Genitourinary Defects Associated with Genomic Deletions in 2p15 Encompassing OTX1

Carolina J. Jorgez\(^1,2\)*, Jill A. Rosenfeld\(^6\), Nathan R. Wilken\(^7\), Hima V. Vangapandu\(^8\), Aysegul Sahin\(^2\), Dung Pham\(^2\), Claudia M. B. Carvalho\(^4\), Anne Bandholz\(^6\), Amanda Miller\(^7\), David D. Weaver\(^7\), Barbara Burton\(^8\), Deepti Babu\(^9\), John S. Bamforth\(^9\), Timothy Wilks\(^10\), Daniel P. Flynn\(^11\), Elizabeth Roeder\(^12\), Ankita Patel\(^1\), Sau W. Cheung\(^4\), James R. Lupski\(^4,5\), Dolores J. Lamb\(^1,2,3\)*

\(^1\)Center for Reproductive Medicine, Baylor College of Medicine, Houston, Texas, United States of America, \(^2\)Scott Department of Urology, Baylor College of Medicine, Houston, Texas, United States of America, \(^3\)Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, United States of America, \(^4\)Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, United States of America, \(^5\)Department of Pediatrics, Baylor College of Medicine, Houston, Texas, United States of America, \(^6\)Signature Genomic Laboratories, PerkinElmer, Inc., Spokane, Washington, United States of America, \(^7\)Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana, United States of America, \(^8\)Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, United States of America, \(^9\)University of Alberta, Edmonton, Alberta, Canada, \(^10\)Madigan Army Medical Center, Department of Pediatrics, Tacoma, Washington, United States of America, \(^11\)Department of Children's Endocrinology, St. Luke's Children's Specialty Center, Boise, Idaho, United States of America, \(^12\)Department of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States of America

Abstract

Normal development of the genitourinary (GU) tract is a complex process that frequently goes awry. In male children the most frequent congenital GU anomalies are cryptorchidism (1–4%), hypospadias (1%) and micropenis (0.35%). Bladder extrophy and epispadias complex (BEEC) (1:47000) occurs less frequently but significantly impacts patients’ lives. Array comparative genomic hybridization (aCGH) identified seven individuals with overlapping deletions in the 2p15 region (66.0 kb–5.6 Mb). Six of these patients have GU defects, while the remaining patient has no GU defect. These deletions encompass the transcription factor OTX1. Subjects 2–7 had large de novo CNVs (2.39–6.31 Mb) and exhibited features similar to those associated with the 2p15p16.1 and 2p15p14 microdeletion syndromes, including developmental delay, short stature, and variable GU defects. Subject-1 with BEEC had the smallest deletion (66 kb), which deleted only one copy of OTX1. Otx1-null mice have seizures, prepubescent transient growth retardation and gonadal defects. Two subjects have short stature, two have seizures, and six have GU defects, mainly affecting the external genitalia. The presence of GU defects in six patients in our cohort and eight of the thirteen patients reported with deletions within 2p14p16.1 (two with deletion of OTX1) suggest that genes in 2p15 are important for GU development. Genitalia defects in these patients could result from the effect of OTX1 on pituitary hormone secretion or on the regulation of SHH signaling, which is crucial for development of the bladder and genitalia.

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Data Availability: The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. The data is from clinical genetic laboratories databases Signature Genomics and Baylor College of Medicine. Due to patient confidentiality the data is not publicly available but if more information is required you can contact Jill Rosenfeld (Signature Genomics) and Ankita Patel (Baylor College of Medicine).

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* Email: cj129804@bcm.edu (CJJ); dlamb@bcm.edu (DJL)

Introduction

The genitourinary (GU) tract is a multicomponent organ system in which mesenchymal-epithelial interactions play a critical developmental role [1]. Imbalance of this tightly regulated interaction can cause congenital birth defects. GU defects are among the most common male congenital anomalies and include cryptorchidism (1%–4%) [2], hypospadias (1%) [3,4], and micropenis (0.35% of full-term newborns) [4]. Vesioureteral reflux (VUR) is another common GU defect affecting 1% of newborns [5]. In contrast, bladder extrophy and epispadias complex (BEEC) occurs in 1:47,000 newborns [6,7]. It is a more severe urologic malformation characterized by abnormal invagination of the bladder through the abdominal wall and an abnormal urethral opening.

These GU defects can have long-term sequelae. Cryptorchidism is associated with infertility and testicular cancer [8–13]. Azospermia is reported in men with unilateral cryptorchidism (13%) and untreated bilateral cryptorchidism (89%) [8]. Orchidopexy performed before 2 years of age minimizes germ cell loss, but there is a significant difference in the ability and time required to father a child in men with bilateral cryptorchidism (65.3%).
This decline in fertility is not evident in men with unilateral cryptorchidism (89.7%), when compared to controls (93.7%) [14,15]. Nearly 5–10% of men who develop germ cell tumors have a history of cryptorchidism [11,13]. BEEC significantly affects patients' lives psychologically, socially, and sexually with patients reporting anxiety and low self-esteem due to abnormalities of the genitalia and erectile and orgasmic dysfunction [16,17]. In addition, some BEEC patients exhibit elevated FSH and spermatogenic failure [18]. While GU defects frequently occur as isolated defects, they present together with multiple GU defects, usually known as CAKUTs (congenital anomalies of the kidney and urinary tract), that occur in 1:500 live births [19]. Emerging evidence suggests that genetic and genomic changes can result in susceptibility to abnormal GU tract development [20].

