Isolated Bladder Exstrophy Associated with a De Novo 0.9 Mb Microduplication on Chromosome 19p13.12

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BACKGROUND: The exstrophy-epispadias complex (BEEC) is a urogenital birth defect of varying severity. The causes of the BEEC are likely to be heterogeneous, with individual environmental or genetic risk factors still being largely unknown. In this study, we aimed to identify de novo causative copy number variations (CNVs) that contribute to the BEEC. **METHODS**: Array-based molecular karyotyping was performed to screen 110 individuals with BEEC. Promising CNVs were tested for de novo occurrence by investigating parental DNAs. Genes located in regions of rearrangements were prioritized through expression analysis in mice to be sequenced in the complete cohort, to identify high-penetrance mutations involving small sequence changes. **RESULTS**: A de novo 0.9 Mb microduplication involving chromosomal region 19p13.12 was identified in a single patient. This region harbors 20 validated RefSeq genes, and in situ hybridization data showed specific expression of the *Wiz* gene in regions surrounding the cloaca and the rectum between GD 9.5 and 13.5. Sanger sequencing of the complete cohort did not reveal any pathogenic alterations affecting the coding region of *WIZ*. **CONCLUSIONS**: The present study suggests chromosomal region 19p13.12 as possibly involved in the development of CBE, but further studies are needed to prove a causal relation. The spatiotemporal expression patterns determined for the genes encompassed suggest a role for *Wiz* in the development of the phenotype. Our mutation screening, however, could not confirm that *WIZ* mutations are a frequent cause of CBE, although rare mutations might be detectable in larger patient samples.
19p13.12, microduplication, bladder exstrophy-epispadias complex, array-based molecular karyotyping, in situ hybridization analysis, copy number variations, *WIZ* gene *Birth Defects Research (Part A) 97:133–139, 2013.*

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INTRODUCTION

The clinical presentation of the bladder-exstrophy-epispadias complex (BEEC) ranges from epispadias (E) and classical bladder exstrophy (CBE), to the most severe form, cloacal exstrophy (CE), often referred to as the OEIS complex (omphalocele, exstrophy, imperforate anus, and spinal defects) (Carey, 2001; Gearhart, 2002). The incidence for the complete spectrum has been reported to be 1 in 10,000 live births, with multiple surveys stating a male-to-female ratio of 2.4:1 (Ebert et al., 2009). Although its etiology is still unclear, there is vast evidence that the BEEC has a strong genetic contribution: (i) a 400-fold increase in the recurrence risk for offspring of BEEC affected individuals (Shapiro et al., 1984), (ii) the existence of multiplex families (Ludwig et al., 2009), and (iii) higher concordance rates among monozygous compared with dizygous twins (62% vs. 11%; Reutter et al., 2007). Furthermore, instances of associated numeric or structural chromosomal aberrations currently in 12 cases support a genetic background involvement in the BEEC (Rotmensch et al., 1991; Battaglia et al., 1999; Ludwig et al., 2009; Draaken et al., 2010; El-Hattab et al., 2010; Lundin et al., 2010; Zaki et al., 2012).

The aim of the present study was to identify causative de novo microaberrations characterized by loss or gain of genomic material (i.e., copy number variations [CNVs]), which may contribute to the BEEC at a genome-wide level. Molecular karyotyping was performed in 110 cases exhibiting BEEC. This analysis and subsequent quantitative PCR (qPCR) analysis of parental DNA revealed a heterozygous de novo 0.9 Mb 19p13.12 microduplication harboring 20 validated RefSeq genes in a single patient. Whole mount in situ hybridization (WISH) analysis was performed on mouse embryos to investigate ventrocaudal expression of the transcripts encoded at gestational days 9.5 and 10.5. This period is the equivalent of human gestational weeks 4 to 6 (O'Rahilly, 1979), which is the postulated time of BEEC organogenesis in humans (Wood et al., 2003). According to its expression pattern, the *WIZ* (widely interspaced zinc finger motifs) gene seemed to be the most promising candidate encompassed by the duplication observed, and, hence, we investigated all the 110 patients for the presence of *WIZ* mutations.

