Translational Research for Pediatric Lower Urinary Tract Dysfunction

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This review provides a comprehensive view of translational research aimed at elucidating the pathophysiology of pediatric lower urinary tract dysfunction (LUTD). A web search was conducted according to combinations of keywords, and the significance of each article was defined by the author. The dramatic evolution of the mass analysis method of genomes, transcripts, and proteins has enabled a comprehensive analysis of molecular events underlying diseases, and these methodologies have also been applied to pediatric LUTD. In genetic analyses of syndromes underlying daytime incontinence, urofacial (Ochoa) syndrome may be creating a prototype of a new research approach. Nocturnal enuresis has long been studied genetically, and several candidate loci have been reported. However, the pursuit for enuresis genes has been abandoned partly because genetic association and enuresis phenotype (bladder or renal type) could not be linked. Enuresis associated with diabetes insipidus has provided new insights into the etiology of the diseases. A chronobiological approach may shed new light on this area. Posterior urethral valves and neurogenic bladders have attracted the interest of pediatric urologists to the smooth muscle biology of the bladder. Bladder extrophy and cloacal anomalies are rare but major anomalies caused by defective urorectal development and have recently been studied from a genetic standpoint. Translational studies for pediatric LUTD may be extended to adult bladder disease, or to application of precision medicine for diseased children.

Keywords: Pediatrics; Lower Urinary Tract Symptoms; Genomics; Enuresis; Urinary Incontinence

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**INTRODUCTION**

Pediatric lower urinary tract dysfunction (LUTD) involves a broad spectrum of conditions. Daytime urinary urgency, incontinence, and nocturnal enuresis are documented in 5%–15% of school children [1,2], but the majority of them are self-resolving and can be conceived as failure to achieve appropriate coordinated micturition behavior or day-and-night micturition cycles. More severe cases requiring medical care usually involve daytime incontinence and may show detrusor overactivity or impaired sphincter function as an underlying cause. Most severe cases are usually associated with underlying or concomitant disorders such as spinal disorders, psychological or cerebral disorders, bladder outlet obstruction, and very rare congenital anomalies including cloacal anomaly, bladder and cloacal extrophy, and others.

This review covers basic research findings involving pediatric LUTD, with special emphasis on the research findings that recent genomics, proteomics, and cell biology has provided for elucidation of the pathophysiology of these diseases. We excluded research aimed at ‘regenerative medicine’ including cell therapy, tissue engineering, novel devices, and pharmaceutical therapies, because there have already been many reviews on these topics including our own [3,4].

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METHODS

A PubMed search was conducted according to a combination of keywords including, 'congenital,' 'pediatric,' 'bladder,' 'enuresis,' 'incontinence,' 'gene,' 'genome,' 'genetics,' 'proteomics,' 'posterior urethral valve' and 'translational,' but NOT 'cancer' or 'neoplasm.' Of them, 'regenerative medicine' reviews were excluded. Those treating pure animal experiments were kept to a minimum, and were limited to those having relevant clinical implications. Retrieved articles considered relevant were classified according to subject topics, and the significance or each article was defined by the author.

RESEARCH METHODOLOGY

The dramatic evolution of mass analysis methods of genomes, transcripts, and proteins is allowing for comprehensive analysis of molecular events underlying various diseases.

Genomic analysis has been employed to investigate inherited genetic disorders, and is often able to detect causative genetic defects of diseases. Single nucleotide polymorphisms (SNPs) have also been implicated for candidate genes of diseases. Recently, sequencing technology has rapidly advanced in terms of time and cost, making whole genome sequencing (WGS) relatively common. The cost issue is crucial, since funding support required for application of such innovative methods is not easily obtained for relatively minor diseases, like pediatric LUTD. Through the use of this technology, whole genome SNP mapping has become more feasible. Copy number variation (CNV) is a relatively new field. It is defined as a phenomenon in which sections of the genome are repeated and the number of repeats in the genome varies between individuals in the human population [5,6]. Reports utilizing CNV data have been increasing as WGS has become more feasible.