Array-comparative-genomic-hybridization (aCGH) is a powerful genetic tool for detecting copy-number variants (CNVs), identifying new genetic syndromes, and correlating genotype and phenotype abnormalities more precisely than routine karyotyping. Two newly discovered syndromes involve CNV abnormalities within chromosome 2p. The first, chromosome 2p13p16.1 microdeletion syndrome, was reported in ten individuals with deletions ranging from chr2:55.48-63.33 Mb (UCSC-hg18 build of the human genome). These patients have common facial features and intellectual disability, and 70% of them present with GU defects. The GU defects include those involving the testis (4/4), external genitalia (2/10), and kidneys (4/10). The three individuals without GU defects were females [21–29]. The second syndrome, chromosome 2p14p15 microdeletion syndrome, was reported in three individuals with deletions ranging from chr2:61.97-65.98 Mb [30,31]. These patients have mild intellectual disability; one has cryptorchidism.

Located between the two microdeletion syndromes' critical intervals and deleted in three patients identified in clinical databases is the orthodenticle-homolog-1 (OTX1) gene (63.13–65.14 Mb). OTX1 is a transcription factor with important roles in controlling specification, maintenance, and regionalization of the vertebrate brain. Otx1 null mice suffer from spontaneous epilepsy with focial, as well as generalized seizures [32]. Also, they display a transient decrease in gonadal size with profound architectural changes [33].

This study identified six subjects with GU defects harboring a deletion in 2p15 who share an interval of minimal overlap corresponding with the coding regions of EHBPI, OTX1, and WDPIC in all but one subject, whose deletion only involved OTX1. The presence of these defects suggests that this region has a role in GU development (Fig. 1).

Materials and Methods

Selection of Study Subjects

Three different protocols were used. Two were approved and overseen by the Institutional Review Board of Baylor College of Medicine (BCM) and the other by the Institutional Review Board-Spokane at Signature Genomics (SG). The first protocol from BCM included a cohort of 30 BEEC probands and 83 controls analyzed using a research aCGH from NimbleGen containing 720,000 probes from 2008–2012. The second protocol from BCM covers 18,734 probands tested by clinical aCGH at BCM Medical Genetic Laboratories from 2008–2012. The SG protocol included 30,183 probands tested by clinical aCGH from 2007–2010. The probands referred for clinical aCGH display a range of clinical conditions. Probands were recruited independent of race, ethnicity, and age. Approved informed consents were obtained from the parents or legal guardians. Blood was collected from subjects and controls with normal genitourinary development. Blood or saliva was collected from the subjects' immediate family when available. DNA was extracted using the Qiagen Puregene DNA extraction kit (Valencia, CA) according to the manufacturer's protocol.

Clinical Reports

Subject-1 (63.13 Mb-63.20 Mb): A 10-year-old male was the first child of healthy, non-consanguineous parents. Family history showed no significant problems. He was delivered vaginally at 40 weeks following a pregnancy complicated by maternal hypertension. At birth, the patient had BEEC and underwent bladder exstrophy repair followed by epispadias repair. He later developed a urethral cutaneous fistula and bilateral VUR. He underwent bladder neck closure, urethral-cutaneous fistula repair, Mitrofanoff creation, and bilateral ureteral re-implant. The urologist’s report indicated that the patient’s development appeared grossly normal without dysmorphic features. A formal examination was not performed by a clinical geneticist.

Subject-2 (62.82 Mb-67.37 Mb) was a 4-year-old male born via vaginal delivery at term to a 36-year-old G7P2 mother. The mother took progesterone for the first 11 weeks of pregnancy. Birth weight was 3.28 kg (25th percentile) and length was 53.3 cm (75th–90th percentile). He required supplemental oxygen for several minutes after birth. Genital anomalies included unilateral right cryptorchidism (surgically repaired) and congenital absence of the foreskin. At age 2, he had an adenoïdectomy and ear tube placement. He is developmentally delayed and has speech apraxia. At age 4, he uses 3–4 word phrases, and his receptive language is better than his expressive language. He has delayed visual maturation and a history of mild hypotonia. Dysmorphic features include metopic ridging, hooded eyelids, a prominent nose, a thin upper lip, a slightly protruding tongue, and slightly protruding ears. He also has hirsutism on his back and a single pigmented macule on his penis.