MATERIALS AND METHODS

Patients and DNA Isolation

The sample originally contained 112 BEEC patients. In two of these patients, a 22q11.2 microduplication was identified (Draaken et al., 2010), and these patients were excluded from the present study. All patients (n = 110; E n = 8, CBE n = 95, CE n = 7) were of Central European origin and all reported an unremarkable family history. Written informed consent was obtained from all patients and parents before study entry, and the study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn.

Blood or saliva samples were obtained from the patients and (if available) their parents. In 91 patients, both parents were available; in 15 patients, only one parent agreed to participate; and in four patients, no parental sample could be obtained.

Isolation of genomic DNA was carried out by a Chemagic Magnetic Separation Module I (Chemagen, Baesweiler, Germany) or, in the case of saliva samples, the Oragene DNA Kit (DNA Genotek, Kanata, Canada) was used.

Array-Based Molecular Karyotyping

Human660W-Quad-v1 and HumanOmni1-v1-Quad BeadChip (Illumina, San Diego, California), which contain 657,366 and 1,140,419 markers, respectively, were used for molecular karyotyping. QuantiSNP (v2.2, www.well.ox.ac.uk/QuantiSNP/) was used to identify potential CNVs. All CNV calls with a size >30 kb and a log Bayes factor of <7 (Engels et al., 2009), or which included less than three single nucleotide polymorphisms (SNPs), were discarded. Detected aberrations were then compared with the CNVs identified in our internal datasets using Cartagenia Bench software (v4.0, Leuven, Belgium). We used CNV data from a total of 730 anonymous individuals with diagnoses unrelated to BEEC, who had been genotyped using the same arrays. All CNV regions showing complete overlap with more than three controls were discarded. The remaining CNVs were screened for gene content by accessing the UCSC human genome browser assembly hg18, and by entire coverage with known CNVs listed in the Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/) or Decipher (http://decipher.sanger.ac.uk/). Following these filter steps, the remaining CNVs were checked visually in GenomeStudio v2011.2, (Illumina, San Diego, CA).

Quantitative PCR

Confirmation of the identified CNVs as well as parental genotyping was carried out by qPCR. All reactions for qPCR were performed on an ABI Prism 7900HT Fast Real-Time PCR System with SYBR Green (Applied Biosystems, Foster City, USA) for detection as previously described (Draaken et al., 2011). Primers used for verifying the 19p13.12 duplication were directed against the genes *ILVBL* (1F-5′-TGGTTAGGGTGGATCAGGAC-3′, 1R-5′-CTTTGGGACTCTGGGAGTTG-3′), *AKAP8* (2F-5′-GTAGCTGTAGGTTGCGCCTG-3′, 2R-5′-CTCGTGGCTGCTGAAGTCT-3′), and *CYP4F11* (3F-5′-AGGCACTGAGCTCCAAGATG-3′, 3R-5′-CTAGCTGCTGGTGAGAGGTG-3′).

Paternity Testing

Paternity testing was performed with the PowerPlex 16 System (Promega, Madison, WI) allowing co-amplification and three-color detection of 16 loci according to the manufacturer's specifications.

WISH of Mouse Embryos

Mouse embryos of a wildtype NMRI background were dissected into ice-cold phosphate-buffered saline (PBS), fixed overnight in 4% paraformaldehyde (PFA)/ PBS, and then processed for in situ hybridization as described by Chotteau-Leliévre et al. (2006). Digoxigenin-labeled antisense RNA probes were transcribed in vitro from PCR products generated either from a mouse E10.5 cDNA library or from our in-house MAMEP collection (Molecular Anatomy of the Mouse Embryo Project (http://mamep.molgen.mpg.de). For murine *Wiz*, a 946 bp PCR product covering transcript variants 1, 2, and 3 (nucleotides 1610–2555 of GenBank accession number NM_212438.3) was used. The primers contained T7 and

T3 promoter sites that facilitated the generation of antisense and sense probes, respectively. Riboprobes were synthesized using the appropriate RNA polymerase and a nucleotide mix containing digoxigenin-11-UTP (Roche). Probes were purified using G-50 sephadex columns (GE-Healthcare). Following probe hybridization and washes, an anti-DIG antibody conjugated to alkaline phosphatase (AP; Roche) was incubated with embryos overnight at 4°C, and detection of AP activity was carried out using BM Purple (Roche). For each probe, embryos were processed concurrently and staining reaction times were maintained between embryos, to limit variation in signal intensity. At least three embryos were examined for each gene and stage. Figures depict representative staining. Images were captured using AxioVision software (Zeiss) with a Zeiss AxioCam and SteREO Discovery.V12 microscope.

