



Published in final edited form as:

*J Pediatr.* 2011 November ; 159(5): 825–831.e1. doi:10.1016/j.jpeds.2011.04.042.

## Phenotype severity in the bladder exstrophy-epispadias complex: analysis of genetic- and non-genetic contributing factors in 441 families from North America and Europe

Heiko Reutter, MD<sup>1,2</sup>, Simeon A. Boyadjiev, MD<sup>3,4</sup>, Lisa Gambhir, MD<sup>5</sup>, Anne-Karoline Ebert, MD<sup>6</sup>, Wolfgang H. Rösch, MD<sup>6</sup>, Raimund Stein, MD<sup>7</sup>, Annette Schröder, MD<sup>7</sup>, Thomas M. Boemers, MD<sup>8</sup>, Enrika Bartels, MD<sup>1</sup>, Hannes Vogt, MD<sup>8,9</sup>, Boris Utsch, MD<sup>10,11</sup>, Martin Müller, MD<sup>11</sup>, Birte Detlefsen, MD<sup>8</sup>, Nadine Zwink<sup>12</sup>, Sebastian Rogenhofer, MD<sup>13</sup>, Rita Gobet, MD<sup>14</sup>, Goedele M.A Beckers, MD<sup>15</sup>, Arend Bökenkamp, MD<sup>16</sup>, Abdol-Mohammad Kajbafzadeh, MD<sup>17</sup>, Enrique Jaureguizar, MD<sup>18</sup>, Markus Draaken<sup>1,19</sup>, Yegappan Lakshmanan, MD<sup>4</sup>, John P. Gearhart, MD<sup>4</sup>, Michael Ludwig, PhD<sup>5</sup>, Markus M. Nöthen, MD<sup>1,19</sup>, and Ekkehart Jenetzky, MD<sup>12</sup>

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany <sup>2</sup>Department of Neonatology, Children's Hospital, University of Bonn, Bonn, Germany <sup>3</sup>Section of Genetics, Department of Pediatrics, University of California Davis, Sacramento, CA, USA <sup>4</sup>Department of Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University, Baltimore, MD, USA <sup>5</sup>Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany <sup>6</sup>Department of Pediatric Urology, St. Hedwig Hospital Barmherzige Brüder, Regensburg, Germany <sup>7</sup>Department of Urology, University of Mainz, Mainz, Germany <sup>8</sup>Department of Pediatric Surgery and Pediatric Urology, Children's Hospital of Cologne, Cologne, Germany <sup>9</sup>Department of Pediatric Surgery, University of Mainz, Mainz, Germany <sup>10</sup>Department of Pediatric Nephrology, Charité Clinical Center, Berlin, Germany <sup>11</sup>Department of Pediatrics, University of Erlangen-Nürnberg, Erlangen, Germany <sup>12</sup>Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany <sup>13</sup>Department of Urology, University of Bonn, Bonn, Germany <sup>14</sup>Department of Pediatric Surgery, University of Zurich, Zurich, Switzerland <sup>15</sup>Department of Pediatric Urology, VU Medical Center, Amsterdam, The Netherlands <sup>16</sup>Department of Pediatric Nephrology, VU Medical Center, Amsterdam, The Netherlands <sup>17</sup>Department of Urology, Pediatric Urology Research Center, Children's Hospital Medical Center, Tehran University of Medical Sciences, Tehran, Iran <sup>18</sup>Department of Pediatric Urology, University Hospital La Paz, Madrid, Spain <sup>19</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany

### Abstract

**Objective**—To identify genetic and non-genetic risk factors contributing to the severity of the bladder exstrophy-epispadias complex (BEEC).

© 2011 Mosby, Inc. All rights reserved.

**Corresponding author** Dr. med. Heiko Reutter, Institute of Human Genetics & Department of Neonatology, Children's Hospital, University of Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany; Tel: +49 (0) 228 287 51012; Fax: +49 (0) 228 287 51011; reutter@unibonn.de.  
Edited by TW and WFB

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare no conflicts of interest.

**Study design**—Patients with BEEC from North America (n=167) and Europe (n=274) were included. The following data were collected: associated anomalies, parental age at conception, mode of conception, periconceptional folic acid supplementation, maternal risk factors during pregnancy, and environmental risk factors. Patients were divided into three subgroups according to phenotype severity: (i) mild - epispadias (E, n=43); (ii) intermediate - classic bladder exstrophy (CBE, n=366); and (iii) severe - cloacal exstrophy (CE, n=31). These subgroups were then compared to identify factors which contribute to phenotype severity.

**Results**—Males were overrepresented in all subgroups. A relatively high prevalence of cleft lip with or without cleft palate was observed. Maternal smoking and medical radiation during the first trimester were associated with the severe CE phenotype. Compliance with periconceptional folic acid supplementation was associated with the mildest phenotype (E).

**Conclusions**—Periconceptional folic acid supplementation appears to prevent the development of the severe phenotype of BEEC.