Subject-3 (60.98 Mb-63.37 Mb) is an 11-year-old male born at 37 weeks via Cesarean section to a 20-year-old G1P0 mother following a pregnancy complicated by placenta previa (which resolved) and maternal hypertension beginning at 34 weeks of gestation. Birth weight was 2.56 kg and length (47 cm) were between the 3rd and 10th percentiles. Duodenal web and malrotation were diagnosed neonatally and surgically repaired. He had slow growth during the neonatal period. Endocrine studies were normal. Genital anomalies included a hypoplastic scrotum, unilateral right cryptorchidism (testes were each 1cc), discontinuous prominent raphe, micropenis (phallus measuring 1.8 cm at 4 months, <3rd percentile for his age), and a 1 cm cyst on the penis. Orchidopexy was performed. He had kidney stones. He required a cholecystectomy at age 4. He had moderate to severe developmental delays, intellectual disabilities, vision problems, ptosis, recurrent ear infections, febrile seizures, chronic nosebleeds, mixed hypo/hypertonia, and GI motility problems resulting in vomiting, diarrhea, and constipation. Dysmorphic features include a flattened occiput, persistence of hair on the lateral forehead, metopic ridging, prominent superior orbital ridges, telecanthus, reverse epicanthal folds, long eyelashes, downsloping palpebral fissures, hypoplastic alae nasi with bulbous tip, a narrow palate, a prominent upper lip, micrognathia, and prominent ears with underdeveloped helices. He has pes planus, mild finger tapering, tightness of the knees, hypoplastic pectoralis major, and hypotrophic lower leg muscles. His growth measurements at 9 years, 5 months were height 115.5 cm (3rd percentile), weight 19.3 kg (3rd percentile), and OFC 47.6 cm (-2 SD). At age 10, he started having seizures, which are controlled with medications. At age 11,
he attended special education and regular classes without behavioral problems.

Subject-4 (59.92 Mb-66.23 Mb) is a 21-month-old male delivered vaginally at 39\+6 weeks to a 24-year-old G4P2SAb1 mother with a history of uterine prolapse. Prenatal ultrasound was remarkable for “stomach debris”, which resolved on subsequent ultrasounds. His birth weight was 2.98 kg (25th–50th percentile) and length 47.3 cm (50th percentile). At birth he had hypotonia and dysmorphic features including a very small anterior fontanelle with ridged sutures, a beaked prominent nose with short columella, micropenis with bilateral testes palpable in a small scrotum, and rocker-bottom feet. His karyotype was normal. He had small, downslanting palpebral fissures with epicanthal folds, sparse eyebrows, and bilateral elbow dimples. He had microcephaly and poor growth. His brain MRI revealed diffuse cerebral atrophy, prominent ventricles suggestive of colpocephyaly, and an enlarged cisterna magna. Further workup revealed mesocardia and right-sided cross-fused renal ectopia. At 17 months he had motor skills in the 6–8 month range and language/social skills in the 9–10 month range. His failure to thrive and global developmental delay were complicated by frequent upper respiratory infections and gastroesophageal reflux.

Subject-5 (60.91 Mb–65.51 Mb) is a 16-year-old male born at term with a birth weight of 3.01 kg (10th–25th percentile). At birth microcephaly was present and his face was asymmetrical. He had small, downslanting palpebral fissures with epicanthal folds, sparse eyebrows, and bilateral elbow dimples. He had microcephaly and poor growth. His brain MRI revealed diffuse cerebral atrophy, prominent ventricles suggestive of colpocephyaly, and an enlarged cisterna magna. Further workup revealed mesocardia and right-sided cross-fused renal ectopia. At 17 months he had motor skills in the 6–8 month range and language/social skills in the 9–10 month range. His failure to thrive and global developmental delay were complicated by frequent upper respiratory infections and gastroesophageal reflux.

Subject-6 (61.42 Mb–64.17 Mb) is a 14-year-old male born after induction at 38 weeks. Pregnancy was complicated by severe nausea gravidarum. His birth weight was 2.84 kg (10th–25th percentile) with a length of 49.5 cm (50th percentile). Apgar scores were 7 and 8 at 1 and 5 minutes, respectively. In the first week he had jaundice that spontaneously resolved. He was referred to a pediatric endocrinology clinic at age 14 to evaluate short stature (height 146 cm, (3rd percentile) and weight 31.9 kg (1st percentile)). He had pervasive developmental and speech delay. He has a history of chronic and significant sinusitis and generalized hypoponia. Dysmorphic features include a small bitemporal diameter, dolichocephaly with a prominent forehead, epicanthal folds, long eyelashes, hypertelorism, right otosclerosis, and a very high-arched mouth palate. He has some degree of clinodactyly. The chest wall is asymmetric. He has small testes (5 ml), but his penile length and girth are considerably larger than expected with his current development. He has a history of leukopenia and possible cyclic neutropenia. He has had significant infections, especially involving his teeth over the years. He had 2...
ears (custom-designed, exon targeted (probands were confirmed by fluorescence in situ 105K, whole-genome, oligonucleotide-based array (SignatureChipOS described methods [36]. DNA from subject-3 was analyzed using a 135K, whole genome, oligonucleotide-based array (SignatureChipOS v2.0 [subjects 2 & 5] or v3.0 [subject 4], custom-designed by whole-genome, oligonucleotide-based array (SignatureChipOS) were used as a control. Deletions in the probands were confirmed by fluorescence in situ hybridization (FISH) with BAC clones from deleted regions using previously published methods [38]. Long-range PCR was performed using TaKaRa-LA-Taq (TAKARA-Bio). A 25 μl PCR reaction was performed using 1.25U TaKaRa enzyme, 0.4 mM dNTP, 0.2 μM of primer forward (ccttgacttgccctcacact) and reverse (gcctaatcccctttgcctta), 1.25U TaKaRa enzyme, 0.4 mM dNTP, 0.2 μM of primer forward (ccttgacttgccctcacact) and reverse (gcctaatcccctttgcctta), 0.25M of primer 

Subject-7 (62.74 Mb–65.76 Mb) is a 4-year-old male born at term to a 24-year-old G2P1-2 mother. His birth weight was 3.29 kg (25th percentile). At 30 months the child had developmental delay and dysmorphic features. He rolled at 8–9 months, sat at 12 months, walked at 18 months, and spoke at 26 months. His growth has been normal, with weight in the 90th percentile. Facial features include right ptosis, short palpebral fissures (<5th percentile), large ears (>95th percentile), a long nose, a smooth and somewhat long philtrum, and a thin upper lip. He also has a left Sydney crease, mild right third and fourth finger camptodactyly, pes planus, prominent heels, and bilateral esotropia. Behavior is abnormal with significant hyperactivity. At 30 months the left testicle was nonpalpable in the scrotum or inguinal canal and the right testicular volume seemed small for age. At age 4 he is essentially nonverbal.

