Bladder Exstrophy-Epispadias Complex

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The bladder exstrophy-epispadias complex (BEEC) represents an anterior midline defect with variable expression comprising a spectrum of anomalies involving the abdominal wall, pelvis, urinary tract, genitalia, and occasionally the spine and anus. The vast majority of BEEC cases are classified as non-syndromic, and the etiology of this malformation is still unknown. This review presents the current state of knowledge on this multifactorial disorder, including historical retrospect, phenotypic and anatomical characterization, epidemiology, proposed developmental mechanisms, existing animal models, and implicated genetic and environmental components. These published lines of evidence argue strongly that BEEC occurs as a result of strong genetic predisposition that is yet to be deciphered. Birth Defects Research (Part A) 85:509–522, 2009. © 2009 Wiley-Liss, Inc.

Key words: bladder exstrophy-epispadias complex; OEIS; genetics; animal model; multifactorial

INTRODUCTION

The bladder exstrophy-epispadias complex (BEEC) represents an anterior midline defect with variable expression comprising a spectrum of anomalies involving the abdominal wall, pelvis, urinary tract, genitalia, and occasionally the spine and anus. The vast majority of BEEC cases are classified as nonsyndromic, and the etiology of this malformation is still unknown. This review presents the current state of knowledge on this multifactorial disorder, including historical retrospect, phenotypic and anatomical characterization, epidemiology, proposed developmental mechanisms, existing animal models, and implicated genetic and environmental components. These published lines of evidence argue strongly that BEEC occurs as a result of a genetic predisposition that is yet to be deciphered.

DISCUSSION

History of BEEC

The term exstrophy, derived from the Greek work ekstriphein, which literally means “turn inside out,” was first used by Chaussier in 1780 (Online Mendelian Inheritance in Man [OMIM] database entry 600057). The earliest known report was the description on an Assyrian tablet from approximately 2000 BC that is now in the British Museum in London (Ives et al., 1980). The first medical description is probably Aldovrandus’ Historia Monstrum, published in 1646, which references scientific details documented by Scheuke von Gräfenberg in 1595 (Hall et al.,...
A dried pelvis specimen of a 6-year-old boy showing marked diastasis of the pubic rami was recorded by Gerardus and Willem Vrolik in their “Tabulae” of the Museum Vrolik (Oostra et al., 1998). Vrolik further portrayed an ectopic bladder that was visible through an infraumbilical defect of the abdominal wall, a phenotype characteristic of classic bladder exstrophy.

**Range of Phenotypes in the BEEC**

BEEC is one of the most severe urologic birth defects, with a profound impact on continence, sexual function, and morbidity because of the effect of chronic and recurrent infections on renal function. Several urological malformations (e.g., ectopic pelvic kidney, renal agenesis, hydronephrosis) are present in approximately one third of all cases. Furthermore, vesicoureteral reflux, obstruction of the ureters and the failed closure of the urethral fold. The pelvic bones are excessively flared, with wide separation of the hips. Many patients have extensive infraumbilical fascial defects in regard to the open laying bladder plate. Further associated malformations are omphalocele, lipomeningocele or myelomeningocele, and bilateral inguinal hernias. Boys may have undescended testes (Satsuma et al., 2006). Split or duplicated bladder is common and unilateral kidney agenesis and intestinal prolapse may also be present (Chisholm and McParland, 1979).

**Anatomic Classification**

In the BEEC spectrum, the degree of extroversion of the bladder varies considerably.

*Grade I* is of minimal degree: the urethra is completely epispadiac, the diastasis of the bony symphysis and of the rectus muscles is minimal, and only the bladder sphincter is exstrophic (Fig. 1, *left*). This might be overlooked frequently in girls unless the labia are separated and the introitus is carefully inspected (Chisholm and McParland, 1979). The smallest amount of prolapsed bladder mucosa is seen upon straining.

*Grade II* represents a milder variant of CBE in which epispadias and diastasis of symphysis pubis are present together with an overly split bladder neck over the trigone. The ureteral orifices are exposed (Chisholm and McParland, 1979). In a few cases, only part of the bladder is exposed, at either the upper or lower end, or a part of exstrophic bladder may be split from the remainder of the bladder that is covered (Ives et al., 1980). With straining, substantial bladder mucosa will prolapse (Chisholm and McParland, 1979).

*Grade III* represents CBE (Fig. 1, *middle*) with epispadias split phallus or bifid clitoris, wide separation of the pubic symphysis, and anteriorly placed ectopic and often stenotic anus (Ives et al., 1980). In addition, the pelvic wings are flared, the angles of the hips are rotated posteriorly, and the feet are pronated (Chisholm and McParland, 1979). Upon straining, the bladder tends to balloon forward, but with relaxation, the bladder mucosa may be indented into the abdomen by gentle digital pressure. Associated anomalies include renal defects such as kidney agenesis or kidney dysgenesis, respectively (Anonymous, 1987).

*Grade IV* is CE or OEIS manifesting with severe exstrophy of the entire bladder with widely separated abdominal rectus muscles and imperforate anus (Fig. 1, *right*). The pelvic bones are excessively flared, with wide separation of the symphysis and severe rotational displacement of the hips. Many patients have extensive infraumbilical fascial defects in regard to the open laying bladder plate. Further associated malformations are omphalocele, lipomeningocele or myelomeningocele, and bilateral inguinal hernias. Boys may have undescended testes (Satsuma et al., 2006). Split or duplicated bladder is common and unilateral kidney agenesis and intestinal prolapse may also be present (Chisholm and McParland, 1979).

**BEEC and Associated Syndromes and Malformations**

Unlike other congenital midline defects, such as hypoplasia or cleft lip with cleft palate, that are often reported in association with recognizable syndromes (i.e., 22q11 deletion syndrome [OMIM #192430], CHARGE-syndrome [OMIM #214800], or the hand-foot-genital syndrome), the vast majority of BEEC cases are nonsyndromic. Nonetheless, occurrence of the BEEC has been reported as part of various syndromes (London Dysmorphology Database, http://www.lmdatabases.com/index.html). Associated syndromes, malformations, and sequences and other congenital diseases have been described in approximately 30 reports (Table 1).