Histological Analyses of Mouse Embryos

Embryos were dissected and fixed overnight in 4% PFA/PBS as above, followed by processing into paraffin wax. In situ hybridization was performed on paraffin sections (5 μm) using the respective antisense probe according to the protocol from Chotteau-Leliévre et al. (2006) with minor modifications, and detection of AP activity was visualized using BM Purple (Roche). Following staining, slides were quickly dehydrated in 80% and then 100% ethanol, cleared twice for 1 min in xylene (Roth) and coverslips were mounted with Entellan mounting medium (Merck). Photographs were taken using AxioVision software (Zeiss) with a Zeiss AxioCam and SteREO Discovery.V12 microscope. Three sections from at least two different embryos were analyzed for each stage shown and representative images are provided.

Sequence Analysis

Analysis of the coding exons of the human *WIZ* gene was performed using polymerase chain reaction (PCR), and PCR-amplified DNA products were subjected to direct automated sequencing (3130XL Genetic Analyzer, Applied Biosystems, Foster City, CA) according to the manufacturer's specifications. The two strands of each amplicon were sequenced with specific primers (sequences are obtainable on request for PCR and sequencing primers). WIZ amino acid positions were numbered according to Ensembl transcript ID ENST00000389282.

RESULTS

Array Analysis

Molecular karyotyping was performed in 110 BEEC patients, and the initial results from QuantiSNP identified 22,586 CNVs. After Cartagenia bench filtering, 221 variations remained. A stringent filtering step to remove all known CNVs deposited either in the databases or our own controls, along with a manual check of the aberrations in the chromosome view in Genome-Studio, reduced the list to 14 candidate variations. To verify these remaining heterozygous 14 CNVs (eight deletions, six duplications), qPCR was performed. These analyses established four false positive deletions and revealed transmission from a healthy parent in eight patients (maternally transmitted, five cases; paternally transmitted, three cases). In one patient, the duplication was transmitted either from his mother or from his father, as both parents were heterozygous for the duplication. A de novo origin of a duplication could be proven in one female with CBE.

This de novo duplication involving chromosomal region 19p13.12 had an estimated size of 0.9 Mb and comprised 697 consecutive markers on the array (Fig. 1). The first and last duplicated markers were SNP19-14944640 at genomic position 14,944,640 and rs6512075 at genomic position 15,888,067, respectively, according to UCSC human genome browser assembly build 18 (hg18). The flanking markers were rs10401807 at genomic position 14,932,009, and rs12973181 at genomic position 15,903,258. The breakpoint regions can, therefore, be defined as ranging from position 14,932,010 to 14,944,639 and 15,888,068 to 15,903,257, respectively. qPCR analysis confirmed this observation and revealed that this rearrangement was absent in the parents. Paternity testing revealed no incompatibility with the genotypes in this family, thus indicating a de novo event (data not shown).

There were gene interruptions at both breakpoints, affecting the excitatory amino acid transporter gene (*EAAT4* or *SLC1A6*) and the gene encoding cytochrome P450 4F11 (*CYP4F11*). Array data revealed two additional imbalances in this patient. However, both a 1 Mb duplication on chromosome 17q25.1 (first and last duplicated markers: rs1585804, rs1699607), and a 95.5 kb deletion at 1q32.1 (first and last deleted markers: rs17006055, rs2245510) turned out to be of maternal origin (data not shown).