DNA from subjects-2, -4, and -5 was analyzed using a 135K, whole-genome, oligonucleotide-based array (SignatureChipOS v2.0 [subjects 2 & 5] or v3.0 [subject 4], custom-designed by whole-genome, oligonucleotide-based array (SignatureChipOS) were used as a control. Deletions in the probands were confirmed by fluorescence in situ hybridization (FISH) with BAC clones from deleted regions using previously published methods [38]. Long-range PCR was performed using TaKaRa-LA-Taq (TAKARA-Bio). A 25 μl PCR reaction was performed using 1.25U TaKaRa enzyme, 0.4 mM dNTP, 0.2 μM of primer forward (ccttgacttgccctcacact) and reverse (gcctaatcccctttgcctta), 0.25M of primer 

Long-range PCR Amplification

Long-range PCR was performed using TaKaRa-LA-Taq (TAKARA-Bio). A 25 μl PCR reaction was performed using 1.25U TaKaRa enzyme, 0.4 mM dNTP, 0.2 μM of primer forward (ccttgacttgccctcacact) and reverse (gcctaatcccctttgcctta), 1 M betaine and 250 ng of DNA template. The PCR conditions were as follows: 98°C (30 s); then 32 cycles of 94°C (20 s), 52°C (20 s) and 68°C (20 s); finally 68°C (10 min). One fraction of the amplification product was electrophoresed on a 1% agarose gel. The other fraction was purified using the Exo-SAP-IT kit (USB Scientific, Cleveland, OH). Purified products were sequenced using Sanger sequencing combined with ABI 3730x DNA analyzers for capillary electrophoresis and fluorescent dye terminator detection (Genewiz). Data were analyzed using bioinformatics databases (http://blast.ncbi.nlm.nih.gov and http://genome.ucsc.edu).

Results

Identification of CNVs in 2p15 in Subjects with GU defects

We identified seven subjects with deletions of the 2p15 region ranging from 66 kb to 6.3 Mb in size (Fig. 1). Four subjects’ deletions extend distally, at least partially overlapping the proposed critical region for the 2p15p16.1 microdeletion syndrome, and five subjects’ deletions extend proximally into 2p14. Three subjects’ deletions extend in both directions and only subject-1 (smallest deletion) lacks these other regions. Subject-1 was identified in a cohort of 30 BEEC patients using NimbleGen 3×720 aCGH (Fig. 2A). His deletion was validated using a custom aCGH from Agilent (Fig. 2B) and qPCR CNV-Taqman assays (Fig. 2C). None of the remaining 29 BEEC subjects or the 85 controls without GU defects have CNVs in OTXI. Subjects 2–5 were identified among 30,183 probands tested by aCGH at SG (Fig. 3A and data not shown) and validated by FISH (Fig. 3C–D and data not shown). Two additional subjects with OTXI deletions...
were identified at SG, but their clinical information was unavailable for publication. FISH testing on the parents of subjects 3–5 showed the children's deletions to be de novo. Subjects 6–7 were detected from 18,734 probands tested by aCGH at BCM Medical Genetic Laboratories (Fig. 3B and data not shown) and validated by FISH (data not shown). Parental testing for subjects 6–7 showed the children’s deletions to be de novo. The common region deleted in these subjects included only OTX1 at 2p15 (Fig. 1). No additional CNVs of known or suspected clinical significance were detected in these subjects. Subject-7 had three CNVs: a 2p13 microdeletion and two small microduplications. The two microduplications were maternally inherited and of unclear significance, one at 17q24.2 (62550305-62819345 encompassing the HELZ, PSMD12 and PITPN1 genes) and another at Xp22.31 (7980296-8075153) that involves only MIR651.

Among these individuals, clinical features varied with some phenotypic overlap with the currently described 2p15p16.1 and 2p14p13 microdeletion syndromes. However, all subjects with the exception of subject-5 are reported to have GU defects (Table 1) that mainly involve the genitalia (testes (4/7), penis (3/7) and scrotum (2/7)). Five subjects had multiple GU defects (Table 1).

Analysis of Exon Sequence and Deletion Breakpoints

To assess whether OTX1 was the only gene deleted in subject-1, long-range PCR was used to define the breakpoints of the deletion. Subject-1’s deletion was located at chr2:63,130,672–63,196,654 (data not shown), a region that only encompasses OTX1. There are two OTX1 variants that encode the same protein but differ in the 5’UTR, NM_014562.3 (chr2:63,131,441–130,696–63,138,470) and NM_001199770.1 (chr2:63,130,696–63,138,470), and both variants are deleted. The two genes flanking OTX1, EH domain binding protein 1 (EHBP1) (chr2:62,754,749–690,125,125) and WD repeat containing planar cell polarity effector (WDPCP) (chr2:63,202,039–63,669,371), were not included in the deleted region. Long range PCR analysis of the mother’s DNA did not show the deletion and the father’s DNA was unavailable for testing; consequently, inheritance could not be established.