Although genomic variation should entail phenotypic change through transcription to mRNA and translation to protein level, transcriptome is less easy to investigate than genomes, because of ethical and clinical hurdles against obtaining human materials for extracting RNA and protein samples. Application of these new methods to humans requires ethical justification for obtaining samples in a way as least invasive as reasonably achievable. For the same reason, proteomics analysis of human organs is not easily attainable [7]. Pediatric LUTD is functional disease, and except for special circumstances like bladder augmentation [8], pathological specimens are usually not obtained. On the other hand, urine can be readily obtained and urinary proteins may reflect certain pathological conditions in the urinary tract. One major problem is that such proteins may most likely have renal origins, and only a small portion of them may derive from the bladder. For example, groups investigated the expression level of prostaglandin E2 and nerve growth factor (NGF) for overactive bladder (OAB) in children, but in both cases it is difficult to prove whether these molecules are secreted from the bladder or from the kidney [9-11]. Comprehensive proteomics using mass spectrometry has been investigated for upper urinary tract diseases, but not applied to LUTD yet [12,13].

There are also other omics approaches that may have clinical significance for pediatric diseases like microbiomics and metabolomics, although clinical investigation is yet to be done [14].

DISEASE CONDITIONS

Daytime Incontinence

Involuntary urination (incontinence) during the daytime is not uncommon in school children, and usually resolves with the age [1]. Since urinary continence is accomplished by a complex coordination of bladder detrusor, urethral sphincter, afferent and efferent innervation, various diseases can be underlying causes. Of those, Ochoa syndrome (urofacial syndrome) has attracted the interest of basic scientists. Since the first report by Ochoa on association between characteristic facial features and urinary incontinence in 1987 [15,16], a genomic analysis reported in 1997 designated the association of a chromosomal region in 10q23-q24 [17], whole genome SNP mapping identified HPSE2 gene coding heparanase-2 and LRIG2 as causative genes [18,19], and recently a model mouse was created [16].

Such an approach may become a research prototype, which can be adopted for other rare diseases having distinct genetic causes. Noonan syndrome [20], Fragile X syndrome [21], or 21 trisomy (Down syndrome) [22] can all be causes for daytime urinary incontinence. Association of these diseases with bladder problems has only been descriptive, and few causal mechanisms have been proposed thus far, but these diseases may be candidates for further translational research by the recent OMICS methodology. In contrast, the more common and well-known cause of refractory daytime incontinence, attention deficit disorder with hyperactivity [23], may be more difficult to analyze, because of the multifactorial and socio-psychological nature of the disease.
For sporadic OAB cases, expression level of related molecules, such as prostaglandin E2 and NGF are investigated in clinical samples [9-11].

**Nocturnal Enuresis and Diabetes Insipidus**

Nocturnal enuresis, which means involuntary urination during sleep, is experienced by 5%-15% of school children. Bed-wetting is harmful for a child's self-esteem, but fortunately, nocturnal enuresis subsides in most children by adolescence [24,25]. The triad etiology of nocturnal enuresis is nocturnal polyuria, decreased nocturnal bladder capacity, and impaired arousal during sleep by bladder distention. Abnormal diurnal rhythm of plasma vasopressin is reported in patients with enuresis [26], and desmopressin, a synthesized antidiuretic hormone, is now a standard medication for enuretic children showing nocturnal polyuria. Reduction in nocturnal functional bladder capacity is another common feature in pathogenesis of refractory nocturnal enuresis [27]. Impaired arousal during sleep is now treated by alarm therapy.

Although the etiology of nocturnal enuresis is multifactorial, it is probably the most prevalent inherited disease in humans. Since 1995, a Danish group and a Dutch group has reported association of 4 genomic loci with enuresis from analyses of enuretic pedigrees, and named them ENUR1 (13q 13q13-q14.3) [28], ENUR2 (12q) [29], ENUR3 (22p11) [30] and ENUR4 (8q) [31]. However, all likely candidate genes in these loci, like aquaporin or dopamine receptors, have been excluded so far [32]. The pursuit for enuresis genes has been stranded partly because genetic association and enuresis phenotype (bladder or renal type) could not be linked in general [33,34].

Besides these studies in enuretic pedigrees, some groups investigated the polymorphism of candidate genes like 5-hydroxytryptamine receptor 2A and nitric oxide synthase in sporadic enuretic patients [35,36].

In rare cases, renal diabetes insipidus (DI) can be a cause of enuresis. Although these are exceptional cases compared to common enuresis cohort, causative molecules like vasopressin 2 receptor (V2R) [37], aquaporin 2 (AQP-2) [38-40], arginine vasopressin-neurophysin II [41], and Wolfram syndrome gene 1 (WFS1) [42] have been identified. Although rare, these cases provide further insights on the pathophysiology and treatment of enuresis. For example, cases of enuresis with DI including those with defective V2R or AQP-2 genes, still show positive response to desmopressin, a synthetized vasopressin-like drug, indicating that the desmopressin may have an extrarenal pathway for vasopressin affecting nocturnal enuresis [40,43,44].

