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Mutation Analysis and Cloning of Childhood Renal Disease

Dear Colleagues,

Thank you for your interest in the mutational screening of patients with nephrotic syndrome (NS), branchio-oto-renal syndrome (BOR), or patients that have a congenital or developmental abnormality of the urinary tract.

We are performing mutational analysis in the *NPHS2*-gene (podocin) and *WT-1*-gene. Our aim is to find out whether there is any correlation between the occurrence of mutations in the *NPHS2*-gene and the clinical outcome of these patients (e.g. response to steroids and cytotoxic drugs, relapse after transplantation) (Karle et al. *J Am Soc Nephrol* 13:388, 2002). This genetic analysis is investigational and is performed in the setting of a research laboratory and there are no universal standards for the performance of these studies. The investigators endeavor to attain the highest standards in their analysis, but these analyses should not be considered diagnostic tests, rather investigational genetic tests, not intended to replace other clinical or laboratory evaluations or treatments that would otherwise be considered the standard of care.

Identification of new genes causing branchio-oto-renal syndrome will offer new insights into the pathomechanisms of hearing defects, urinary tract malformations (UTM), as well as kidney and ear development. Congenital developmental abnormalities of the human genitourinary (GU) tract account for a significant degree of morbidity seen in children possessing such lesions. Clinically these abnormalities comprise the most common causes of infant and childhood chronic renal insufficiency and ultimately renal transplantation. The purpose of this proposal is to provide critical data needed to elucidate the genetic causes that underlie these various syndromes and provide a potential screening tool for families at high risk. Additionally, insights gained from this study will provide us and the research community with new information involving the abnormal and normal development of the GU tract, which will have a potentially larger patient application in the future.

Branchio-oto-renal syndrome (BOR) is an autosomal dominant disorder with the features of renal anomalies, hearing loss, and branchial arch defects. The prevalence of BOR approximates 1:40,000 in the general population. In 1997, mutations in the *EYA1* gene were identified as causing BOR. Although disease-causing mutations in *SIX1* and *SIX5* have also recently been identified in patients, a large number of cases are still unaccounted for, suggesting that several more BOR genes are present in the genome.

Congenital urinary tract malformations (UTM) are the most common cause of end stage renal disease in the pediatric population, accounting for 32% of primary etiologies at the time of renal transplantation. Over 90% of UTM cases are non-syndromic, but congenital malformations of the urinary tract can also occur as part of over 200 syndromes. Urinary tract malformation loci are present on chromosomes 1p13, 6p21, 10q26, and a number of genes including *HNF1 β* , *PAX2*, *EYA1* and *UPIIIa* have been shown to be mutated in UTM patients. Further, BOR genes that are identified through this study may also contribute to non-syndromic cases of UTM.

These genetic tests are presently considered investigational and are part of a research protocol. There is no cost for the blood draw, shipping or processing of the samples to the patients or family members of

the patients who agree to participate in the study. Office visits for physicians or genetic counselors are not paid for by this study, nor are any other laboratory tests. Results of genetic analyses are generally available 3-6 months following the receipt of a sample. Results are transmitted directly to the corresponding physician and not to individual participants. Participants will therefore need to depend upon their local physician to communicate and explain the results of the genetic tests. The investigators would be happy to discuss the results of the genetic testing with any local physician who wishes to do so. **No results will be reported for individual participants who do not have a diagnosis of nephrotic syndrome, BOR or UTM at the time of enrollment.**

If an individual is found to have nephrotic syndrome, BOR or UTM after enrolling in the study a local physician may contact the investigators, at which time results of any genetic testing which has been performed can then be released to the local physician. Local physicians, or their representatives, are expected to review the consent document with prospective participants and indicate that they feel the participant understands the nature of the study by signing the consent document before the participant signs the consent document. In addition to the copy that is returned to the investigators, the participant and the local physician should also keep a signed copy of the consent.

We also kindly ask you to fill out a clinical questionnaire which includes not only important information on the family history, the clinical picture, the response to treatment, and extrarenal associations, but also on the ethnicity of your patient. Recent studies and our own data suggest that ethnic groups are affected differently by mutations in genes causing nephrotic syndrome, such as podocin and nephrin. Our group is interested in elucidating genotype/phenotype correlations in this disease. We, therefore, want to encourage you to describe your patient's ethnicity in as detailed a way as possible. Please feel free to check more than one box and/or use the "other" checkbox with a more detailed description.

Please return the following items to the investigators:

1. Signed consent document.
2. Health questionnaire.
3. Blood sample: 3-10ml EDTA or Na-Heparin blood for each participant.
4. Outside the U.S.: Customs Invoice (see end of document)

Blood samples without a signed consent document cannot be processed or analyzed.

As in the past, we are happy to provide free shipping of your blood samples. However, there has been abuse of our customer numbers. Therefore, we would like to kindly ask you to contact our laboratory for information on free shipping. Virginia Vega-Warner will be happy to help you and can be contacted by e-mail at vvegaw@umich.edu. DNA samples can be shipped by regular mail.