There are at least two reports of patients with frontonasal dysplasia and CE, suggesting a common etiology (Neidich et al., 1988; Robin et al., 1996) owing to disturbed development of the midline structures. Evidence for an X-chromosomal locus comes from two patients presenting with X-linked Goltz syndrome and CE and one infant exhibiting CBE in combination with the Opitz G/BBB syndrome (Jacobson et al., 1998), a defect of midline development, caused by mutations in the MIDI-gene on Xp22 (Quaderi et al., 1997). One of two unrelated patients with the combination of congenital cutaneous aplasia and epibulbar dermoids was also reported to have CBE (Lees et al., 2000). Another association, axial mesodermal dysplasia, combining epibulbar dermoids and CBE (Russell et al., 1981) suggests that axial mesodermal dysplasia and BEEC share a common developmental defect (Haldar et al., 1994). The genes responsible for all of these observations remain to be identified and
Developmental Mechanisms and Animal Models of BEEC

Failure of mesodermal reinforcement of the abdominal wall and/or the cloacal membrane (CM) has been postulated to play a major role in the pathogenesis of BEEC (Muecke, 1964; Marshall and Muecke, 1970; Mildenberger et al., 1988; Mollohan, 1999; Park, 2002; Wood et al., 2003). Often, other anomalies based on mesodermal maldevelopment are inherent to BEEC, including omphalocele, hernias, kidney agenesis, and imperforate anus.

A detailed report on the current knowledge of the embryology of the genitourinary tract is beyond the scope of this review, and the interested reader is referred to the full descriptions given by Baskin et al. (1996), Lerman et al. (2002) and Park (2002). Wood et al. (2003) proposed that BEEC arises in conjunction with other associated malfor-
motions as the result of an overall deficiency of the mesoderm (somatic and lateral plate) to the infraumbilical region during the first half of the first trimester. Because this mesodermal cell population forms both muscular and skeletal components of the bladder and the infraumbilical body wall, it is plausible that a deficiency of this cell population would account for the phenotype observed. Consequently, more severe phenotypes (multiple anomalies) would be associated with an overall deficiency of the mesoderm (Russell et al., 1981; Moore and Weaver, 1990; Winter, 1991). To demonstrate this hypothesis, animal models have been generated to mimic the deficient mesodermal emigration from the primitive streak toward the abdominal midline (human equivalent of gestational weeks 3–4).

For example, lateral plate mesoderm, which forms the external genitalia and musculoskeletal elements of the median infraumbilical region, arises from the caudalmost aspect of the primitive streak (Selleck and Stern, 1991), and as such is the last extraembryonic mesodermal cell population to be formed during gastrulation. Accordingly, formation of this cell population is vulnerable to a number of early primitive streak events, including anomalous foreshortening of the posterior streak, precocious closure of the posterior neuropore, and/or mechanical obstruction of mesoderm migration from the dorsally located streak to the ventromedial abdominal region. Such mechanisms have been proposed for mesoderm-derived anomalies (Griffith and Wiley, 1991; Griffith and Zile, 2000).

Whereas multisystem malformation complexes that comprise BEEC may be caused by a very early deficiency of primitive streak generated mesoderm, it is likely that isolated BEEC is caused either by a later pathogenic event or by one more localized to the developmental field of the defect (Opitz, 1993). Localized obstruction of mesenchyme migration, owing to aberrant development of the CM or body stalk and/or regional alterations in cell death or proliferation, has been hypothesized to play a role in the formation of isolated BEEC. Deviations in temporal initiation of migration or spatial accessibility along the mesoderm migration route, for example, can irreversibly affect the appropriate "seeding" of the ventral body wall with critical mass of lateral plate/somite-derived mesoderm cells necessary to form lower body wall musculoskeletal elements and bladder musculature. Consequently, as the bladder and other pelvic viscera acquire critical bulk during fetal stages, the wall can no longer contain these viscera and ruptures, as does the ventral wall of the forming bladder.

Muecke (1964) first reported that mechanical disruption or enlargement of the cloacal membrane in chicks prevents the invasion of mesodermal cells along the infraumbilical midline, thereby resulting in exstrophy. In support of this hypothesis, Austin et al. (1998) provided evidence in humans that anomalous overgrowth of the cloacal membrane is associated with bladder exstrophy. One pervasive view on its etiology is that premature rupture of CM may be the cause of BEEC (Muecke, 1964). Support for this idea arises from animal models of cloacal exstrophy showing that abnormal partitioning of the CM results in displacement of the genital tubercle, thus in the formation of epispadias. Accordingly, a study of the development of hereditary anorectal malformations in pig embryos was performed, and the authors concluded that agenesis of the dorsal part of the CM may form the basis of congenital malformations of cloaca-derived orifices such as hypospadias, epispadias, bladder and cloacal exstrophy, double urethra, and cloacal membrane agenesis (Van der Putte, 1986). In addition, Thomalla et al. (1985) performed a series of elegant experiments on chick embryos in which a laser was used to incise the CM in the lower abdominal wall, thus creating a hernia defect. The resulting chicks were born with CE, suggesting that premature rupture of the CM results in CE. Nevertheless, no urorectal septation takes place in birds, leaving a common cloaca in any case. The timing of CM disruption in this model determined the resulting variant of the BEEC, with an earlier disruption (4–6 weeks’ gestation, before fusion of the urorectal septum to the CM) leading to the more severe CE (Gearhart, 2002). The authors postulated that BEEC would form if CM rupture occurred just after the urorectal septum completed its descent (6 weeks), but before the initial formation of the genital tubercle (Russell et al., 1981; Greene et al., 1991; Jones, 1997). Mechanical obstruction of mesoderm migration to the lower ventral abdominal wall has also been reported in association with abnormal caudal insertion of the body stalk (umbilical ring), which results in failure of the normal mesodermal interposition in the lower midline (Mildenerberger et al., 1988; Vermeij-Keers et al., 1996). The resultant placement of the umbilical cord directly adjacent to the CM/cloaca, with no intervening supportive connective tissues, results in a superficially placed, unstable CM/cloaca that is prone to rupture. Abnormal formation of the body stalk was also reported as a mechanism for BEEC in patients conceived by in vitro fertilization (Wood et al., 2003). In addition to mechanical disruption, it has been proposed that localized alterations in cell death will act to reduce the ventral mesenchymal cell population leading to infraumbilical midline deficiencies in mice, including BEEC (Wei and Sulik, 1993, 1996; Vermeij-Keers et al., 1996; Van der Werrff et al., 2000).