WISH Analysis

The duplicated region on chromosome 19, harbors 20 validated RefSeq genes (including the two genes interrupted by the rearrangement) and three noncoding RNAs. To obtain a comprehensive overview of their transcriptional activity during development of the external genitalia, we performed WISH for the mouse orthologous genes on embryos at gestational days (GD) 9.5 and 10.5, with particular emphasis on the ventrolateral trunk. We were unable to amplify mouse genes *Olfr63* (human (h) *OR10H3*), *Cyp4f40* (*hCYP4F11*), *Cyp4f15* (*hCYP4F2*), *Ephx3* (*hEPHX3*), and *Rasal3* (*hRASAL3*) from embryonic cDNA libraries between E9.0 and E11.0, and, hence, it is unlikely that these genes are expressed at significant levels during embryogenesis; so they were not investigated further. In addition, the human genes *CYP4F24*, *UCA1*, and *CYP4F1* in this region have no mouse orthologs and thus could not be analyzed by WISH. Table 1 summarizes the results obtained from successful WISH analyses on the remaining genes. Although some genes showed no expression at these stages, several transcripts could be ubiquitously detected (verified using probes to detect the sense transcript). Only transcripts for orthologs of human *CASP14*, *AKAP8*, *SYDE1*, *BRD4*, *CYP4F22*, and *WIZ* were found to be specifically expressed in the hindgut and/or caudal region. Here, *Akap8*, *Syde1*, and *Brd4* were all expressed mainly ubiquitously at GD 9.5, and in cloacal and other tissues at GD 10.5. *Casp14* and *Cyp4f14* (*h4F12*) were expressed in the hindgut region at stage GD 9.5, however, no expression in this region was observed at stage GD 10.5. Similarly, *Cyp4f39* (*h4F22*) was also expressed in the hindgut region at GD 9.5, whereas at GD 10.5 it was not expressed.