In addition, we performed Sanger sequencing of OTX1 on subject-1 as well as 29 BEEC subjects. Seven subjects had a synonymous SNP p.Leu264Leu (rs17850223). No additional SNPs were present in the coding region of OTX1.

Discussion

During the past several years many microdeletion syndromes have been identified using microarray methods [40]. This is especially true for subjects with intellectual disability who commonly have other abnormalities, such as GU defects. We identified seven male subjects, six with GU defects involving the genitalia, who share a commonly deleted region in 2p15 involving only OTX1 (Fig. 1). The 2p15p16.1 microdeletion syndrome was described in ten subjects focusing mainly on their neurodevelopmental phenotypes without emphasizing that seven of them also have identifiable GU defects, predominantly affecting the testes and kidneys. The first report of the 2p15p16.1 microdeletion was in two individuals: a boy with small testes and penis, hydrenephrosis, and VUR and a girl with a multicystic kidney and hydronephrosis [28]. To date, three of six female patients reported with 2p15p16.1 microdeletions (not including OTX1) have GU defects, one presenting with hypogonadism and two with hydronephrosis [22,24,26-28]. All four male patients with 2p15p16.1 microdeletions (only one of which includes OTX1) have GU defects including testicular (100%), kidney (50%), and penile (25%) defects [21,23,28,29] (Fig. 1). Four genes are present in the minimally deleted region of these patients. AHSA2 and SNR70B have unknown functions. The other two, USP34 and XPO1, are better characterized, but no role in GU development is attributed to them. USP34 functions downstream of the β-catenin complex to control the stability of axin as well as enhance NF-kB activation [41,42]. XPO1 is over-expressed 2–4 fold in cancer [43]. More recently, a second 2p14p15 microdeletion syndrome was described in three subjects. The first description is of a boy and a girl with mild intellectual disability and no GU defects with OTX1 deleted only in the girl [30]. In an additional case, OTX1 is deleted in a boy with intellectual disability and cryptorchidism [31]. A small deletion in subject-1 (encompassing only OTX1) and the minimal overlapping region between subjects-2 and -3 (which includes EHB1, OTX1 and WDPCP), allows us to suggest that OTX1 is involved in normal genitourinary development.

We cannot exclude the possibility that the two additional genes deleted in the minimal region of subjects 2–7, EHB1 and WDPCP, could also be implicated in the diverse GU phenotypic defects observed. This is particularly true for WDPCP since mutations may be associated with Bardet-Biedl syndrome 15 (BBS15) [44]. BBS15 is characterized by rod-cone dystrophy, truncaal obesity, postaxial polydactyly, cognitive impairment, hypogonadism, cryptorchidism, micropenis, and renal abnormalities in which renal disease is a major cause of morbidity and mortality. On the other hand, EHB1 is a putative genetic susceptibility loci for prostate cancer with no association with GU defects [45]. In our cohort subjects 2–7 exhibit larger deletions involving additional genes that may contribute to their GU and other phenotypic anomalies.

OTX1 and OTX2 are transcription factors with important roles in controlling specification, maintenance, and organ regionalization. The Gudmap database indicates that both OTX1 and OTX2 are expressed in murine urogenital ridge, testis, and ovary [46,47]. In the prepubescent stage, Otx1-deficient mice have seizures along with growth retardation and gonadal defects attributed to low levels of pituitary hormones (growth hormone, FSH, and LH), which dramatically affect ovary and testis size and architecture. Nevertheless, Otx1’s role in modulation of pituitary hormones is transient, and four-month-old mice show normal hormonal levels and gonadal size [33]. Otx2 null embryos die embryonically because of major body abnormalities, including absence of the neuroectoderm [48]. However, Otx2 heterozygous male mice display compromised fertility (reduced LH levels and testicular weight) due to a defect in the development, number, and migration of GnRH neurons [49].

OTXs are important in cell fate differentiation, and a specific threshold of OTX proteins is required for proper SHH signaling.
Table 1. Comparison of the clinical features of male patients with deletion in 2p14-p16.1 that encompasses OTX1.

