadias among 107,481 male and female live births in 2012,[28] suggesting approximately 11 cases per 10,000 male live births, because hypospadias in females is exceedingly rare. Thus, although the frequency in this study was similar to that in the nationwide survey, the number of cases was indeed small. Having incorporated an abundance of covariate information, we observed that the low intake group had a tendency to show unhealthy behaviour and low socioeconomic status (Table 1). Thus, we included these factors in the multivariable model, and confirmed the association between isoflavone intake and hypospadias (Table 2). In addition, we thoroughly tested the robustness of the association, and found a clear association throughout the subgroup and sensitivity analyses (Table 3). Moreover, when we performed an analysis mutually adjusted for genistein intake in early and mid-late pregnancy, genistein intake in early pregnancy was associated with hypospadias, but no association was observed with intake in mid-late pregnancy. The combination of these results suggested that the association observed in this study could not be dismissed as mere coincidence.

Another advantage of the study is that the participants were Japanese pregnant women in general. We previously reported that selected characteristics of the mothers and children in the JECS were essentially the same as those of the general Japanese population.[12] Also, the distribution of maternal and infant characteristics between the subpopulation with participating fathers and the total population was comparable.[12] Thus, our finding has a high probability of extrapolation to Japanese pregnant women in general. Despite these advantages, some limitations of the study also merit consideration. First, the FFQ we used was validated for Japanese women aged 40-74 years (mean genistein intake = 28.57 mg/day),[13] but not specifically for the pregnant women. Nonetheless, our estimate of genistein intake (mean 18.8 mg/day) is considered reasonable, because older age groups tend to consume more soya foods than younger age groups.[29] Second, we did not collect information on hypospadias phenotypes, though some studies reported that exposure-hypospadias association may differ by phenotype.[30] Further studies exploring the association of isoflavone intake with different hypospadias phenotypes are required. Thus, we could not estimate the influence by misclassifying some cases of distal hypospadias, which is not conductive to early detection, as noncases. Finally, as a weakness common to observational studies, residual confounding due to unmeasured factors (in this case, such as family history of hypospadias[30]) may well have been presented.

CONCLUSION

We observed, in a prospective birth cohort study, that low isoflavone intake in early pregnancy was associated with an elevated risk of hypospadias. This epidemiologic finding may help elucidate the embryologic mechanism of hypospadias.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.urology.2018.11.008.

APPENDIX

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