In view of the shortcomings of the mechanically-induced animal models, transgenic or spontaneous animal models are needed to unravel the morphogenetic events leading to BEEC in more detail. Naturally occurring cases of CBE in animals are rare, and this might depend on the considerable differences in the early development of the CM, such as in rodents and higher vertebrates (Lendon and Forbes, 1971). Apart from the first report of an affected male cat (Geoffroy-Saint-Hilaire, 1832), the offspring of breeding experiments in hares were described with separated pubic bones, but without malformations of the urogenital tract (von Degenhardt, 1964). Stec et al. (2002) described the natural occurrence of CBE in a rhesus monkey: here, the phenotype was identical in both external appearance and internal anatomy when compared to CBE in human. Apart from the models mentioned previously (Muecke, 1964; Thomalla et al., 1985) CBE was also created in lambs by infraumbilical incision (Slaughenhoup et al., 1996). The embryology of CBE was further studied in a rat model (Mildenerberger et al., 1988), and artificial exstrophy in female dogs was used to study renal function separately (Fein, 1969).

All of these models support the concept that the morphogenesis of the BEEC malformation spectrum is primarily attributable to an abnormal early embryonic development of the CM. The models provide, however, only partial explanations for the phenotypic features in children with exstrophy, implying that mimicking of human genetics and embryology, as well as an unequivocal pathogenetic interpretation, are not to be expected.
mainly when dealing with different species. Moreover, a careful analysis of these cases illustrates several shortcomings; as outlined (Stec et al., 2002; Männner and Kluth, 2003), it is questionable whether they actually imitate the genetics and embryology of human BEEC-as mentioned by Männner and Kluth (2003), none of these models have clearly demonstrated that the experimental interventions really affect the CM, and the observations made do not allow an unequivocal pathogenetic interpretation (Thomalla et al., 1985). Moreover, cloacal exstrophy in chick embryos (Muecke, 1964; Thomalla et al., 1985) was not associated with malformations of the caudal neural tube (e.g., myelocystocele) frequently observed in human CE cases. The assumption that CE might be due to premature rupture of the CM during early embryogenesis is also challenged by observations from Langer et al. (1992) and Kaya et al. (2000), reporting prenatal diagnosis of CE by ultrasonographic findings before rupture of the CM. On the other hand, a completely different mechanism has been postulated for the organogenesis of CBE (Beaudoin et al., 2004). Here, observations of the pelvic development in the rabbit embryo imply that bladder and pelvic bone anomalies are related in temporal-spatial development in extrophic malformations. Given their results, the authors propose a novel mechanism to explain exstrophies that should be regarded as occurring as early as secondary gastrulation and in no way involve the cloacal membrane fate.

In addition to two reports describing the incidental occurrence of CE in chick embryos after the administration of nigericin and ochratoxin A (Vedel-Macrandre and Hood, 1986; Wei and Sulik, 1996), future work by Männner and Kluth (2003) possibly provides new insights, as these authors were able to generate CE in six Leghorns by treating chick embryos with suramin or trypan blue. Knockout mice exhibiting a BEEC-like phenotype are extremely rare. Only one gene, p63 (apart from causing congenital defects of the extremities and skin) has been shown to completely reproduce human bladder exstrophy in p63−/− mice (Cheng et al., 2006), although this was not mentioned in an earlier report by Mills et al. (1999). As noted by Ince et al. (2002), female p63−/− mice exhibited abnormal genital morphogenesis with hypoplastic genitalia, a single cloacal opening, and persistence of columnar epithelium at lower genital tract sites.

**Variable Expressivity and Penetrance of BEEC**

Variable expressivity has been observed in several patients showing an intermediate phenotype between the recognized clinical entities of E, CBE, and CE (Martínez-Frias et al., 2001; Boyadjiev et al., 2004). Moreover, in

<table>
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<tr>
<th>Familial cases</th>
<th>References</th>
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<tr>
<td>Two CBE male cousins</td>
<td>Köhler, 1928&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Uncle and nephew with CBE</td>
<td>Sorrentino, 1958; Reutter et al., 2003</td>
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<tr>
<td>Two CBE sisters</td>
<td>Shapiro et al., 1984</td>
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<tr>
<td>Sister/brother with CBE</td>
<td>Shapiro et al., 1984; Messelink et al., 1994; Froster et al., 2004</td>
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<tr>
<td>Mother with E, offspring with CBE</td>
<td>Smith et al., 1992</td>
</tr>
<tr>
<td>Mother and son with CBE</td>
<td>Messelink et al., 1994</td>
</tr>
<tr>
<td>Mother with E, son with CBE</td>
<td>Keppler-Noreuil, 2001</td>
</tr>
<tr>
<td>Sister/brother with CE, both stillborn</td>
<td>Reutter et al., 2003</td>
</tr>
<tr>
<td>Male/female cousins with CBE</td>
<td>Reutter et al., 2003</td>
</tr>
<tr>
<td>CE male, maternal CBE grandfather, paternal great-great aunt with CE</td>
<td>Reutter et al., 2003</td>
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<td>Two male third degree cousins with CBE</td>
<td>Reutter et al., 2003</td>
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<td>Brother with CBE/brother with E</td>
<td>Reutter et al., 2003</td>
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<tr>
<td>Male/female third degree cousins with CBE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Reutter et al., 2003</td>
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<tr>
<td>Uncle with E, nephew with CBE</td>
<td>Reutter et al., 2003</td>
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<tr>
<td>Brother with E, brother with CBE</td>
<td>Boyadjiev et al., 2004</td>
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<tr>
<td>Sister with CE, half-brother with E</td>
<td>Boyadjiev et al., 2004</td>
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<tr>
<td>Uncle with CBE, niece with E</td>
<td>Boyadjiev et al., 2004</td>
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<tr>
<td>Three male second degree cousins, two brothers with E, one with CE</td>
<td>Boyadjiev et al., 2004</td>
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<tr>
<td>Brother with CBE, sister with E&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Kajbafzadeh et al., 2006</td>
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<tr>
<td>Two CBE male cousins, one maternal CBE uncle&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Reutter et al., 2007b</td>
</tr>
<tr>
<td>Two male third degree cousins with CBE&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Gambhir et al., 2008a; Ludwig et al., 2008</td>
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<sup>a</sup>If not mentioned otherwise, each citation refers to a single observation.