| Subjects | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Rajcan et al. | Hancarova et al. |
|----------|---|---|---|---|---|---|---|---------------|------------------|
| Size of Deletion (Mb) | 0.07 | 5.05 | 2.39 | 6.31 | 4.59 | 2.75 | 3.02 | 7.89 | 3.72 |
| Chromosome Localization | 2p15 | 2p15p14 | 2p16.1p15 | 2p16.1p14 | 2p16.1p14 | 2p15p14 | 2p16.1p15 | 2p15p14 |
| Genomic Region (Mb, hg18) | 63.13–63.20 | 62.82–67.87 | 60.98–63.37 | 59.92–66.23 | 60.91–65.51 | 61.42–64.17 | 62.74–65.76 | 55.33–63.23 | 62.01–65.73 |
| General | | | | | | | | | |
| Age at Evaluation (years) | 10 | 4 | 9 | 1.75 | 16 | 14 | 4 | 7 | 4 |
| Sex | Male | Male | Male | Male | Male | Male | Male | Male | Male |
| Developmental Delay | - | + | + | + | + | + | + | + |
| Seizures | - | - | + | - | + | - | - | - |
| Feeding Problems | - | - | + | + | - | - | - | + |
| Vision Problems | - | + | + | - | - | + | + | + |
| Recurrent Ear Infections | - | + | + | - | - | + | - | - |
| Short Statue | - | - | + | - | - | + | - | - |
| Microcephaly | - | - | + | - | + | - | + | + |
| Hypotonia | - | + | + | - | - | + | - | + |
| Urological Features | | | | | | | | | |
| Bladder Exstrophy | + | - | - | - | - | - | - | - |
| Epispadias | + | - | - | - | - | - | - | - |
| VUR | + | - | - | - | - | - | - | - |
| Cryptorchidism | - | + | + | - | - | + | - | + |
| Small Testes | - | - | + | - | - | + | - | + |
| Absent Foreskin | - | + | - | - | - | - | - | - |
| Scrotal anomalies | - | - | + | - | - | - | - | - |
| Micropenis | - | - | + | - | - | - | - | - |
| Kidney Abnormalities | - | - | + | - | - | - | - | - |
| Facial Features | | | | | | | | | |
| Flattened Occiput | NM | - | + | - | + | - | NM | + | + |
| Metopic Ridging | NM | + | + | NM | NM | - | NM | + | - |
| Ptosis | NM | - | + | - | - | + | + | - |
| Slanted Palpebral Fissures | NM | - | + | + | NM | - | + | + |
| Epicantinal Folds | NM | + | + | NM | NM | + | NM | + | - |
| Prominent Nose | NM | + | + | + | + | - | + | + |
| Long, Straight Eyelashes | NM | - | + | NM | NM | + | NM | + | - |
| Large Ears | NM | + | + | NM | - | - | + | + |
| Thin Upper Lip | NM | + | - | - | - | + | - | + |
| Other | | | | | | | | | |

2p15 Deletions and Genitourinary Defects
The SHH signaling pathway coordinates the formation of the bladder, internal urethra, and genitalia [51]. Heterozygous Otx1 mice are not fully characterized, but correct dosage of Otx2 is critical for normal fertility and testis size. Otx1 and Otx2 have functional similarity and interchangeable roles [52]. OTX2 could compensate for OTX1 deficiency in levels that vary among subjects. Since OTX1 haploinsufficiency could have a direct effect on the SHH signaling pathway, which is crucial for development of the bladder and genitalia, this may explain the range of GU defects seen in our patients and the bladder phenotype present in subject-1. Six of our subjects and two previously reported cases have genital defects including cryptorchidism, hypogonadism, micropenis, epispadias, and foreskin and scrotal anomalies. There is no mention of abnormal testicular descent in Otx1-null mice, but in our experience, unless the testes are beside the kidneys (abdominal cryptorchidism), an abnormal testis position is often overlooked and lesser degrees of cryptorchidism just above the inguinal ring are not reported.

Regardless, we cannot exclude the possibility that the hypogonadism and cryptorchidism present in some subjects may be secondary to a pituitary defect similar to that occurring in the Otx1 null mice [33]. Fetal defects in the pituitary-Leydig cell axis are associated with cryptorchidism [53]. The hypothalamic-pituitary axis regulates testicular hormone secretion in the second half of fetal life and FSH controls the Sertoli cell proliferation responsible for testis volume increase upon the onset of spermatogenesis. LH regulates the Leydig cell androgen and INSL3 secretion required for testis growth and descent [54]. Hypogonadism resulting from OTX1 haploinsufficiency could lead to micropenis and cryptorchidism in these patients. In addition, Otx1 null mice suffer from generalized seizures and growth retardation [32]. In concordance with these mouse findings, two of our subjects, as well as two additional cases reported in the literature, have seizures and two have short stature (although this did not correlate with age in our cohort).

One caveat of our study is that two of the seven pregnancies were complicated by maternal hypertension (Subject-1 and -3) and another by progesterone supplementation for the first 11 weeks of pregnancy (Subject-2). These additional conditions/medical interventions may have impacted normal GU development. In addition to genetic factors, placental insufficiency, low birth weight, and twinning are implicated as contributing to the etiology of cryptorchidism [55,56]. The impact of preeclampsia, in vitro fertilization, and exposure to endocrine disrupters on normal GU development is controversial and the results vary between studies [55,56]. With respect to BEEC, preconception or first trimester exposure to alcohol, environmental toxins, or maternal disease are not associated with the anomaly [57]. A direct relationship between maternal hypertension and progesterone intake is not obvious in boys born with cryptorchidism or BEEC; accordingly, OTX1 microdeletion remains a strong candidate genomic condition associated with the GU defect observed in these patients.