Enuretic patients show loss in day-and-night change of urine production from the kidney and functional bladder capacity, but a precise mechanism has not been well-investigated. On the other hand, the recent evolution of molecular chronobiology has demonstrated that several genes in the kidney show circadian oscillation is controlled by the circadian clock, genetic ‘machinery’ controlling day-and night changes in cellular function. The circadian clock exists in most organs of the body including the kidney, and may contribute to day-and-night change in urine output. Also, our group discovered that the circadian clock exists in the bladder too, and may contribute to day-and-night change in the bladder capacity [45,46]. It is highly likely that the circadian clock may play a key role in enuresis, and ideally, reciprocal studies with clinical molecular epidemiology and laboratory biological investigations will clarify the true genetic mechanism of nocturnal enuresis.

**Posterior Urethral Valves and Neurogenic Bladder**

Although incontinence and enuresis are highly prevalent conditions, they are not life-threatening. On the other hand, more severe forms of LUTD in children are seen in patients with posterior urethral valves (PUVs) and neurogenic bladder, typically seen in those with congenital spinal disorders. In these diseases, massive fibrosis and remodeling of the bladder smooth muscle is seen, leading to end-stage renal disease if mistreated. The devastating effects of these diseases have attracted the interest of pediatric urologists to the smooth muscle biology, and more specifically, the effect of stretch injury to the bladder smooth muscle cells [47]. However, little extension of these laboratory findings into a clinical context has been done. Genetic or proteomic approach of clinical materials is still scarce in this area, and molecular analysis of clinical end-stage bladder disease occurs only in limited cases like those in which bladder augmentation is indicated. For example, our group performed an immunohistochemical study of the specimen of augmented bladder for parathyroid hormone (PTH) 1 receptor, a receptor of PTH related protein, which is a potent endogenous relaxant of the bladder smooth muscle under distention [8,48].

Recently, a genome-wide study revealed possible association of 47 loci with copy number variants in 32 cases of PUVs [49]. Genetic etiology of neurogenic bladder may belong to neurological investigators.
Rare Anomalies: Bladder Exstrophy, Cloacal Anomalies, and Others

Bladder exstrophy and cloacal anomalies are extremely rare, having a profound impact on patients’ continence, sexual life, and renal function, requiring complex surgical repair. Since they are major anomalies caused by defective urorectal development, they have recently drawn the attention of basic scientists [50-52]. For bladder exstrophy, a genome-wide association study followed by a whole exome sequencing study revealed the importance of the ISL1-pathway in humans and mice and proposed SLC20A1 and CELSR3 as candidate genes [51,52]. For cloacal anomalies, comparative genomic hybridization revealed CNVs in 7 patients (41%), including 5 gains and 2 losses [50].

There are much rarer diseases like megacystis microcolon intestinal hypoperistalsis syndrome, which has implicated ACTG2 as a causative gene [53,54]. Investigation of such rare diseases should not be underrated, since the causative genes related to these diseases could also be involved in more common and prevalent conditions.

CONCLUSIONS

Pediatric bladder diseases might be perceived as ‘orphan’ diseases, attracting the attention of few clinicians. On the other hand, lower urinary tract symptoms are quite prevalent in elderly patients, represented by OAB and nocturia. Interestingly, nocturnal enuresis in children and nocturia in the elderly sometimes share the same etiology; increase in nocturnal urine output, decrease in nocturnal bladder volume. In such a sense we may advocate that investigation of genetic etiology of pediatric diseases may elucidate the pathophysiology of a broader range of patients, including adults with LUTD. Since pediatric diseases may have less comorbidity like diabetes mellitus, prostate hypertrophy, and sexual hormonal changes, they may provide researchers opportunities to analyze the effect of genetic alteration per se.

In the interest of pediatric patients themselves, since pediatric LUTD is more likely to be associated with genetic background than is adult LUTD, it may be more suitable for development and application of precision medicine. The reduction of research costs in terms of funding, time, and human effort may hopefully lead to more scientific knowledge that would ultimately contribute to improved health care for pediatric LUTD.