<sup>b</sup>This family was also reported in Gambhir et al. (2008a), but failed to be mentioned as initially reported in Reutter et al. (2003).

<sup>c</sup>Consanguineous family.

BEEC, bladder exstrophy-epispadias complex; CBE, classic bladder exstrophy; E, epispadias; CE, exstrophy of the cloaca.
some multiplex families (Table 2) affected members exhibit different forms of BEEC.

Familial occurrence of BEEC has been reported to occur in 0.4 to 2.7% of the cases (Ives et al., 1980; Shapiro et al., 1984; Boyadjiev et al., 2004; Gambhir et al., 2008a), probably dependent on the proper collection of generation history or the size of the study group. Keppler-Noreuil (2001) reported a family with a CE male, a maternal CBE grandfather, and a maternal great-great aunt with CE, each separated by a nonaffected generation. In two pedigrees reported, CBE was observed in male/female third-degree cousins and male/male third-degree cousins, respectively, after having skipped several generations (Reutter et al., 2007b); however, the possibility of monogenic inheritance in some BEEC families needs to be corroborated by additional reports.

### Epidemiological Data, Teratogenic Agents, and BEEC

Because familial occurrence is rare, environmental factors are thought to play a role in the etiology of BEEC. However, the existing epidemiologic studies of BEEC have not identified evidence of major teratogenic factors (Ives et al., 1980; Boyadjiev et al., 2004; Gambhir et al., 2008a). Descriptive epidemiologic and clinical data suggest male sex, race, advanced parental age (Boyadjiev et al., 2004), and increased parity after adjusting for age (Byron-Scott et al., 1998) as predisposing risk factors. Gambhir et al. (2008a) described that periconceptional maternal exposure to smoking was significantly more common in patients with CE than in a combined group of patients with E and CBE ($p = 0.009$).

Several reports have associated BEEC with periconceptional maternal exposure to teratogens or infections. An apparent infection has been reported in two cases (Wood, 1869; Jordan et al., 1968) and exposure to drugs (Kepler-Noreuil, 2001; Wakefield et al., 2002) or alcohol (Finette et al., 1996; Robin et al., 1996) has also been reported twice. Medication with substances ascribed to harbor teratogenic effects has been reported rarely (“flush-shot” in one case and diphenylhydantoin, phenobarbital, and misonidine in an additional case [Carey et al., 1978]; diazepam [Lizcano-Gil et al., 1995]; misoprostol [Orioli and Castilla, 2000]; heparin [Kepler-Noreuil, 2001]; valproic acid [Kepler-Noreuil et al., 2007]). In one case, in which epispidias has been noted in coincidence with Al Awadi/Raas-Rothschild syndrome, the authors mentioned an exposure to x-rays during pregnancy (Mollica et al., 1995).

Several reports described the occurrence of BEEC in infants resulting from in vitro fertilization (IVF) (Shanske et al., 2003; Wood et al., 2003, 2007; Yokoyama et al., 2007) and, as noted by Wood et al., (2007) the incidence of BEEC children conceived by IVF appears to be higher than expected. This finding, combined with an estimated recurrence risk of 0.3 to 0.8 in children born to patients with BEEC (Ludwig et al., 2005), might influence the counseling of adult patients for assisted reproduction (AR). However, as reviewed in two recent reports (D’Hauwers et al., 2008; Gambhir et al., 2008b), successful reproductive outcomes were achieved in at least nine male patients with BEEC using AR, including IVF and intracytoplasmic sperm injection (ICSI).

Contrary to observations in chickens, where CE can be induced by nigericin, ochratoxin A, suramin, or trypan blue (Vedel-Macrander and Hood, 1986; Wei and Sulik, 1996; Männner and Kluth, 2003; Männner et al., 2003; see above), no specific teratogenic agents causally related to the formation of BEEC have been identified in humans to date.

### Occurrence Rates, Recurrence Risk, and Twin Studies

**Occurrence rates of the BEEC.** Varying data have been reported not only on the incidence of the various BEEC forms and among different ethnic groups but also on the male-to-female ratio observed, pointing toward a higher occurrence in males than in females, ranging from 1.5:1 to 6.0:1 (Anonymous, 1987; Bennett, 1973; Ives et al., 1980; Boyadjiev et al., 2004).

An average rate for E has been estimated to be 2.4 per 100,000 in a communication from the International Clearinghouse for Birth Defects Monitoring Systems (Anonymous, 1987), and all but 4 of these 148 cases were males. However, it is possible that a proportion of the incontinent females with epispidias remain undiagnosed (Allen et al., 2004). In this sense, a much lower male-to-female ratio (1.4:1) has been reported most recently (Gambhir et al., 2008a). The observed rate in Europe ranged from 0.6 per 100,000 (Paris) to 4.7 per 100,000 (Denmark) (Anonymous, 1987) and the highest rate of 8.1 per 100,000 has been observed in Native American Indians, whereas an incidence of 1 per 100,000 was found for Americans of Asian origin (James et al., 1999).