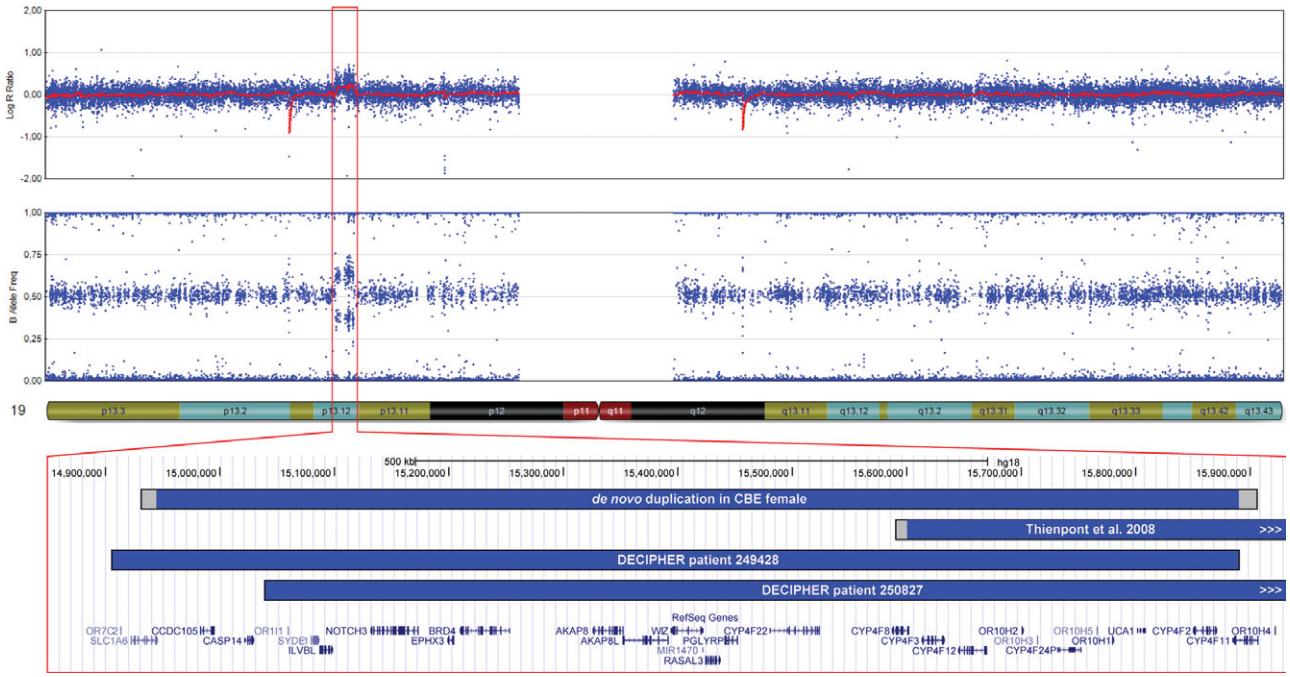

Figure 1. Results of molecular karyotyping for one female CBE patient. GenomeStudio plot of whole chromosome 19 showing the duplication (red frame) involving 697 consecutive SNPs on 19p13.12 (top). Log *R* ratio (log *R*; top) represents a measure of signal intensity for each SNP and red dots indicate the average intensity. B allele frequency (BAF; middle) denotes an allelic intensity ratio for each SNP. The combination of increasing log *R* intensity and a shifted BAF suggests duplication. The RefSeq Genes (bottom) within the de novo duplicated region identified in our patient (blue) and the flanking sides (gray) are shown from UCSC Browser (hg18). Overlapping duplications reported by Thienpont et al. (2008) and DECIPHER (v5.1; GRCh37) are also shown.

Wiz expression was widely detected in the embryo at GD 9.5 with stronger expression observed in the neural tube, branchial arches, frontonasal process, and forelimb bud (Fig. 2A). WISH at GD10.5 showed a similar pattern to GD 9.5 in the maxillary and mandibular branchial arches, fore and hindlimb buds, and tail (Fig. 2B), with a close-up of the ventrocaudal region illustrating *Wiz* expression in the developing genital region. Given this observation, we also analyzed midsaggital sections at GD 12.5 (Fig. 2D), GD 13.5 (Fig. 2E), and GD 14.5 (not shown). All stages showed a similar pattern, with strong *Wiz* expression detectable in the brain, frontonasal process, tail, genital tubercle, neural tube, and lung.