### Keywords

Bladder exstrophy-epispadias complex; BEEC; epidemiology; teratogenity

---

The bladder exstrophy-epispadias-complex (BEEC) occurs on a spectrum of severity. This includes the mildest form, epispadias (E), the intermediate form classic bladder exstrophy (CBE), and the most severe form, exstrophy of the cloaca (CE). The latter is also termed the OEIS (omphalocele, exstrophy, imperforate anus, and spinal defects) complex.<sup>1</sup> The overall birth prevalence for the complete BEEC spectrum in children of European descent has been estimated to be 1 in 10,000.<sup>2</sup> Birth prevalences (including terminated pregnancies) for the specific subtypes have been estimated to be 1 in 117,000 in males and 1 in 484,000 in females for E,<sup>3</sup> 1 in 37,000 for CBE,<sup>4</sup> and 1 in 200,000 to 1 in 400,000 for CE.<sup>5</sup> Although BEEC can occur as part of a complex malformation syndrome, the majority (~98.5%) of cases are classified as isolated.<sup>6-8</sup> Isolated BEEC is assumed to be multifactorial, involving both genetic and environmental factors.<sup>6-8</sup> Although progress has been made in the analysis of genetic risk factors,<sup>9,10</sup> the identification of environmental factors is problematic. Due to the rarity of the phenotype, prospective population-based studies have little power to identify possible environmental factors. Birth registries provide access to larger samples of patients and controls, but information on phenotype expression and environmental risk factors is limited.<sup>6,11-13</sup> The recruitment of BEEC cohorts at major treatment centers enables the collection of detailed clinical information and data on risk factors. However, a carefully matched control group is usually unavailable. An alternative approach is to identify genetic- and non-genetic factors in patient-subgroups formed according to phenotype severity. These risk factors also represent promising candidates for the risk of developing the disease per se.

In a previous study, we used the latter approach to investigate a sample of 214 unrelated patients from Europe. Periconceptional maternal exposure to smoking was identified as a risk factor for severe BEEC (CE).<sup>8</sup> For the purposes of the present study, this sample was enlarged by adding 60 new patients and combining it with a sample of 167 patients from North America. The aims of the present study were to corroborate our previous findings with improved statistical power, and to identify additional risk factors by comparing potential demographic and other risk factors between BEEC subgroups with mild, intermediate, and severe phenotypes.

## Methods

The study was conducted in accordance with the Declaration of Helsinki, and ethical approval was obtained from the respective ethics committees. All participants provided written informed consent. Consent for minors was obtained from legal guardians. All of the included patients had BEEC. The presence of additional malformations or medical diseases was documented in order to identify syndromal forms.

### European Study (ES-Cohort)

The ES-Cohort was recruited between 2003 and the end of 2008. Families with BEEC were identified through patient support groups in Austria, France, Germany, Italy, Spain, Switzerland, and The Netherlands, and through pediatric urology clinics in Austria, France, Germany, Switzerland, and The Netherlands. Parental interviews, clinical examinations, review of clinical documentation, and blood sampling were performed during routine clinical appointments, in accordance with the procedures used for the NAS Cohort (see below). Ethnicity was assessed according to the origin of the four grandparents. A total of 313 families agreed to participate in the study. All 313 families were asked to complete the epidemiological questionnaire. The questionnaire was completed by a total of 274 families.

### North American Study Cohort (NAS-Cohort)

The NAS-Cohort was recruited between 2001 and the end of 2005 through the Pediatric Urology Clinic at the James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine. A total of 285 families with BEEC were identified through the local institutionally approved BEEC database of 815 patients, the website of an internet support group (<http://www.bladderexstrophy.com>), or external physicians. Of these, 232 consented to participate in the present study. All 232 families were asked to complete the epidemiological questionnaire. The questionnaire was completed by a total of 167 families. Patients in the NAS-Cohort were classified as Caucasian, African American, American Indian, Hispanic, or Asian.

### Control data

To determine the prevalence-ratio of Down syndrome and congenital anomalies beyond the BEEC spectrum, data from the European Surveillance of Congenital Malformations (EUROCAT) network (<http://eurocat-network.eu>) were used as reference data from the general population. EUROCAT is a European network of population-based registries, which was initiated in 1979 for the epidemiological surveillance of congenital anomalies. The EUROCAT data were collected between 1980 and the end of 2007. Within this period, EUROCAT surveyed approximately 12 million births in Europe.

### Epidemiological questionnaire

The epidemiological questionnaire is based upon the National Birth Defect Prevention Study questionnaire of the CDC (Center of Disease Control and Prevention) (<http://www.nbdpn.org>). The questions concern the exstrophy phenotype and associated anomalies. Most of the epidemiological questionnaire items address details of the pregnancy including parental age at conception; mode of conception (e.g. assisted reproductive techniques); and periconceptional folic acid supplementation. The questionnaire also addresses maternal factors during the pregnancy including disease; intake of drugs of abuse, alcohol, tobacco, and medications; exposure to possible environmental risk factors, such as chemical toxins at work and at home; and medical radiation. Drugs of abuse and medications taken by mothers during the periconceptional and/or first trimester period were

classified according to the Anatomical Therapeutic Chemical (ATC) Classification System ([www.whooc.no](http://www.whooc.no)).

For the ES-Cohort, the epidemiological questionnaire also included items from the EUROCAT questionnaire concerning folic acid supplementation. The epidemiological questionnaire used in the NAS-Cohort did not specifically enquire about maternal periconceptual folic acid supplementation, since flour in the USA has been routinely fortified with folic acid since 1998. The analyses of maternal periconceptual folic acid supplementation were therefore restricted to families from the ES-Cohort.