The reported incidence of CBE varies from 2.1 to 4.0 per 100,000 live births (Anonymous, 1987; Yang et al., 1994; Martinez-Frias et al., 2001; Nelson et al., 2005; Caton et al., 2007). White infants were significantly more likely to have CBE than nonwhites ($p < 0.0001$), and the incidence again varied by geographic region, as well as by socioeconomic status and insurance status (Nelson et al., 2005). White, non-Hispanic maternal ethnicity was also found to be associated with CBE in a survey from a 17-year period (1983–1999) in New York State (Caton et al., 2007). In this study, CBE showed a statistically significant downward linear trend by year, and these authors also identified summer conception and male sex as possible risk factors. For CBE, Nelson et al. (2005) found an almost even male-to-female ratio and a similar ratio (1.32) was reported by Martinez-Frias et al. (2001), whereas it is 2.4:1 summarized from multiple surveys (Harvard and Thompson, 1951; Higgins, 1962; Bennett, 1973; Jeffs et al., 1982; Gambhir et al., 2008a). However, two series reported a 5:1 to 6:1 male-to-female ratio (Ives et al., 1980; Anonymous, 1987).

CE, with a prevalence ranging from 0.5 to 1 per 200,000 live births (Soper and Kilger, 1964; Belman, 1976; Carey, 1978; Hurwitz et al., 1987; Moore and Weaver, 1990; Martinez-Frias et al., 2001; Caton et al., 2007), has been reported to be more common in females (Martinez-Frias et al., 2001). This finding has also been observed in the New York State survey in which additional factors associated with CE were preterm birth, low birth weight, multiple births, and not residing in New York City (Caton et al., 2007). A sex ratio close to unity was found in a series...
described by Boyadjiev et al. (2004) and a male-to-female ratio of 2:1 was reported by Gambhir et al. (2008a). As reviewed by Keppler-Noreuil (2001), CE seems to be under-ascertained in stillborns and may have an incidence ranging from 1 in 10,000 to 1 in 50,000. Also, an inclusion of cases that were terminated would give more accurate incidence data as outlined in a recent report where the authors established a prevalence of CE in the state of Iowa of approximately 1 in 27,174 in cases ascertained from 2002 to 2006 (Keppler-Noreuil et al., 2007).

Given this, the combined incidence of the BEEC spectrum can be approximated to be 1 in 10,000. The still-apparent discrepant male-to-female ratio observed argues for genetic determinants of BEEC, but it might also be explained by anatomic differences, as well as underascertainment in females in some cases (as mentioned previously).

Recurrence Risk

Further support for genetic factors impacting on the expression of BEEC can be drawn from an increased recurrence risk for offspring of affected individuals. Among siblings, Ives et al. (1980) estimated the recurrence risk to be approximately 1% in nonconsanguineous and nonaffected parents of CBE cases, whereas other reports established a risk of 2.9% (Messelink et al., 1994), 0.8% (Boyadjiev et al., 2004), 0.25% (Reutter et al., 2005) and of 0.3% (Shapiro et al., 1984), respectively.

According to Risch (2001), the simplest way to measure genetic effects is through familial risk ratios, defined as the risk (\( \lambda \)) to a given type of relative of an affected individual divided by the population prevalence. For isolated CBE, the recurrence-risk ratio of disease in siblings (\( \lambda_s \)) has been estimated to be 108, based on a prevalence of 3.3:100,000 (Reutter et al., 2007a). Likewise, Ludwig et al. (2005) concluded from a review of the available literature that \( \lambda_s \) for BEEC is approximately 350 to 500. In their series, Shapiro et al. (1984) also described a 400-fold increased risk to offspring (\( \lambda_o \)) of the affected compared to the general population. Interestingly, only affected females—the less frequently affected sex seen in BEEC cases—produced affected offspring. Among other explanations, this may indicate a higher recurrence risk to offspring of the less commonly affected sex as a result of the fact that affected females have higher genetic liability for BEEC than affected males (so-called Carter effect; Carter, 1976).

Familial Recurrences

Familial recurrences further support the idea of genetic susceptibility underlying BEEC. Although familial occurrence is rare, 30 multiplex families have been reported to date (Table 2). All but three of these families have two affected members. In two families described, there are three affected members of both genders with BEEC defects of variable severity. In a unique Moroccan family, three males (two cousins and maternal uncle) were affected with CBE (Reutter et al., 2007b). This report suggests that in rare families the inheritance of BEEC may be consistent with autosomal dominant inheritance with reduced penetrance (Reutter et al., 2003) or with autosomal recessive trait or X-linked transmission.

These observations indicate that at least one gene with a major effect on the phenotype exists, yet in the majority of cases additional causal factors are necessary for the phenotype to occur. A better understood birth defect, cleft lip with or without cleft palate (CL/P) follows a similar paradigm. Although the majority of the CL/P cases are sporadic and nonsyndromic, a small number of families are affected by a similar autosomal dominant condition, van Der Woude syndrome, because of interferon regulatory factor 6 (IRF6) gene mutations. Interestingly, it has become clear that variants within IRF6 contribute to susceptibility of the isolated CL/P (Zucchero et al., 2004; Park et al., 2007). Similarly, a small subgroup of cases may follow Mendelian inheritance, whereas in the majority, BEEC is mainly inherited as a complex trait with multiple genetic factors (heritable or de novo somatic or germline mutations) and complex gene-gene or gene-environment interactions contributing to its formation. This implies that concentrated research effort on the few multiplex families may yield a clue regarding the major BEEC susceptibility genes. In the absence of such families, incidental chromosomal rearrangements or large scale association studies will be needed to uncover the genetic determinants of BEEC.

Twin Studies

Apart from the observation of familial recurrences, twin studies provide an efficient method to detangle the influences of genetic and environmental factors on a trait (Neale et al., 1994; Martin et al., 1997; Risch, 2001). Although monozygotic (MZ) twins are essentially genetically identical, dizygotic (DZ) twins share on average half of their genes, like ordinary siblings. Based on the assumption that intrauterine environment is similar among MZ and DZ twins, a higher concordance rate (CR) among MZ twins (as compared to DZ twins) would point to genetic factors involved in the etiology of BEEC, whereas similar CRs obtained for the two groups of twins would point to environmental causes (Martin et al., 1997; Risch, 2001). CR, which can be described as pairwise or probandwise, is a standard measure of similarity used in twin studies. The pairwise CR simply gives the proportion of affected pairs being concordant for the disease, whereas the probandwise CR (defined as 2 No. of concordant pairs ÷ No. of discordant pairs + No. of discordant pairs) represents the proportion of affected individuals among the cotwins of previously ascertained index cases. The probandwise CR has the advantage of being interpretable as the recurrence risk in a cotwin of an affected individual.