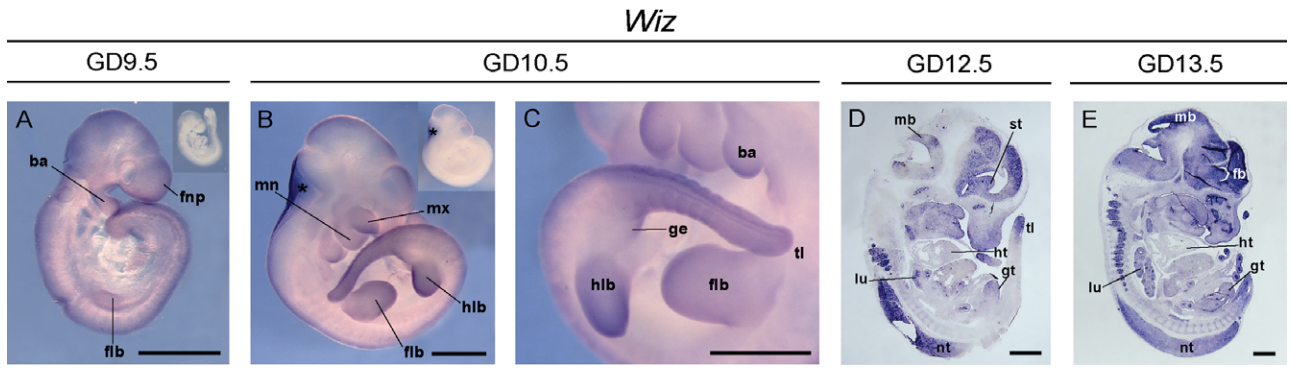

Figure 2. Analysis of *Wiz* expression in midgestational mouse embryos by in situ hybridization. (**A**) Whole-mount of a gestational day (GD) 9.5 mouse embryo. Expression is widely detected in the embryo with stronger expression observed in the neural tube, branchial arches (ba), frontonasal process (fnp), and forelimb bud (flb). (**B**) Whole-mount at GD 10.5 shows a similar pattern to GD 9.5 in the maxillary (mx) and mandibular (mn) branchial arches, fore and hindlimb buds (flb and hlb), and tail. (**C**) A close-up of the ventrocaudal region illustrating expression in the developing genital region (ge). (**D** and **E**) Midsaggital sections at GD12.5 (D) and GD13.5 (E) show strong expression of *Wiz* in the brain, frontonasal process (fnp), tail (tl), genital tubercle (gt), neural tube (nt), and lung (lu). *Wiz* transcripts were undetected in the heart (ht) at all stages examined. Insets in (A) and (B) show embryos hybridized with a sense probe. Note that the area of staining marked by an asterisk in B is due to trapping. The scale bar corresponds to 400 μm. fb, forebrain; mb, midbrain; st, striatum.

Table 1
Expression Patterns of Duplicated Genes Contained on 19p13.12 in Mouse Embryos at GD9.5 and GD10.5

Gene	Mouse ortholog	Expression at GD9.5	Expression at GD10.5
SLC1A6	*Slc1a6*[#]	None	None
CASP14	*Casp14*	Hindgut	Eye
SYDE1	*Syde1*[#]	Ubiquitous	Restricted to limb buds, tail (cloacal membrane included), branchial arches, frontonasal process (FNP)
ILVBL	*Ilvbl*	None	None
NOTCH3	*Notch3*[#]	Ubiquitous, stronger in neural tissues	Ubiquitous, stronger in neural tissues
BRD4	*Brd4*[#]	Ubiquitous, stronger in FNP, branchial arches, and limb buds	Ubiquitous, stronger in limb buds, tail (cloacal membrane included), branchial arches, and FNP
AKAP8	*Akap8*[#]	Ubiquitous	Restricted to limb buds, tail (cloacal membrane included), branchial arches, FNP
WIZ	*Wiz*	Ubiquitous with higher expression in the caudal region	Higher expression in the caudal region, neural tube, branchial arches, and limb buds
PGLYRP2	*Pglyrp2*[#]	ubiquitous	Ubiquitous
CYP4F22	*Cyp4f39*	Hindgut	None
CYP4F8	*Cyp4f17*	None	None
CYP4F16	*Cyp4f16*[#]	Ubiquitous	Ubiquitous
CYP4F12	*Cyp4f14*	Weak ubiquitous, tailgut	Weak ubiquitous, intersomitic
CYP4F11	*Cyp4f40*[#]	None	None

[#]Expression data listed were either obtained using probes from the MAMEP database (#) or from probes generated during the course of this study.

Candidate Gene Analysis

Examination of all *WIZ* exons and their adjacent splice sites failed to reveal any likely causative sequence variant in the *WIZ* genes from our patient samples.

A total of nine sequence variants were detected, which were all unlikely to be causative for the occurrence of BEEC. Five rare variants which had previously been identified and deposited in dbSNP Build 136 (synonymous coding: rs62115899, rs141499743, rs117240920; non-synonymous coding: rs79917926, rs113267381) were each detected once among our patients, and one previously known common variant (rs929613) was found. With the exception of rs117240920, all variants displayed similar frequencies as reported in the databases. For rs117240920 a minor allele (T) frequency of 0.04 was found in our patient sample, compared to reported frequencies of 0.008 to 0.022. The rare variants present in databases were considered to be unlikely causative for BEEC given the low prevalence of the malformation and they were, therefore, not followed up for parental inheritance.

Four rare variants were not present in databases: three nonsynonymous variants (c.G544A, p.Asp182Asn; c.G1132C, p.Glu378Gln; c.C4436T, p.Pro1479Leu), with one of them inherited from a healthy father (c.G544A) and two inherited from a healthy mother (c.G1132C; c.C4436T), and a synonymous variant (c.C4377T, p.Gly1459=) for which the transmission was not investigated. Using several SNP function prediction algorithms, all non-synonymous variants (previously known and newly identified) were classified as benign.