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## Appendix. Summary of the translational research cited in the article

| Disease                     | Reference          | Year | Category       | Methodology          | Molecules and findings                                      |
|-----------------------------|--------------------|------|----------------|----------------------|------------------------------------------------------------|
| **Incontinence/OAB**        |                    |      |                |                      |                                                            |
| Urofacial syndrome          | Ochoa [15]         | 1987 | Clinical Study | Clinical Study       | N/A                                                        |
| Wang [17]                   | 1997               |      | Genetic locus  | Genome mapping       | 10q23-q24                                                  |
| Daly [18]                   | 2010               |      | Genomic mutation | Genome mapping   | HPSE2 (heparanase-2)                                       |
| Stuart [19]                 | 2014               |      | Bialleric mutation | Genetic sequencing | LRIG2                                                      |
| **Sporadic OAB**            |                    |      |                |                      |                                                            |
| Aoki [11]                   | 2009               |      | Urinary Eicosanoid | ELISA             | Prostaglandin E2                                            |
| Oktar [9]                   | 2013               |      | Urinary protein | ELISA               | Nerve growth factor                                         |
| Telli [10]                  | 2015               |      | Urinary protein | ELISA               | Nerve growth factor                                         |
| **Nocturnal enuresis**      |                    |      |                |                      |                                                            |
| Familial enuresis           | Eiberg [28]        | 1995 | Genetic locus  | Genome mapping       | 13q13-q14.3 (ENUR1)                                        |
| Anell [29]                  | 1997               |      | Genetic locus  | Genome mapping       | 12q ( ENUR2)                                               |
| Eiberg [30]                 | 1998               |      | Genetic locus  | Genome mapping       | 22q11 ( ENUR3)                                             |
| Eiberg [31]                 | 2001               |      | Genetic locus  | Genome mapping       | 4p16.1 (ENUR4)                                             |
| Loeys [34]                  | 2002               |      | Genetic locus  | Genome mapping       | 22q11, 13q13-14, 12q                                        |
| **Sporadic enuresis**       | Wei [35]           | 2010 | Polymorophism | PCR-RFLP             | 5-hydroxytryptamine receptor 2A gene                       |
| Balat [36]                  | 2007               |      | Polymorophism | PCR-RFLP             | Nitric oxide synthase gene polymorphisms                  |
| **Diabetes insipidus**      | Müller [40]        | 2002 | Genetic mutation | Targeted sequencing | Aquaporin-2 gene                                           |
| Kanemitsu [41]              | 2002               |      | Genetic mutation | Targeted sequencing | Arginine vasopressin-neurophysin II                        |
| Shalev [38]                 | 2004               |      | Genetic mutation | Targeted sequencing | Aquaporin-2                                                |
| Robben [43]                 | 2007               |      | Genetic mutation | Targeted sequencing | V2R gene deficiency                                        |
| Marshall [42]               | 2013               |      | Clinical Study | Clinical Study       | WFS1 gene, encoding wolframin                              |
| Yamashita [37]              | 2016               |      | Genetic mutation | Targeted sequencing | V2R gene deficiency in partial DI                          |
| **Posterior urethral valve**| Boghossian [49]    | 2016 | DNA CNV        | WGS                  | 47 rare candidate CNVs in 32 cases                        |
| Neurogenic bladder          | Nishikawa [8]      | 2015 | Protein        | Immunostaining       | PTH-PTH related protein receptor 1                          |
| Exstrophy                   | Draaken [51]       | 2015 | Genetic mutation | GWAS                | ISL1                                                       |
| von Lowtzow [52]            | 2016               |      | DNA CNV        | WGS                  | Rare CNVs identified                                        |
| Cloaca                      | Harrison [50]      | 2014 | DNA CNV        | WGS                  | CNV in 41% of cases, 5 gain and 2 loss                      |
| MMHIS                       | Wangler [53]       | 2014 | Genetic mutation | WGS                 | ACTG2 gene missense variants                               |
| Thorson [54]                | 2014               |      | Genetic mutation | WGS                 | ACTG2 gene mutation                                         |

OAB, overactive bladder; N/A, not applicable; ELISA, enzyme-linked immunosorbent assay; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; DNA CNV, DNA copy number variant; DI, diabetes insipidus; WGS, whole genome sequencing; GWAS, genome-wide association study; MMHIS, megacystis microcolon intestinal hypoperistalsis.