In a review of the literature, Reutter et al. (2007a) identified a total of 72 twin pairs and added eight previously unreported BEEC twin pairs. Together with four twin pairs reported most recently (Ben-Neriah et al., 2007; Keppler-Noreuil et al., 2007), this brings the number of reported BEEC twin pairs up to 84 (Table 3). Of these twin pairs, 24 were not mentioned in the table because of the lack of data on zygosity (five twin pairs: Chisholm and McParland, 1979; Lowry and Baird, 1982; Hesser et al., 1984; Pinette et al., 1996), concordance (five twin pairs: Schinzl et al., 1979), zygosity and concordance (10 twin pairs: Shapiro et al., 1984; Martinez-Frias et al., 2001), or according to the presence of conjoined twins (four twin pairs: Ornoy et al., 1980; Goldfischer et al., 1997; Corona-Rivera et al., 2003; Casale et al., 2004). Of the remaining 60 twin pairs, 39 were MZ twins (concordant, 17; discordant, 22) and 21 were DZ twins (concordant, 1; discordant, 20).
Reutter et al. (2007a) compared CRs among BEEC twin pairs and established 7.5-fold and 5.6-fold higher pairwise and probandwise CRs, respectively, among MZ twins compared with DZ twins. The resultant MZ/DZ probandwise CR of 5.6:1 depicts the risk among MZ twins to be both affected, which is 5.6-fold the risk compared with DZ twins. This value, by exceeding 2, indicates that genetic influences from multiple loci may be nonadditive, but rather multiplicative, and that epistasis (interaction between contributing genes) may exist. This assumption is supported by values obtained for the familial risk ratios, which are defined as the risk of BEEC for a relative of an affected individual divided by the population prevalence (Risch, 2001). Here, Reutter et al. (2007a) estimated familial risk ratios for MZ and DZ twins of 4500 and 600, respectively, resulting in an MZ/DZ ratio of 7.5.

Monozygotic concordance rates less than 100% (here, 43.6%) provide evidence of environmental effects on the formation of BEEC. As outlined by Reutter et al. (2007a), incomplete CRs could be due to somatic events (single gene or chromosomal mutations) occurring after the splitting of the two embryos (Puck, 1998; Sommer et al., 2002). Alternatively, epigenetic changes during development can create variation in heterochromatin structure and distribution, even among MZ twins, resulting in variable gene expression (Choi and Kim, 2007). On the other hand, it has been speculated that the twinning process with its inherent changes for asymmetry, cytoplasmic deficiency, and competition may favor a discordant expression of midline defects, like CE (Nance, 1981). CE in a fetus of a 30-week triplet pregnancy conceived by IVF and evaluated by chorionic villi sampling (CVS) led Shanske et al. (2003) to speculate about possible pathogenetic mechanisms, in this case including IVF, multiple gestation, trauma to the uterus or uterine vessels following CVS, and placenta accreta. They concluded that the cumulative effects of all or some of these factors may have resulted in uteroplacental insufficiency adequate to produce this phenotype. Also, Keppler-Noreuil et al. (2007) suggested a nongenetic etiology responsible for CE, including uterine vascular pathogenesis, based on the observation of various sets of discordant twins. These factors may indeed contribute to formation and strength of BEEC though multiple data emphasize the importance of genetic factors.

### Molecular Genetics of BEEC

**Folate-related genes and BEEC.** Insufficient periconceptional folic acid intake and deficient folate metabolism in mothers and fetuses have been acknowledged as risk factors for several midline defects (Botto et al., 2002). Methylenetetrahydrofolate is required for the conversion of homocysteine to methionine and of deoxyuridine monophosphate to deoxythymidine monophosphate in support of DNA synthesis, and it also serves as a major source of one carbon unit for purine biosynthesis. All of these pathways might be affected by the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism that causes an A222V substitution in the protein, thereby giving rise to a thermolabile variant with reduced activity (C/T, 35% reduction; T/T, 70% reduction) (Frost et al., 1995). In that context, Mills et al. (2005) tested, apart from the *MTHFR* (A222V) variant, several polymorphisms in genes encoding proteins (*MTHFD1*: methylenetetrahydrofolate dehydrogenase