DISCUSSION

Based on genome-wide analyses, recent studies have identified a broad number of CNVs associated with human disorders (Vermeesch et al. 2011). However, the great majority of CNVs are neutral variants and it often proves difficult to implicate a specific CNV in disease development. Most important evidence for a causal relationship is de novo occurrence in a patient. Further important support is provided through independent observation of an identical or overlapping CNV in additional patients. Even more straightforward to the level of the causative gene is the identification of mutations in a gene from the CNV region in patients.

The de novo 19p13.12 duplication detected in our female patient is associated with isolated CBE without any other abnormal features. We are aware of only one published report on a chromosomal rearrangement involving band p13.12 (Thienpont et al. 2008). In this report, the affected female presented with microcephaly, velopharyngeal insufficiency, mental retardation, dysmorphism, and a muscular ventricular septal defect but without any genitourinary involvement (Table 2). Compared with the duplication detected in our CBE patient, only one region of overlap could be identified which comprises the proximal part, ranging from the loci encoding *CYP4F8* to *CYP4F11* (Fig.1). However, in the aberration established by Thienpont et al. (2008), this fragment was found to be triplicated, followed by duplication and again a triplication. Furthermore, the Database of Chromosomal Imbalance and Phenotype in Humans (DECIPHER; v5.1) lists two patients showing a similar duplication to that observed in our case. Here, a completely different phenotype including macrocephaly and cognitive and behavioral impairments was reported, and one of these patients also showed craniofacial and limb anomalies (Table 2). These phenotypic differences may be attributable to the orientation of the duplication (in tandem or inverse), which is still unknown in all four cases. Conversely, the completely different features in patients sharing a similar aberration reflect the observations made for various other CNVs (e.g., duplication 22q11.21) showing extreme variability in expression and penetrance (Draaken et al., 2010).

In our case, two further heterozygous aberrations were identified in the patient, which we assumed were not causally related to the CBE phenotype. Aside from their maternal inheritance in both the cases, the 1 Mb

Table 2
Genetic and Clinical Findings in Patients with a Gain Involving 19p13.12

	Thienpont et al. 2008	Decipher patient 249428	Decipher patient 250827	This study
Rearrangement de novo	dup 19p13.11 (1.3 Mb) yes	dup 19p13.12 (0.98 Mb) no	dup 19p13.12 (1.28 Mb) yes	dup 19p13.12 (0.9 Mb) yes
Additional aberration de novo	Two flanking triplications (1.2 Mb and 0.8 Mb) yes	−	−	dup 17q25.1 (1 Mb); del 1q32.1 (0.1 Mb) maternal origin
Short stature (or IUGR[a])	+	Tall stature, proportionate	−	−
Brain	Normal MRI	nr[b]	nr[b]	nd[c]
Cranium	Microcephaly	Macrocephaly	Macrocephaly	−
Craniofacial	Broad nasal bridge, thin upper lip, mild retrognathia, bilatral ptosis	High palate, frontal bossing, long philtrum, small ears, small nose, thin lips, full cheeks, open mouth appearance	−	−
Behavioral / cognitive impairment	+	+	+	−
Ophthalmologic	Strabismus	−	−	−
Heart	Muscular VSD[d]	−	−	−
Limb	Long fingers, talipes valgus	Tapering fingers,short phalanges, short and broad toes, flat arches of feet	−	−
Genitourinary	−	−	−	Bladder exstrophy
Additional findings	Velopharyngeal insufficiency, sacral dimple	Advanced bone age, large epiphyses, soft skin, joint laxity, gynaecomastia	Scoliosis	−

(+) Present; (−) absent.
[a]IUGR, intrauterine growth retardation.
[b]nr, not reported.
[c]nd, not done.
[d]VSD, ventricular septal defect.

duplication on chromosome 17q25.1 seems to be a benign variant, since studies using various control population cohorts have observed chromosomal gains comprising this region (Pinto et al., 2007; Simon-Sanchez et al., 2007; Zogopoulos et al., 2007; Itsara et al., 2009). The observed 95.5 kb deletion at 1q32.1 interrupts the gene encoding cathepsin E, an endolysosomal aspartic proteinase, predominantly expressed in cells of the immune system (Yanagawa et al., 2007), gastric mucosal cells, and epidermal keratinocytes (Kawabuko et al., 2011). However, the complete cathepsin E knockout in mice resulted only in a lysosomal storage disorder in macrophages and an increased susceptibility to bacterial infection (Yanagawa et al., 2007). Taken together, these data support the assumption that a dosage effect of a gene distal to *CYP4F11* (Fig. 1) might be causally related to BEEC.