The deleted region shared by our subjects is between the minimum deletion region of the 2p15p16.1 [28] and 2p14p15 microdeletion syndromes [31]. The presence of GU defects in 86% of our cohort, in 70% of subjects with 2p15p16.1 deletions, and in 33% of the subjects with 2p14p15 deletions suggests that this region contains genes important in GU development. Our results may associate deletions of OTX1 with GU anomalies, possibly through alterations of the SHH signaling pathway. Larger deletions in many of these subjects (including multiple genes) result in additional features, such as developmental delay, intellectual disability, and dysmorphic features. Of note, OTX1 may not be the only gene involved in GU development in this region since

| Subjects | Nipple Abnormalities | Pes Planus | Attention Deficit Behavior | VUR = vesicoureteral reflux; NM = not mentioned. | doi:10.1371/journal.pone.0107028.t001 |
|----------|----------------------|------------|---------------------------|-------------------------------------------------|-------------------------------------|
| 1        | NM                   | NM         | NM                        | VUR = vesicoureteral reflux; NM = not mentioned. |                                     |
| 2        |                      |            |                           |                                                 |                                     |
| 3        |                      | +          |                           |                                                 |                                     |
| 4        |                      |            |                           |                                                 |                                     |
| 5        |                      |            |                           |                                                 |                                     |
| 6        |                      |            |                           |                                                 |                                     |
| 7        |                      |            |                           |                                                 |                                     |

Table 1. Cont.

[50].
three patients with 2p15p16.1 deletions that do not include OTX1 also display GU defects. OTX1 deletion could affect neighboring genes, in particular the gene WDFP1 in which mutations were associated with hypogonadism, cryptorchidism, micropenis, and renal abnormalities [44]. OTX1 microdeletions are rare in population surveys tested by aCGH, varying from 0.010–0.019% in BCM and SG clinical laboratories, respectively. In addition, the B5 normal controls tested in our laboratory and the 8329 controls analyzed by Cooper et al. [58] did not exhibit CNVs in OTX1. The ISCA database includes three individuals with CNVs containing OTX1, but only one individual has a microdeletion (5.3 Mb). Decipher has four subjects with microdeletions ranging from 0.51–3.21 Mb. Two of them are boys with no phenotype recorded and two are girls with no GU defects reported. In addition, Decipher lists seven subjects with microduplications. Four have large duplications (15.32–89.01 Mb) and no reported GU defects. The other three have small duplications (0.72–4.66 Mb) including a female (0.72 Mb) with an enlarged kidney, a male with cryptorchidism (4.66 Mb), and a third male with no reported phenotype. The absence of controls with CNVs in OTX1 and the phenotypes present in patients with microdeletions suggests the importance of proper dosage of OTX1 and 2p15 genes in GU tract development. Understanding the molecular mechanisms behind the pathogenesis of GU defects is important for genetic counseling and the implementation of therapeutic interventions. The present study suggests that chromosomal region 2p15 and OTX1 are involved in GU tract development, but further detailed studies are needed to identify a causal relationship.

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Author Contributions
Conceived and designed the experiments: CJJ DJL. Performed the experiments: CJJ NRV HVV AS CMBC AB AP AM DDW BB DB JSB TW DF ER SWC JRL DJL. Contributed reagents/materials/analysis tools: CJI JAR NRW DP DMW DB DB JSB TW DF ER SWC JRL DJL. Contributed to the writing of the manuscript: CJI. JAR NRW HVV AS CMBC AB AP AM DDW BB DB JSB TW DF ER SWC JRL DJL.