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### Table 3

**BEEC Twin Reports from the Literature**

<table>
<thead>
<tr>
<th>Twin pairs</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>Monozygotic concordant twin pairs</strong></td>
<td></td>
</tr>
<tr>
<td>2 pairs of CBE twins, gender not mentioned</td>
<td>Enderlen, 1904; Higgins, 1962&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 pair of CBE male twins</td>
<td>Uson et al., 1959; Shapiro et al., 1984 (5 pairs); Lattimer and Smith, 1966; Boyadjiev et al., 2004</td>
</tr>
<tr>
<td>1 pair of female CE twins</td>
<td>Redman et al., 1981; McLaughlin et al., 1984; Chitrit et al., 1993; Lee et al., 1999</td>
</tr>
<tr>
<td>1 pair of CE twins, gender not mentioned</td>
<td>Timor-Tritsch et al., 2000</td>
</tr>
<tr>
<td><strong>Monozygotic discordant twin pairs</strong></td>
<td></td>
</tr>
<tr>
<td>1 pair of female twins, one twin with CBE</td>
<td>Coates, 1805; Reule and Arsell, 1967; Shapiro et al., 1984 (3 pairs); Reutter et al., 2003</td>
</tr>
<tr>
<td>1 pair of male twins, one twin with CBE</td>
<td>Bugge, 1981; Langer et al., 1992; Reutter et al., 2003; Reutter et al., 2007a (2 pairs)</td>
</tr>
<tr>
<td>1 pair of twins, one twin with CBE, gender n.m.</td>
<td>Nance, 1981; Ahmed, 2003</td>
</tr>
<tr>
<td>1 pair of female twins, one twin with CE</td>
<td>Boyadjiev et al., 2004; Reutter et al., 2007a (2 pairs)</td>
</tr>
<tr>
<td>1 pair of female twins, one twin with CE</td>
<td>Meizner et al., 1995 (2 pairs); Karellas et al., 2005</td>
</tr>
<tr>
<td>1 pair of twins, one twin with CE, gender n.m.</td>
<td>Hiett et al., 1992; Keppler-Noreuil et al., 2007</td>
</tr>
<tr>
<td>1 pair of female twins, one twin with PE</td>
<td>Cerrah Celayır et al., 2000</td>
</tr>
<tr>
<td><strong>Dizygotic concordant twin pairs</strong></td>
<td></td>
</tr>
<tr>
<td>1 pair of twins, female CE twin, male twin with omphalocele</td>
<td>Bruch et al., 1996</td>
</tr>
<tr>
<td><strong>Dizygotic discordant twin pairs</strong></td>
<td></td>
</tr>
<tr>
<td>1 pair of twins, one CBE affected male twin</td>
<td>Marshall and Muecke, 1962; Ives et al., 1980; Reutter et al., 2003 (2 pairs); Reutter et al., 2007a (3 pairs)</td>
</tr>
<tr>
<td>1 pair of twins, one CBE twin, gender n.m.</td>
<td>Lattimer and Smith, 1966; Shapiro et al., 1984 (5 pairs)</td>
</tr>
<tr>
<td>1 pair of twins, one CE affected male twin</td>
<td>Lakshmanan et al., 2001; Boyadjiev et al., 2004; Noack et al., 2005; Reutter et al., 2007a</td>
</tr>
<tr>
<td>1 pair of twins, one CE twin, gender n.m.</td>
<td>Ben-Neriah et al., 2007; Keppler-Noreuil et al., 2007 (2 pairs)</td>
</tr>
</tbody>
</table>

<sup>a</sup> If not mentioned otherwise each citation refers to a single observation.

BEEC, bladder extrophy-epispadias complex; CBE, classic bladder extrophy; CE, extrophy of the cloaca; PE, pseudoextrophy; n.m., not mentioned.
Chromosomal Aberrations and Molecular Genetic Findings in Patients with BEEC

Cytogenetic and molecular analyses have revealed chromosomal anomalies in 20 patients with BEEC (Table 4), although none of these anomalies appear to be causative. Here, numerical chromosomal aberrations (47,XXX [observed twice]; 47,XXY; 47,XYY; 47, [no sex reported], +18; 45,X0/46XX [mosaic]) were observed in six patients and, interestingly, were found in association with Down syndrome in an additional four CBE males, one CBE female, and one girl with CE. Aneuploidy of sex chromosomes in five of these cases might point to a gonosomal loci, or locus, involved in the formation of BEEC.

Table 4

<table>
<thead>
<tr>
<th>BEEC</th>
<th>Karyotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>47,XXY</td>
<td>Raboch, 1975c</td>
</tr>
<tr>
<td>E</td>
<td>47,XY,dup(9p)</td>
<td>Chipail et al., 1976</td>
</tr>
<tr>
<td>E</td>
<td>46,XY,del(4)(p2-p7?)</td>
<td>Nicholls and Duffy, 1998</td>
</tr>
<tr>
<td>CBE</td>
<td>46,XX,4p-</td>
<td>Battaglia et al., 1999</td>
</tr>
<tr>
<td>CBE</td>
<td>47,XXY</td>
<td>Boyadjiev et al., 2004</td>
</tr>
<tr>
<td>CBE</td>
<td>46,XY,(8;9)(p11.2;q13)</td>
<td>Boyadjiev et al., 2004</td>
</tr>
<tr>
<td>CBE</td>
<td>46,XY,(2;9)(q13;q32)</td>
<td>Ludwig et al., 2005</td>
</tr>
<tr>
<td>CBE</td>
<td>47,XY,+21</td>
<td>Reutter et al., 2006b, in press; Ebert et al., 2008 (two cases)</td>
</tr>
<tr>
<td>CBE</td>
<td>47,XX,+21</td>
<td>Reutter et al., 2008</td>
</tr>
<tr>
<td>CE</td>
<td>47,XXX</td>
<td>Husmann and Vandersteen, 1999</td>
</tr>
<tr>
<td>CE</td>
<td>47,XX</td>
<td>Lin et al., 1993; Husmann and Vandersteen, 1999</td>
</tr>
<tr>
<td>CE</td>
<td>Trisomy 18 (no sex reported)</td>
<td>Carey et al., 1978</td>
</tr>
<tr>
<td>CE</td>
<td>45,X0/46,XX (mosaic)</td>
<td>Husmann and Vandersteen, 1999</td>
</tr>
<tr>
<td>CE</td>
<td>46,X,der(Y)(Y;9)(q11.23q34.1)-del(Y)</td>
<td>Thauvin-Robinet et al., 2004</td>
</tr>
<tr>
<td>CE</td>
<td>46,XY,del(5)(q12.2q13.2)</td>
<td>Kosaki et al., 2005</td>
</tr>
<tr>
<td>CE and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypomelanosis Ito</td>
<td>Mosaic: diploid/tetraploid/t(1;6); [in fibroblasts: 16% (3 cells) 92,XXXX, 11% (2 cells) 46,XXt(1;6)p32,q13, 73% (14 cells) 46,XX]</td>
<td>Leonard and Tomkins, 2002</td>
</tr>
</tbody>
</table>

*If not mentioned otherwise, each citation refers to a single observation.

BEEC, bladder exstrophy-epispadias complex; E, epispadias; CBE, classic bladder exstrophy; CE, exstrophy of the cloaca.