### Statistical analysis

A binomial test was performed to compare male-to-female ratios. To assess the impact of potential risk factors on phenotype severity, two types of analysis were performed: (i) three-group comparisons (E vs. CBE vs. CE); and (ii) two-group comparisons (E/CBE vs. CE). ANOVA was used for parental age, and the Chi<sup>2</sup>-test or Fischer's exact test was used for all other factors. In the two-group comparisons for maternal age, smoking, and radiation exposure, adjusted odds ratios were calculated using logistic regression. Unadjusted odds ratios are reported for the two-group comparisons, since only paternal age appeared to be confounded by maternal age. All significant p-values ( $p < 0.05$ ) should be considered explorative, since no adjustment for multiple testing was performed. For some results, the number of patients does not total 441, since some questionnaires were incomplete.

### Results

As expected, CBE was the most common BEEC phenotype (Table I). The male-to-female ratio for BEEC was 2.3:1. The highest male-to-female-ratio (2.4:1) was observed for CBE. Lower ratios were observed for E (1.7:1) and CE (1.6:1) (Table II). Three patients had Down syndrome. None of the other patients fulfilled the criteria for any known malformation syndrome. All patients with Down syndrome and BEEC belong to the ES-cohort. Mothers in the ES-cohort were slightly older than those in the NAS-cohort (NAS:29 years; ES:30 years; Table I), and this may have contributed to the occurrence of Down syndrome in patients of the ES-cohort. However, of the children with Down syndrome and EEC, one mother was only 24 years of age at the time of childbirth (the other two mothers were 36 and 45 years of age). To determine whether the age of the mothers in our cohorts differs from the age of the mothers in the EUROCAT cohort, it would be necessary to perform a detailed comparison with matched (e.g. according to the year of birth) cases from the EUROCAT data. Since the present year of birth distribution ranges from 1951 to 2008, an adequately matched control cohort cannot be generated from the EUROCAT sample, which only began in 1979. Thus, no such comparison was attempted. Given the reported prevalence of 0.11% in the EUROCAT data, the prevalence of Down syndrome in the present BEEC cohort (0.68%) is 6-fold (prevalence ratio = 6.10, 95% CI: 2.08; 17.77) higher than that in the EUROCAT survey (Table III). The three patients with Down-syndrome were excluded from the analyses of associated congenital anomalies.

Across the three phenotypic subgroups, ventricular septal defect (VSD) was the most commonly associated non-BEEC-spectrum congenital anomaly. This was present in five of the 438 patients (1 E, 3 CBE, and 1 CE). Hence, the risk for VSD in the present BEEC cohort was 1.1%. In the EUROCAT survey, the much lower birth prevalence of 0.25% was observed for VSD. The ratio of the prevalence of VSD in the present BEEC cohort compared to that of the EUROCAT survey was thus 4.47 (95% CI: 1.91; 10.36) (Table III). Since echocardiography is required to detect a VSD, the prevalence figures for VSD in the present and in the EUROCAT samples may be incorrect. Our questionnaire for patients and parents did not enquire about the performance of echocardiography, and so we do not know

how many patients with BEEC underwent this investigation and how many patients with BEEC with VSD may have been missed. However, since BEEC requires surgical correction, echocardiography may have been requested as part of the pre-operative assessment, in particular for children born within the past two decades. As a consequence, more VSDs may have been detected in our BEEC population than in the EUROCAT survey, in which, to our knowledge, the great majority of cases were not assessed with echocardiography.

Cleft lip with or without cleft palate (CL/P) was observed in three patients with BEEC (2 CBE and 1 CE). The risk for CL/P in a patient with BEEC was thus 0.68% (95% CI: 0.014%; 0.197%). In the EUROCAT survey, a prevalence of 0.085% was found for CL/P. Thus the prevalence ratio was 7.98 (95% CI: 2.72; 23.25) (cf. Table III).

Each of the following co-morbid anomalies was observed in one patient only: gastroschisis, hemivertebrae, maxillary hypoplasia, congenital heart tumor, bilateral pre-auricular fistulas, and syndactyly of the toes. No further anomalies were reported. The most common co-morbid disease was childhood epilepsy. This was reported in three patients (2 E and 1 CBE). It was impossible to obtain detailed information on the precise nature of the childhood epilepsy, and no further analysis was performed. One patient with reported childhood epilepsy had developmental delay. However, he did not display any other features of a recognizable syndrome. This patient underwent routine array based analysis to screen for gain- or loss of genetic material, and molecular genetic testing for fragile-X-Syndrome. The results were normal.

### **Three- (E vs. CBE vs. CE) and two- (E/CBE vs. CE) group comparisons of periconceptual and first trimester risk factors**

For each risk factor, the absolute number of patients in whom the risk factor was present and the relative frequencies are shown for each subgroup (Table IV; available at [www.jeds.com](http://www.jeds.com)). Significant findings from the three- (E vs. CBE vs. CE) and two-group (E/CBE vs. CE) comparisons are shown (cf. Table IV).

### **Maternal intake of medications and/or drugs of abuse**

For the patients with E and CE, a significantly higher exposure to antacids was reported compared with patients with CBE in the three-group comparison.

### **Maternal exposure to tobacco, alcohol, and soft drinks**

During the first trimester, patients with CE (31%) had a significantly higher exposure to maternal smoking (any amount) compared with patient with CBE (14%) and E (7%) (Table IV). However, this association was not replicated in an analysis of the NAS-Cohort only, or in a separate analysis of the 60 ES-Cohort families that had not been previously analyzed. Since the mothers of patients with CE were significantly younger than the mothers of patients with E or CBE, an analysis was performed to identify a possible association between maternal smoking and maternal age. This showed an equal distribution of smoking-mothers across all maternal age groups (OR 0.99 (0.93; 1.05). Interestingly, mothers who smoked reported a significantly higher intake of antacids ( $\chi^2= 4.66$ ;  $p=0.036$ ) than those who did not.