References
1. Chunha GR, Alarid ET, Turner T, Donjacour AA, Bousin EL, et al. (1992) Normal and abnormal development of the male urogenital tract. Role of androgens, mesenchymal-epithelial interactions, and growth factors. J Androl 13: 465–473.
2. Sijstermans K, Hack WW, Meijer RW, van der Voort-Doedens LM (2008) The frequency of undescended testis from birth to adulthood: a review. Int J Androl 31: 1–11.
3. Pauluzzi LJ, Erickson JD, Jackson RJ (1997) Hypoacusis trends in two US surveillance systems. Pediatrics 100: 831–834.
4. Guaspari L, Paris F, Jaeli C, Kafiti N, Orsini M, et al. (2011) Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: a nested case-control study. Hum Reprod 26: 3155–3162.
5. Williams G, Fletcher JT, Alexander SL, Craig JC (2008) Vesicoureteral reflux. J Am Soc Nephrol 19: 847–862.
6. Caton AR, Bloom A, Druschel CM, Kirby RS (2007) Epidemiology of bladder and cloacal exstrophies in New York State, 1983–1999. Birth Defects Res A Clin Mol Teratol 79: 781–787.
7. Nelson CP, Dunn RL, Wei JT (2005) Contemporary epidemiology of bladder exstrophy in the United States. J Urol 173: 1728–1731.
8. Hadziselimovic F, Herzog B (2001) The importance of both an early psychosexual development in childhood and adolescence within the exstrophy-epispadias complex. J Urol 165: 218–222.
9. Lipshultz LI, Caminos-Torres R, Greenspan CS, Snyder PJ (1976) Testicular atrophy following orchidopexy and germ cell maturation for fertility. Lancet 312: 1156–1157.
10. Pike MC, Chilvers C, Peckham MJ (1986) Effect of age at orchidopexy on risk of testicular cancer. Lancet 1: 1246–1248.
11. Kolon TF, Anthony Herron CD, Baker LA, Baskin LS, Baxter CG, et al. (2014) Evaluation and Treatment of Cryptorchidism: AUA Guideline. J Urol 192: 155–165.
12. Rajcan-Separovic E, Harvard C, Liu X, McGillivray B, Hall JG, et al. (2007) Interstitial cell tumor. Int J Urol 11: 640–646.
13. Miller KD, Coughlin MT, Lee PA (2001) Fertility after unilateral cryptorchidism and subsequent fertility after orchidopexy. Lancet 358: 1156–1157.
14. Johnson DE, Woodhead DM, Pohl DR, Robison JR (1968) Cryptorchism and testicular tumorigenesis. Surgery 63: 919–922.
15. Chabchoub E, Vermeesch JR, de Ravel T, de Cock P, Fryns JP (2008) The facial dysmorphism in the newly recognised microdeletion syndrome: molecular characterization and association of the OTX1 and XPO1 genes with autism spectrum disorders. Eur J Hum Genet 16: 1240–1247.
16. Hancarova M, Vejvalkova S, Trkova M, Drabova J, Dleskova A, et al. (2013) Clinical and molecular characterization of two patients with overlapping 2p15p16.1 deletion. J Med Genet 50: 1264–1270.
17. Piccione M, Pio E, Serraino F, Cavani S, Ciccone R, et al. (2012) Interstitial deletion of chromosome 2p15-16.1: report of two patients and critical review of current genotype-phenotype correlation. Eur J Med Genet 55: 230–234.
18. Pinto D, Bernardo I, Grau M, Capalbo A, Rogaia D, et al. (2011) Deletion 2p15-16.1 syndrome: case report and review. Am J Med Genet A 155A: 2473–2478.
19. Liu X, Maleplant P, Rescor C, Lee A, Hudson ML, et al. (2011) 2p15p16.1 microdeletion syndrome: molecular characterization and association of the OTX1 and XPO1 genes with autism spectrum disorders. Eur J Hum Genet 19: 1264–1270.
20. Lipshultz LI, Caminos-Torres R, Greenspan CS, Snyder PJ, et al. (1976) Testicular atrophy following orchidopexy and germ cell maturation for fertility. Lancet 312: 1156–1157.
21. Acampora D, Mazan S, Tuorto F, Avantaggiato V, Tremblay JJ, et al. (1996) New type of microdeletion syndrome involving 2p15-16.1. J Med Genet 33: 1–11.
22. Rajcan-Separovic E, Harvard C, Liu X, McGillivray B, Hall JG, et al. (2007) Interstitial cell tumor. Int J Urol 11: 640–646.
23. Prontera P, Bernardini L, Stangoni F, Capalbo A, Rogaia D, et al. (2011) Deletion 2p15-16.1 syndrome: report of two patients and critical review of current genotype-phenotype correlation. Eur J Med Genet 55: 230–234.
24. Rajcan-Separovic E, Harvard C, Liu X, McGillivray B, Hall JG, et al. (2007) Interstitial cell tumor. Int J Urol 11: 640–646.
34. Jorgez CJ, Weedin JW, Sahin A, Tannour-Louet M, Han S, et al. (2011) Aberrations in Pseudoautosomal Regions (PARs) Found in Infertile Men with Y-Chromosome Microdeletions. J Clin Endocrinol Metab 96: 674–679.
35. Carvalho CM, Zhang F, Liu P, Patel A, Sahoo T, et al. (2009) Complex rearrangements in patients with duplications of MEC2 can occur by fork stalling and template switching. Hum Mol Genet 18: 2208–2201.
36. Duker AL, Ballif BC, Bawle EV, Person RE, Mahadevan S, et al. (2010) Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. Eur J Hum Genet 18: 1196–1201.
37. Ballif BC, Thireis A, McDonald-McGinn DM, Zackai EH, Hersh JH, et al. (2008) Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. Clin Genet 74: 469–475.
38. Traylor RN, Fan Z, Hudson B, Rosenfeld JA, Shaffer LG, et al. (2009) Microdeletion of 6q16.1 encompassing EPHA7 in a child with mild neurological abnormalities and dysmorphic features: case report. Mol Cytogenet 2: 17.
39. Boone PM, Bacino CA, Shaw CA, Eng PA, Hinson PM, et al. (2010) Detection of clinically relevant exonic copy-number changes by array CGH. Hum Mutat 31: 1326–1342.
40. Lupski JR (2009) Genomic disorders ten years on. Genome Med 1: 42.
41. Poalas K, Hatchi EM, Cordeiro N, Dubois SM, Leclair HM, et al. (2013) Negative regulation of NF-kappaB signaling in T lymphocytes by the ubiquitin-specific protease USP34. Cell Commun Signal 11: 25.
42. Lui TT, Lacroix CA, Shaw CA, Eng PA, Hinson PM, et al. (2010) Identification of a previously unrecognized microdeletion syndrome of 15q11.2q12.2. Clin Genet 74: 469–475.
43. Traylor RN, Fan Z, Hudson B, Rosenfeld JA, Shaffer LG, et al. (2008) Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. Clin Genet 74: 469–475.
44. Etchin J, Sanda T, Mansour MR, Kentsis A, Montero J, et al. (2013) KPT-330 inhibitor of CRM1 (XPO1)-mediated nuclear export has selective anti-leukaemic activity in preclinical models of T-cell acute lymphoblastic leukaemia and acute myeloid leukaemia. Br J Haematol 161: 117–127.
45. Kim SK, Shindo A, Park TJ, Ob EC, Ghoosh S, et al. (2010) Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. Science 329: 1337–1340.
46. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, et al. (2011) A copy number variation morbidity map of developmental delay. Nat Genet 43: 838–846.
