Journal of Pediatric Urology (2007) 3, 2—9

ELSEVIER

Journal of Pediatric urology

Mutational analyses of *UPIIIA*, *SHH*, *EFNB2*, and *HNF1β* in persistent cloaca and associated kidney malformations

Dagan Jenkins [a], Maria Bitner-Glindzicz [a], Louise Thomasson [a], Sue Malcolm [a], Stephanie A. Warne [a], Sally A. Feather [b], Sarah E. Flanagan [c], Sian Ellard [c], Coralie Bingham [c], Lane Santos [d], Mark Henkemeyer [d], Andrew Zinn [d], Linda A. Baker [d], Duncan T. Wilcox [d], Adrian S. Woolf [a,*]

[a] *Nephro-Urology Unit, Institute of Child Health, University College London, 30 Guilford Street, London WCIN IEH, UK*
[b] *St James' University Hospital, Leeds, UK*
[c] *Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, UK*
[d] *Univesity of Texas Southwestern Medical Center at Dallas, TX, USA*

Received 6 October 2005; accepted 1 March 2006
Available online 5 May 2006

KEYWORDS
Bladder;
Gene;
Kidney;
Mutation;
Rectum;
Uterus

Abstract *Objectives:* 'Persistent cloaca' is a severe malformation affecting females in which the urinary, genital and alimentary tracts share a single conduit. Previously, a *Uroplakin IIIA* (*UPIIIA*) mutation was reported in one individual with persistent cloaca, and UPIIIA, Sonic Hedgehog (SHH), Ephrin B2 (EFNB2) and Hepatocyte Nuclear Factor 1β (HNF1β) are expressed during the normal development of organs that are affected in this condition. *HNF1β* mutations have been associated with uterine malformations in humans, and mutations of genes homologous to human *SHH* or *EFNB2* cause persistent cloaca in mice.
Patients and methods: We sought mutations of coding regions of *UPIIIA*, *SHH*, *EFNB2* and *HNF1β* genes by direct sequencing in a group of 20 patients with persistent cloaca. Most had associated malformations of the upper renal tract and over half had impaired renal excretory function. The majority of patients had congenital anomalies outside the renal/genital tracts and two had the VACTERL association.
Results: Apart from a previously described index case, we failed to find *UPIIIA* mutations, and no patient had a *SHH*, *EFNB2* or *HNF1β* mutation.

* Corresponding author. Tel.: +44 20 7905 2615; fax: +44 20 7905 2133.
E-mail address: a.woolf@ich.ucl.ac.uk (A.S. Woolf).

doi:10.1016/j.jpurol.2006.03.002

Conclusion: Persistent cloaca is only rarely associated with *UPIIIA* mutation. Despite the fact that *SHH* and *EFNB2* are appealing candidate genes, based on their expression patterns and mutant mice phenotypes, they were not mutated in these humans with persistent cloaca. Although *HNF1β* mutations can perturb paramesonephric duct fusion in humans, *HNF1β* was not mutated in persistent cloaca.

Introduction

A 'cloaca' is a single conduit which links the urinary, genital and alimentary tracts with the exterior of an organism. In the adult duck-billed platypus and echidna, both monotremes, a cloaca is the norm [1], whereas in all other adult mammals, each of these tracts exits the body through a separate opening. About 1:50 000 human births have a 'persistent cloaca' [2]. Persistent cloaca is a potentially devastating disease, generally requiring multiple rounds of corrective surgery and, even with the best current treatments, there can be significant urological and gynaecological sequelae, including incontinence and infertility [3–5]. Upper renal tract malformations are common associations. For example, of 64 patients with persistent cloaca, Warne et al. [6] reported VUR in 36, renal dysplasia in 17, ectopic kidney in nine, solitary kidney in eight, duplex kidney in six and PUJ obstruction in three; about half had chronic renal failure, and 11 had end-stage renal failure when assessed at an average age of 11 years. Malformations of other organ systems can accompany persistent cloaca, with anomalies of the bony sacrum being common [6], and a subset of patients [7] having the VACTERL association (*v*ertebral anomalies, *a*nal atresia, *c*ardiovascular malformations, *t*racheoesophageal fistula, *r*enal and *l*imb anomalies) [8].

The normal development of the metanephros, the precursor of the mature kidney, is integrated with urinary bladder development [9]. Around day 28 of human gestation, the mesonephric duct drains into the urogenital sinus. The epithelia of the sinus and mesonephric duct fuse, and the ureteric bud arises and then interacts with intermediate mesoderm to form the metanephros. The urogenital sinus and the rectum form by partition of the cloaca, with the extension of the urorectal septum and curvature of the caudal part of the embryo; apoptosis is implicated in the breakdown of membranes to open the urogenital sinus and rectum to the amniotic cavity [10–13]. In the female genital tract, the lower part of the vagina forms from the dorsal wall of the urogenital sinus, while the upper vagina, cervix, uterus and fallopian tubes form from the paramesonephric ducts [14,15].

There are two main theories to explain the pathogenesis of persistent cloaca. Teratogen exposure can cause a spectrum of malformations resembling VACTERL in animals [16,17], although overt teratogen exposure has not been a feature in series of patients with persistent cloaca. Possible genetic scenarios that could cause persistent cloaca include de novo dominant mutations, inherited dominant mutations with reduced penetrance, or a disease generated by the interaction of mutations/polymorphisms at more than one locus, since this condition arises sporadically in families. Recessive inheritance is another possibility but perhaps less likely because of the lack of reported recurrences in siblings. Persistent cloaca has been associated with chromosome 7p rearrangements [18]. Furthermore, mutations that disrupt a single gene can cause this malformation: homozygous mutations in the gene *DHCR7* cause persistent cloaca in combination with renal aplasia/hypoplasia/ectopia as part of the Smith-Lemli-Opitz syndrome [19], and a heterozygous de-novo missense mutation in the cytoplasmic domain of *UPIIIA* (human chromosome 22q13.31), a gene expressed in the normal human embryonic urogenital sinus, was found in a girl who had persistent cloaca as well as renal adysplasia [20].

Here, we sequenced *UPIIIA* in 20 females with persistent cloaca, many with associated upper renal tract malformations. In addition, we screened three other genes, *Sonic Hedgehog* (*SHH*: 7q36), *Ephrin B2* (*EFNB2*: 13q33) and *Hepatocyte Nuclear Factor 1β* (*HNF1β*: 17cen–q21.3). As detailed in the Discussion, SHH [8,17,21–23], EFNB2 [24] and HNF1β [25–29] are expressed in the developing renal, genital and alimentary tracts, and have been functionally implicated in the normal development of these structures.

Patients, materials and methods

Patients

The genetic project was approved by the Ethical Committee at the Institute of Child Health, London. Venous blood was collected from index cases