Exposure to alcohol during early pregnancy (any amount) was reported by 76 of the 421 women (18%), and was almost always limited to an occasional alcoholic drink before the confirmation of pregnancy. No differences were found between the three BEEC subgroups. None of the mothers reported excessive drinking or a history of alcohol dependence. No association was found for the consumption of diet Coke (aspartame).

### Maternal exposure to toxins or medical radiation

No association was found for work or household based chemical detergents, hair coloring agents, or other chemical detergents. In the combined ES- and NAS-Cohorts, 5.7% of mothers (25 of 389) reported periconceptional exposure to medical radiation (multiple x-rays during a single examination or computerized tomography). A comparison of all three subgroups showed that a history of periconceptional exposure to medical radiation was significantly more common ( $p=0.013$ ) among mothers of patients with CE (18%) than among mothers of patients with CBE (14%) or E (9%) (Table IV).

### Maternal disease

Descriptive analysis revealed a low incidence of maternal disease during the periconceptional and first trimester periods, e.g. maternal sepsis, epilepsy, thyroid dysfunction, urogenital tract infections, and diabetes. No differences were observed between the three BEEC subgroups.

### Mode of conception (e.g. assisted reproductive techniques)

Previous studies have suggested that assisted reproduction is a risk factor for BEEC.<sup>14,15</sup> To address this, data on the use of IVF (in vitro fertilization) and ICSI (intracytoplasmic sperm injection) were analyzed across the entire cohort. Information was available for 424 pregnancies. Of these, eight had been achieved with the use of ICSI (n=3) or IVF (n=5). These eight patients with BEEC were born between 1997 and 2008. During the same time-period, an additional 209 patients with BEEC from the ES- and NAS-Cohorts had been born. Therefore, 3.6% of the patients with BEEC (8 of 217) were conceived through ICSI or IVF. No differences were found between the three BEEC subgroups ( $p=0.183$ ). It was impossible to determine whether assisted reproduction per se is a risk factor for the development of BEEC because no valid external data were available for comparison.

### Maternal periconceptional folic acid supplementation

Only 18% (34 of 190) of the mothers had followed the current recommendations regarding folic acid supplementation (0.4 mg per day; or 4mg per day in the case of an increased risk of congenital anomaly) (Table IV). During the periconceptional period, mothers of patients with E (24%) had been the most compliant, followed by mothers of patients with CBE (17%), and mothers of patients with CE (14%). However, these differences were not significant.

### Parental age

To investigate a possible association between parental age and BEEC severity, maternal and paternal age were assessed separately for each subgroup (Tables I and IV). Parents of patients with E and CBE were older than parents of patient with CE. As expected, the ages of the two parents were correlated (Spearman  $r=0.711$ ). Adjustment for maternal age showed that the effect of paternal age can be ignored (OR 1.0).

### Discussion

As in previous reports, BEEC was more common in males than in females (Table II).<sup>16,17</sup> A male preponderance was observed in all three phenotypic subgroups. In contrast, previous studies have reported an equal sex distribution, or a female preponderance, among patients with CE.<sup>16,17</sup> These analyses may have been influenced by the former practice of assigning female sex to aphallic 46,XY CE males, in whom reconstruction of the external male genitalia was infeasible.<sup>18</sup>

The prevalence of Down syndrome in the present BEEC cohort was six-fold higher than that reported in the EUROCAT survey. In a previous clinical report, the present authors described three patients with co-morbid BEEC and Down syndrome, and presented a review of the literature.<sup>19</sup> The present study is the first to report the prevalence ratio of Down syndrome in BEEC. Nevertheless, as mentioned above, this observation has not been made on the basis of a detailed comparison with matched datasets. Since our year of birth distribution ranges from 1951 to 2008, an adequately matched control cohort cannot be generated from the EUROCAT sample, which only began in 1979. Thus, no such comparison was attempted and a clear statement about this finding cannot be made. Further research is needed to clarify whether the prevalence of Down syndrome is genuinely increased in the BEEC population. Research is also warranted to determine the exact prevalence of BEEC in individuals with Down syndrome. However, very large samples will be required due to the rarity of BEEC.

In 1987, an epidemiological study by the International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS) reported a higher rate of associated congenital anomalies in BEEC than had been described previously.<sup>6</sup> As well as reporting higher rates of congenital anomalies which are now acknowledged to be part of the BEEC spectrum (e.g. anorectal malformation, neural tube defect, renal agenesis), this study reported higher rates of anomalies beyond the BEEC spectrum, such as CL/P and congenital heart defects.<sup>6</sup> The present study has replicated this observation, at least with regard to CL/P, and is the first to have reported prevalence ratios. The high prevalence of VSD in our BEEC cohort compared to the EUROCAT survey must be interpreted with caution, however. Neither patients nor controls were systematically assessed with echocardiography, which renders prevalence estimates for this specific malformation unreliable. Furthermore, it can be speculated that because echocardiography may have been requested as part of the preoperative assessment in patients with BEEC, this effect of under-reporting may have been stronger than in the EUROCAT sample, in which, to our knowledge, the great majority of cases were not assessed with echocardiography.