**Candidate Gene Approaches, Array Analyses, and Linkage Studies**

Candidate gene approaches with genes derived from observations of chromosomal aberrations seem rational but, as mentioned above, no mutations were found in SF-1 and SET (Thauvin-Robinet et al., 2004; Reutter et al., 2006a) on chromosome 9. The finding of a CBE patient with a de novo reciprocal translocation [46,XY,i(18)[p11.2;q13]] led to the mapping of the breakpoint within the contactin-associ-
ated protein-like 3 (CNTNAP3) gene on chromosome 9q13. The authors identified several CNTNAP3 copies in the pericentric region of chromosome 9, complicating the analysis, and no causal relationship between this gene and CBE was established (Boyadjiev et al., 2005). Utsch et al. (2006) observed an MYH9 mutation in a patient with CBE, duplication of the vagina, and congenital macrocytic thrombocytopenia (MTCP). Defects in MYH9, encoding nonmuscle myosin heavy chain IIa, have been identified in patients with MTCP (Seri et al., 2000). However, there is no molecular evidence as yet that MYH9 is associated with urogenital malformations.

Boyadjiev et al. (2004) considered the HLXB9 homeobox gene a good candidate, because mutations in this gene have been found in patients with the Curranri triad (sacral agenesis, presacral mass, and anorectal malformation [Ross et al., 1998]), but did not detect mutations in blood or bladder DNA in five CE/CBE cases. BEEC candidate genes may be derived from elucidating the function of the protein encoded. In that context the fibroblast growth factor 10 (FGF10) gene has been chosen, as FGF10 plays an important role in regulating growth, differentiation, and repair of the urothelium (Bagai et al., 2002). Complete invalidation of Fgf10 in mice has been shown to result in failure of the urethral plate to fuse ventrally (Yucel et al., 2004). Again, these investigations failed to detect any mutation in 10 patients with CE (Krüger et al., 2008).

Mouse knockouts resulting in a BEEC phenotype are extremely rare and the recently reported ΔNp63−/− murine model represents the sole animal knockout exhibiting the typical features of CBE (Cheng et al., 2006). Given this observation, p63 has been considered a good candidate gene, but no mutations were found in 15 CBE patients and five patients with CE (Ching et al., 2007).

An array-based comparative genomic hybridization (array-CGH) was performed in a consanguineous Moroccan kindred with three CBE members (Reutter et al., 2007b). Analyses of samples from two available patients showed normal karyotype and array-CGH detected one aberrant clone in both, comprising genes AMY1B, AMY2B, and AMY2A that encode salivary and pancreatic amylases. This variation, however, represents a known copy-number variation in patients and five patients with CE (Ching et al., 2007).

Candidate gene–based or genome-wide association studies of samples from two available patients showed normal karyotype and array-CGH detected one aberrant clone in both, comprising genes AMY1B, AMY2B, and AMY2A that encode salivary and pancreatic amylases. This variation, however, represents a known copy-number variation in patients and five patients with CE (Ching et al., 2007).

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Candidate gene–based or genome-wide association methods followed by detailed analyses of the associated genes in a large sample of patients have proved successful in identifying susceptibility genes for other complex disorders (Rao, 2008). However, no association study has been performed to date, because of the lack of a sufficiently large BEEC study cohort. The rarity of large multiplex families also precludes a high-power classic linkage analysis. The first genome-wide linkage study was performed in two pedigrees, each comprising two members affected with CBE (Ludwig et al., in press). Evidence for possible risk/modifying loci (LOD scores >1.50) on chromosomes 2p22.1-p21, 2p25.2-p25.1, 4q23-q32.3, 7q21.3-q33, 7q34-q36.1, 14q31.1-q32.2, and 19q13.33-q13.43 was obtained suggesting the presence of susceptibility genes in these regions. Although underpowered, this study identified potential BEEC candidate regions that need to be corroborated by additional studies.

Summary

Despite substantial effort, the etiology of BEEC remains elusive. However, the existing reports in the medical literature clearly demonstrated the importance of genetic and/or epigenetic factors still to be discovered. Short of an accidental observation of a tale-telling chromosomal rearrangement or co-segregating phenotypic trait in a BEEC patient, the path of uncovering the genetic determinants of BEEC seems to be slow and tedious, but determined, multicenter, collaborative effort. Whole-genome association analysis (WGAS) holds the greatest promise to decipher the genetics of BEEC, but requires a large and well-characterized study population, comprehensive clinical databases, sample repositories, and substantial financial investment. In absence of these resources, a combinatorial approach combining linkage analyses in multiplex families, global expression analyses, studies of rare patients with chromosomal rearrangements, and molecular analyses of high probability candidate genes uncovered by corresponding animal models, may identify genes causally related to BEEC and allow a better understanding of the complex and severe midline defect. Our group has initiated a large international effort to enroll and characterize BEEC families and to secure the biologic specimens for future molecular research. More than 700 families from Western Europe and the United States have been recruited so far, making feasible the prospect of meaningful whole-genome association analysis. It can be predicted that this sample size will not be sufficiently large to detect common predisposing alleles (minor allele frequency, MAF > 0.05) with small effect on the phenotype, but risk variants for complex diseases are almost equally likely to be of minor or major effect (Wray et al., 2008). Thus, one can reasonably expect few risk variants of moderate or large effect (odds ratio > 1.5) to be detected with a sample size of approximately 1000 to 2000 case-parent trios. Although these alleles will likely explain no more than 20% of the familial risk, they will provide a glimpse into the developmental cascades involved in BEEC and will ultimately lead to identification of related risk alleles. In contrast to case-control design, the trio design alleviates the problem with ethnic stratifications as cases are compared to their unaffected parents (Spielman et al., 1995). Again, recruitment of 2000 families for the relatively rare BEEC is a formidable challenge that can be overcome only by the collaborative efforts that we have initiated.

In addition to providing the means for better predicting the genetic risk and for determining potential preventive and therapeutic targets, the identification of BEEC genes will subsequently help to explain more common genitourinary malformations, such as vesicoureteral reflux, ectopic pelvic kidney, renal agenesis, cryptorchidism (Anonymous, 1987) or malignancies (adenocarcinoma, squamous cell carcinoma, or transitional cell carcinoma) of the urinary bladder (Justrabo et al., 1991).
ACKNOWLEDGMENTS

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REFERENCES