Finally, one hypothesis, yet to be verified, may explain the phenotypic differences observed in our patient and others showing a gain of 19p13.12 material. The breakpoints of the duplication in our patient cause gene interruptions for the genes *EAAT4* (*SLC1A6*) and *CYP4F11*. Although both genes affected show no expression at GD9.5 and GD10.5, a fusion protein containing parts of both these genes might interfere with early embryonic development at the time when the morphogenetic events leading to CBE occur. Unfortunately, RNA from the patient was unavailable to perform the analyses required to confirm this hypothesis. It also remains to be elucidated to what degree the postnatal finding of such a fusion-transcript would reflect the situation during embryogenesis.

Spatiotemporal analysis of gene activity in the mouse embryo provides important information regarding the possible function of a gene in a given developmental process. Hence, we performed a detailed expression analysis of the genes comprised by the de novo 19p13.12 duplication. Our analysis (Table 1), which focused on the ventrocaudal trunk region during the developmental stages that are critical for the development of the malformations of the BEEC, showed that *Wiz* is strongly expressed in the relevant tissues—in particular, the cloacal membrane and genital tubercle. The embryonic etiology of bladder exstrophy is unknown, but it has been suggested that it may derive from an abnormally long cloacal membrane (Marshall and Muecke, 1962), or more recently from cranially displaced genital tubercle outgrowth (Kulkarni and Chaudhari, 2008). *Wiz* in its role as a transcription factor is expressed in both the cloacal membrane and genital tubercle during the relevant stages of development for the occurrence of exstrophy. This suggests that an imbalance in expression resulting from duplication/deletion may have relevant phenotypic effects, and, hence, we considered it a reasonable candidate for involvement in normal and abnormal genital formation.

Although one might expect a duplication to result in a subtle increase in transcript level, there is multiple evidence that duplications and deletions as well as point mutations affecting a single gene can result in a similar

phenotype. For instance, *TBX1* truncation mutations as well as *TBX1* missense mutations, resulting in a gain of function, have been observed in patients with DiGeorge syndrome (DGS; Yagi et al., 2003; Zweier et al., 2007). These mutations reflect the data obtained from mouse models, in which the full spectrum of DGS malformations has been elicited in a *Tbx1* dose-dependent manner through either overexpression or underexpression (Liao et al., 2004). Also, an association between microdeletion or microduplication at 16p11.2 and autism has been observed (Weiss et al., 2008), and dysmorphic features and a Rett syndrome-like clinical course were found in the presence of a *FOXG1* duplication as well as a deletion (Florian et al., 2012). These observations prompted us to investigate *WIZ* for the presence of mutations in our patient cohort. However, our screening identified no likely causative mutations in the coding regions or exon–intron boundaries of human *WIZ* in the sample of 110 BEEC patients. It is, therefore, unlikely that mutations affecting the *WIZ* gene are a frequent cause of CBE and/or BEEC; however, our patient cohort might have been too small to detect rare causal mutational events. Furthermore, we may have missed mutations in the promoter region, in as yet unknown regulatory sequences, or in noncoding regions not detectable using the method applied.

In summary, our study showed CBE to be associated with a 0.9 Mb 19p13.12 duplication. WISH analysis of the genes encompassed by the duplication revealed *WIZ* as a plausible candidate to be involved in the development of the urogenital system. Our mutation screening study, however, could not confirm that mutations affecting human *WIZ* are a frequent cause of BEEC. Independent studies are necessary to further verify that a gene dosage effect involving chromosomal region 19p13.12 predisposes to the manifestation of the BEEC.

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