The observation of a higher periconceptual and first trimester maternal intake of antacids among E and CE mothers compared to CBE mothers was unexpected, since the most severe expression of a malformation is typically associated with the highest exposure to a potential teratogenic agent. Interestingly, Acs et al<sup>20</sup> described an association between medical treatment for severe maternal chronic dyspepsia in early pregnancy and congenital anorectal malformations (ARM). This malformation spectrum is closely related to BEEC. Future prospective studies are warranted to investigate the potential teratogenic effect of antacids on the development of the urorectal anatomy.

The present finding of greater exposure to maternal smoking during the first trimester in patients with a severe BEEC phenotype, together with previous reports of associations between periconceptual maternal smoking and various congenital defects,<sup>21,22</sup> raises the question of whether periconceptual maternal smoking impacts on BEEC severity.

No differences in periconceptual or first trimester exposure to alcohol, environmental toxins, or maternal disease were observed across the three phenotype subgroups. These observations are consistent with the findings of previous population based studies.<sup>6,12,13</sup> However, it must be remembered that our data were collected retrospectively. The finding that medical radiation during the periconceptual period may contribute to BEEC severity is interesting. However, this has not been suggested in previous reports, and thus requires independent replication.

Previous reports by Wood et al. suggested a possible association between assisted reproduction and BEEC.<sup>14,15</sup> To determine whether assisted reproduction per se was risk factor for the development of BEEC in the present cohort, it would be necessary to make a comparison with valid external data (proportion of live-born infants conceived through assisted reproduction per year and per country). However, no such data are available. Although no significant differences in the mode of conception were observed between EEC subgroups, the numbers in the individual subgroups were small, and much larger samples are required to draw definite conclusions.

The efficacy of periconceptional folic acid supplementation has been demonstrated for several birth defects e. g. neural tube defects (NTD)<sup>23</sup> and isolated omphalocele.<sup>24</sup> Both anomalies are part of the BEEC spectrum. The present analysis was consistent with our previous observations concerning mothers who commenced folic acid supplementation during the first 10 weeks of gestation. Interestingly, an analysis of the periconceptional period only showed that mothers of patients with E (24%) showed the highest compliance, followed by mothers of patients with CBE (17%), and mothers of CE (14%) patients. Population-based epidemiological studies of NTDs have shown that the preventive effect is greatest when folic acid supplementation is commenced three months prior to conception and then continued throughout early pregnancy.<sup>25</sup> It may therefore be necessary to commence folic acid supplementation before conception to prevent the formation of a severe BEEC phenotype. Although mothers of patients with CE showed the highest compliance during the first 10 weeks of gestation, early embryonic urogenital and neural tube development may have taken place before the commencement of supplementation.

The three-group comparison of parental age showed that parents of patients of E patients were the oldest, followed by parents of patients with CBEs, and parents of patients with CE. No comparison between mothers in the present cohort and mothers of healthy born children was performed, since this may have been biased by heterogeneity in the maternal age at delivery between countries. In general, the occurrence of a severe congenital malformation with a high impact on fertility is likely to be due to a spontaneous *de novo* mutation. Previous investigations have demonstrated that the risk of *de novo* germ cell mutations (including chromosomal aberrations) increases with parental age.<sup>26</sup> The present observation of a higher risk for the severe CE phenotype in patients with younger parents is inconsistent with this generally accepted hypothesis. This may be attributable to differences in life style factors between older and younger parents which have not been investigated in the present study.

Although the present study is one of the most comprehensive descriptive epidemiological analyses of BEEC to date, it had several important limitations. Firstly, the study was a pooled analysis of two previous studies which differed in design (mono-center versus multi-center; and self-help group based). However, virtually identical questionnaires were used. Secondly, the present study is based on parental recall. Depending on the age of the affected family member at inclusion, parental recall bias may influence the accuracy of reporting. Thirdly, it was not possible to adjust for confounders, as these could not be identified with certainty. Finally, it must be remembered that all *p*-values are explorative, and that no adjustment was made for multiple testing.

The results suggest that maternal smoking and medical radiation during the first trimester appear to be associated with a more severe BEEC phenotype. In contrast, periconceptional folic acid supplementation appears to prevent the development of the severe phenotype. An important aim of future studies will be to determine whether these factors also influence the development of BEEC per se.



## Acknowledgments

available at [www.jpeds.com](http://www.jpeds.com).

E.J., H.R., E.B., N.Z., A.-K.E., W.R., R.S., A.S., T.B., M.N., and M.L. are members of the “Network for the Systematic Investigation of the Molecular Causes, Clinical Implications, and Psychosocial Outcome of Congenital Uro-Rectal Malformations (CURE-Net),” which is supported by a research grant (01GM08107) from the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF): <http://www.cure-net.de>. S.B. is partially supported by the Children's Miracle Network, endowed chair in pediatric genetics. This project has been partially supported through NIH grants (R01 DE016886 from the NIDCD/NIH; M01-RR00052 from the NCRR/NIH) and a CMN grant (CMNSB06).

## Abbreviations

<b>BEEC</b>	bladder exstrophy-epispadias complex
<b>CBE</b>	classic bladder exstrophy
<b>CE</b>	cloacal exstrophy
<b>E</b>	epispadias
<b>EUROCAT</b>	European Surveillance of Congenital Malformations network
<b>ES-Cohort</b>	European Study-Cohort
<b>IVF</b>	in vitro fertilization
<b>ICSI</b>	intracytoplasmic sperm injection
<b>NAS-Cohort</b>	North American Study Cohort
<b>OEIS</b>	omphalocele, exstrophy, imperforate anus, and spinal defects complex
<b>VSD</b>	ventricular septal defect

## References

1. Gearhart, JP. Exstrophy, epispadias, and other bladder anomalies. In: Walsh, PC.; Retik, AB.; Vaughan, ED.; Wein, AJ., editors. *Campbell's Urology*. 8th edition. Philadelphia: WB Saunders Co; 2002. p. 2136-2196.
2. Reutter H, Qi L, Gearhart JP, Boemers T, Ebert AK, Rösch W, Ludwig M, Boyadjiev SA. Concordance analyses of twins with bladder exstrophy-epispadias complex suggest genetic etiology. *Am J Med Genet A*. 2007; 143A:2751–2756. [PubMed: 17937426]
3. Gearhart, JP.; Jeffs, RD. Exstrophy-epispadias complex and bladder anomalies. In: Walsh, PC.; Retik, AB.; Vaughan, ED.; Wein, AJ., editors. *Campbell's Urology*. 7th edition. Philadelphia: WB Saunders Co; 1998. p. 1939-1990.
4. Wiesel A, Queisser-Luft A, Clementi M, Bianca S, Stoll D. the EUROSCAN Study Group. Prenatal detection of congenital renal malformations by ultrasonographic examination: An analysis of 709,030 births in 12 European countries. *Eur J Med Genet*. 2005; 48:131–144. [PubMed: 16053904]
5. Hurwitz RS, Manzoni GAM, Ransley P, Stephens FD. Cloacal exstrophy: A report of 34 cases. *J Urol*. 1987; 138:1060–1064. [PubMed: 3656560]
6. Anonymous. Epidemiology of bladder exstrophy and epispadias: a communication from the International Clearinghouse for Birth Defects Monitoring Systems. *Teratology*. 1987; 36:221–227. [PubMed: 3424208]
7. Boyadjiev SA, Dodson JL, Radford CL, Ashrafi GH, Beaty TH, Mathews RI, Broman KW, Gearhart JP. Clinical and molecular characterization of the bladder exstrophy-epispadias complex: analysis of 232 families. *BJU Int*. 2004; 94:1337–1343. [PubMed: 15610117]

8. Gambhir L, Höller T, Müller M, Schott G, Vogt H, Detlefsen, et al. Epidemiological survey of 214 families with bladder exstrophy-epispadias complex. *J Urol*. 2008; 179:1539–1543. [PubMed: 18295266]
9. Draaken M, Reutter H, Schramm C, Bartels E, Boemers TM, Ebert AK, et al. Microduplications at 22q11.21 are associated with classic bladder exstrophy. *Eur J Med Genet*. 2010; 53:55–60. [PubMed: 20060941]
10. Reutter H, Rüschemdorf F, Mattheisen M, Draaken M, Bartels E, Hübner N, et al. Evidence for linkage of the bladder exstrophy-epispadias complex on chromosome 4q31.21-22 and 19q13.31-41 from a consanguineous Iranian family. *Birth Defects Res A Clin Mol Teratol*. 2010; 88:757–761. [PubMed: 20672349]
11. Byron-Scott R, Haan E, Chan A, Bower C, Scott H, Clark K. A population-based study of abdominal wall defects in South Australia and Western Australia. *Paediatr Perinat Epidemiol*. 1998; 12:136–151. [PubMed: 9620564]
12. Tang Y, Ma CX, Cui W, Chang V, Ariet M, Morse SB, Resnick MB, Roth J. The risk of birth defects in multiple births: a population-based study. *Matern Child Health J*. 2006; 10:75–81. [PubMed: 16240077]
13. Caton AR, Bloom A, Druschel CM, Kirby RS. Epidemiology of bladder and cloacal exstrophies in New York State, 1983–1999. *Birth Defects Res A Clin Mol Teratol*. 2007; 79:781–787. [PubMed: 17990338]
14. Wood HM, Trock BJ, Gearhart JP. In vitro fertilization and the cloacal-bladder exstrophy-epispadias complex: is there an association? *J Urol*. 2003; 169:1512–1515. [PubMed: 12629406]
15. Wood HM, Babineau D, Gearhart JP. In vitro fertilization and the cloacal/bladder exstrophy-epispadias complex: a continuing association. *J Pediatr Urol*. 2007; 3:305–310. [PubMed: 18947761]
16. Bennett AH. Exstrophy of bladder treated by ureterosigmoidostomies. Long-term evaluation. *Urology*. 1973; 2:165–168. [PubMed: 4769520]
17. Ives E, Coffey R, Carter CO. A family study of bladder exstrophy. *J Med Genet*. 1980; 17:139–141. [PubMed: 7381870]
18. Reiner WG, Gearhart JP. Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth. *N Engl J Med*. 2004; 350:333–341. [PubMed: 14736925]
19. Reutter H, Bökenkamp A, Ebert A-K, Rösch W, Boemers TM, Nöthen MM, Ludwig M. Possible association of Down syndrome and Exstrophy-Epispadias-Complex: report of two new cases and review of the literature. *Eur J Pediatr*. 2009; 168:881–883. [PubMed: 18923839]
20. Acs N, Bánhidly F, Puhó EH, Czeizel AE. A possible association between maternal dyspepsia and congenital rectal/anal atresia/stenosis in their children: a population-based case-control study. *Acta Obstet Gynecol Scand*. 2009; 88:1017–1023. [PubMed: 19657756]
21. Slickers JE, Olshan AF, Siega-Riz AM, Honein MA, Aylsworth AS. Maternal body mass index and lifestyle exposures and the risk of bilateral renal agenesis or hypoplasia: the National Birth Defects Prevention Study. *Am J Epidemiol*. 2008; 168:1259–1267. [PubMed: 18835865]
22. Karatza AA, Giannakopoulos I, Dassios TG, Belavgenis G, Mantagos SP, Varvarigou AA. Periconceptional tobacco smoking and isolated congenital heart defects in the neonatal period. *Int J Cardiol*. 2010; 31:464–471.
23. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*. 1992; 327:1832–1835. [PubMed: 1307234]
24. Botto LD, Mulinare J, Erickson JD. Occurrence of omphalocele in relation to maternal multivitamin use: a population-based study. *Paediatrics*. 2002; 109:904–908.
25. Van Allen, MI.; McCourt, C.; Lee, NS. Preconception health: folic acid for the primary prevention of neural tube defects. A resource document for health professionals, 2002. Ottawa, Ontario: Minister of Public Works and Government Services Canada; 2002. (Cat. Number H39-607/2002E)
26. Risch N, Reich EW, Wishnick MM, McCarthy JG. Spontaneous mutation and parental age in humans. *Am J Hum Genet*. 1987; 41:218–248. [PubMed: 3618593]

**Table 1**

## Description of the NAS- and ES-Cohorts

<b>Recruitment time-frame</b>	<b>North-American Study (NAS) Cohort (N= 167) 2001 – 2005</b>	<b>European Study (ES) Cohort (N= 274) 2003 – 2008</b>
Ethnicity or country of origin	145 (87 %) Caucasian, 4 African American, 4 American Indian, 7 Hispanic, 1 Asian, 6 families with no data on ethnicity.	258 (94 %) European, 2 Algerians, 3 Nigerians, 1 Afghan, 1 Dubaiian, 1 Indonesian, 2 Iranians, 1 Yemeni, 1 Filipino, 2 Turkish, 1 Ukrainian, and 1 Belarusian
Diagnostic groups	21 (13 %) isolated epispadias (13 males / 8 females) 130 (78 %) classic bladder exstrophy (88 males / 42 females) 15 (9 %) cloacal exstrophy (13 males / 8 females)	22 (8 %) isolated epispadias (14 males / 8 females) 236 (86 %) classic bladder exstrophy (171 males / 65 females) 16 (6%) cloacal exstrophy (10 males / 6 females)
Sex	110 males (66 %); 56 females	195 males (71 %); 79 females
Year of birth	Minimum: 1942; Maximum: 2004 Median: 1995 (1989; 1999)	Minimum: 1953; Maximum: 2008 Median: 1997 (1989; 2002)
Maternal age	Minimum: 16 years; Maximum: 39 years Mean: 29 years (SD= 4.8 years)	Minimum: 17 years; Maximum: 46 years Mean: 30 years (SD= 4.8 years)
Paternal age	Minimum: 18 years; Maximum: 49 years Mean: 31 years (SD= 5.6 years)	Minimum: 19 years; Maximum: 53 years Mean: 33 years (SD= 5.5 years)

**Table 2**

Male-to-female ratio

	<b>Epispadias (E)</b>	<b>Classic bladder exstrophy (CBE)</b>	<b>Cloacal exstrophy (CE)</b>	<b>Complete cohort (BEEC)</b>
Males	27	259	19	305
Females	16	107	12	135
Male-to-female ratio	1.7	2.4	1.6	2.3
Proportion of males (95% CI)	0.63 (0.48; 0.76)	0.71 (0.66; 0.75)	0.61 (0.44; 0.76)	0.69 (0.65; 0.73)
<i>p</i> -value	0.127	<b>&lt;0.001</b>	0.281	<b>&lt;0.001</b>

*p*-values were calculated using the binominal test; CI: confidence interval

**Table 3**

Prevalence of Down syndrome and of congenital malformations in the BEEC cohort compared with the EUROCAT survey

	<b>BEEC cohort</b>	<b>EUROCAT survey</b>	<b>Prevalence ratio (95% CI)</b>	<b>p-value</b>
Down syndrome	3 / 441 (0.68 %)	13 317 / 11 943 497* (0.11 %)	<b>6.10 (2.08; 17.77)</b>	<b>0.014</b>
Ventricular septal defect	5 / 438 (1.14 %)	29 691 / 11 712 426* (0.25 %)	<b>4.47 (1.91; 10.36)</b>	<b>0.006</b>
Cleft lip with or without cleft palate	3 / 438 (0.68 %)	10 470 / 12 288 732* (0.09 %)	<b>7.98 (2.72; 23. 25)</b>	<b>0.007</b>

\* Calculated from reported cases and prevalence rate.

**Table 4**  
 Three- (E vs. CBE vs. CE) and two-(E/CBE vs. CE) group comparisons of periconceptional and first trimester risk factors

	E	CBE	CE	E vs. CBE vs. CE p-value	E/CBE vs. CE p-value	OR	95% CI
<b>Maternal intake of medications and/or drugs of abuse</b>							
Maternal pain relief (over the counter)	8 / 39 (21 %)	84 / 334 (25 %)	9 / 28 (32 %)	0.557	0.382	1.45	0.63; 3.31
Maternal pain relief (prescribed)	1 / 40 (3 %)	14 / 334 (4 %)	1 / 28 (4 %)	1.000	0.909	0.89	0.11; 6.97
Maternal antacid intake	11 / 41 (27 %)	48 / 333 (14 %)	8 / 28 (29 %)	<b>0.028</b>	<b>0.086</b>	<b>2.14</b>	<b>0.90; 5.08</b>
Maternal antihistamine intake	1 / 40 (3 %)	17 / 332 (5 %)	0 / 27 (0 %)	0.697	0.693	-	-
Maternal antibiotic intake	8 / 42 (19 %)	45 / 331 (14 %)	3 / 28 (11 %)	0.552	0.609	0.73	0.21; 2.49
Maternal bronchial inhalations	1 / 40 (3 %)	13 / 334 (4 %)	0 / 28 (0 %)	0.858	0.727	-	-
Maternal steroid intake	0 / 40 (0 %)	10 / 333 (3 %)	0 / 27 (0 %)	0.808	0.769	-	-
Maternal antidepressant intake	1 / 41 (2 %)	3 / 334 (1 %)	0 / 28 (0 %)	0.527	0.801	-	-
Maternal herbal medication	5 / 39 (13 %)	21 / 325 (6 %)	0 / 28 (0 %)	0.112	0.731	-	-
<b>Maternal folic acid intake</b>							
Periconceptional folic acid intake (starting before conception)	5 / 21 (24 %)	27 / 155 (17 %)	2 / 14 (14 %)	0.758	0.716	0.75	0.16; 3.52
Folic acid starting during the first 10 weeks of gestation	4 / 22 (18 %)	27 / 154 (18 %)	5 / 13 (38 %)	0.212	<b>0.075</b>	2.92	0.90; 9.54
<b>Maternal exposure to tobacco, alcohol, and soft drinks</b>							
Maternal smoking	3 / 43 (7 %)	47 / 347 (14 %)	9 / 29 (31 %)	<b>0.012</b>	<b>0.009</b>	<b>3.06</b>	<b>1.32; 7.09</b>
Maternal alcohol	6 / 43 (14 %)	66 / 349 (19 %)	4 / 29 (14 %)	0.601	0.539	0.71	0.24; 2.11
Maternal diet Coke intake (aspartame)	10 / 41 (24 %)	66 / 342 (19 %)	5 / 28 (18 %)	0.717	0.799	0.88	0.32; 2.39
<b>Maternal exposure to toxins or medical radiation</b>							
Maternal exposure to work based toxins	1 / 29 (3 %)	27 / 229 (12 %)	1 / 21 (5 %)	0.317	0.394	0.41	0.05; 3.18
Maternal exposure to chemical detergents	0 / 41 (0 %)	39 / 345 (11 %)	2 / 27 (8 %)	<b>0.040</b>	0.652	0.71	0.16; 3.12
Maternal exposure to hair coloring agents	10 / 43 (23 %)	86 / 347 (25 %)	4 / 27 (15 %)	0.502	0.256	0.53	0.18; 1.58
Maternal exposure to chemical detergents	6 / 41 (15 %)	34 / 342 (10 %)	3 / 27 (11 %)	0.575	0.913	1.07	0.31; 3.72
Medical radiation (multiple x-rays or computer tomography)	4 / 43 (9 %)	16 / 343 (14 %)	5 / 28 (18 %)	<b>0.013</b>	<b>0.011</b>	<b>3.98</b>	<b>1.37; 11.56</b>
<b>Maternal disease</b>							
Maternal sepsis	0 / 40 (0 %)	5 / 333 (2 %)	0 / 27 (0 %)	1.000	0.776	-	-
Maternal epilepsy	1 / 40 (5 %)	0 / 337 (0 %)	0 / 29 (0 %)	0.168	0.817	-	-
Maternal thyroid problems	1 / 41 (2 %)	9 / 338 (3 %)	2 / 29 (7 %)	0.304	0.209	2.73	0.57; 13.10
Maternal bladder/kidney infection	2 / 41 (5 %)	24 / 339 (7 %)	1 / 29 (3 %)	0.853	0.487	0.49	0.06; 3.72

	<b>E</b>	<b>CBE</b>	<b>CE</b>	<b>E vs. CBE vs. CE p-value</b>	<b>E/CBE vs. CE. p-value</b>	<b>OR</b>	<b>95% CI</b>
Maternal diabetes during pregnancy	3 / 41 (7 %)	7 / 383 (2 %)	0 / 29 (0 %)	0.120	0.767	-	-
<b>Mode of conception</b>							
Assisted reproduction (IVS/ICSI)	2 / 43 (5 %)	5 / 353 (1 %)	1 / 29 (3 %)	0.183	0.528	1.26	0.62; 256
<b>Parental age</b>							
Maternal age: Mean (standard deviation)*	30.8 (3.5)	29.8 (5.0)	27.3 (4.1)	<b>0.008</b>	<b>0.005</b>	<b>0.89</b>	<b>0.83; 0.97</b>
Paternal age: Mean (standard deviation)* #	34.3. (5.0)	32.2 (5.6)	30.3 (5.9)	<b>0.012</b>	<b>0.045</b>	<b>0.93</b>	<b>0.87; 1.00</b>

p-values were calculated using ANOVA, Chi<sup>2</sup>, or Fisher-test; no adjustment was made for multiple testing

For all potential risk factors, additional adjusted ORs were calculated using the following significant risk factors: maternal age, radiation, and smoking. With the exception of paternal age<sup>#</sup>, no notable changes in crude ORs were observed. Due to the strong correlation between maternal and paternal age, paternal age became insignificant (OR=1.0) following adjustment for maternal age.

\* unadjusted ORs were calculated using logistic regression; in the case of parental age this was performed separately for each year.