Please e-mail us at the time of shipping with the shipping number, so that we can track the package and ensure safe delivery. Thank you again for your participation. Please do not hesitate to contact us with any questions or concerns.

Best regards,

Friedhelm Hildebrandt, M.D.

Professor of Pediatrics and of Human Genetics
Investigator, Howard Hughes Medical Institute
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Branchio-oto-Renal Syndrome and Congenital Urinary Tract Malformations
Questionnaire, version January 4, 2009

Prof. Dr. F. Hildebrandt

Thank you very much for taking the time to fill out this form.

This form is to be completed by the participant's physician.

General Patient Information		_____MM/_____DD/_____YYYY
Last name: _____	First name: _____	DOB: _____ / _____ / _____ MM DD YYYY
<input type="checkbox"/> M <input type="checkbox"/> F	Height: _____cm	Weight: _____kg
Consanguineous parents	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Relatives with urinary tract malformations	<input type="checkbox"/> Mother <input type="checkbox"/> Brother	
	<input type="checkbox"/> Father <input type="checkbox"/> Sister	
	<input type="checkbox"/> Others: _____	
Ethnicity: <input type="checkbox"/> African <input type="checkbox"/> African American <input type="checkbox"/> American Indian <input type="checkbox"/> Arabic <input type="checkbox"/> Asian <input type="checkbox"/> Caucasian <input type="checkbox"/> Central Slavic		
<input type="checkbox"/> Chinese <input type="checkbox"/> European <input type="checkbox"/> Finnish <input type="checkbox"/> Hispanic <input type="checkbox"/> Indian Subcontinent <input type="checkbox"/> Japanese <input type="checkbox"/> Pacific Islander		
<input type="checkbox"/> Turkish <input type="checkbox"/> Other: _____		

I. Initial Clinical Examination: _____MM/_____DD/_____YYYY

1. Symptoms (initial)

- | | |
|---|--|
| <input type="checkbox"/> Acute event | <input type="checkbox"/> Fever |
| <input type="checkbox"/> During regular examination | <input type="checkbox"/> Urinary tract infection |
| | <input type="checkbox"/> Diminished/increased urinary output |
| | <input type="checkbox"/> Pyelonephritis |
| | <input type="checkbox"/> Hypertension |
| | <input type="checkbox"/> Other: _____ |

2. Laboratory Findings (initial)

- | | |
|--|---|
| Blood studies: <input type="checkbox"/> Creatine: _____mg/dl | Urinalysis: <input type="checkbox"/> Proteinuria _____g/day or g/g crea |
| <input type="checkbox"/> GFR: _____ml/min/1.73m ² | <input type="checkbox"/> Hematuria |
| <input type="checkbox"/> Serum protein: _____g/dl | <input type="checkbox"/> Bacteriuria: _____CFU/ml |
| <input type="checkbox"/> Albumin: _____g/dl | |
| <input type="checkbox"/> CRP: _____mg/l | |

3. Imaging Techniques (initial)

- | | | |
|--|---|---|
| <input type="checkbox"/> Ultrasound | <input type="checkbox"/> Voiding cystourethrography | <input type="checkbox"/> Renal scintigraphy |
| <input type="checkbox"/> Intravenous pyelogram | <input type="checkbox"/> Cystoscopy | <input type="checkbox"/> Other: _____ |

4. Diagnosis (initial)

- | | | | |
|---|--|---|--|
| <input type="checkbox"/> Renal agenesis | <input type="checkbox"/> right <input type="checkbox"/> left | <input type="checkbox"/> Prevesical ureter stenosis | <input type="checkbox"/> right <input type="checkbox"/> left |
| <input type="checkbox"/> Hydronephrosis | <input type="checkbox"/> right <input type="checkbox"/> left | <input type="checkbox"/> Vesico-ureteral reflux | <input type="checkbox"/> right <input type="checkbox"/> left |
| <input type="checkbox"/> Ureteral stenosis | <input type="checkbox"/> right <input type="checkbox"/> left | <input type="checkbox"/> Bladder exstrophy | |
| <input type="checkbox"/> Ureteropelvic junction obstruction | <input type="checkbox"/> right <input type="checkbox"/> left | <input type="checkbox"/> Other: _____ | |

Patient's Name: _____

II. Treatment

- | | |
|---|---|
| <input type="checkbox"/> Dialysis | <input type="checkbox"/> Ureterocystostomy |
| <input type="checkbox"/> Tenckhoff catheter | <input type="checkbox"/> Anti-reflux operation |
| <input type="checkbox"/> Pyelocystostomy | <input type="checkbox"/> Anderson-Hynes pyeloplasty |
| | <input type="checkbox"/> Other: _____ |

III. Extrarenal Association

The patient suffers / suffered from one of the following diseases:

- | | |
|---|---|
| <input type="checkbox"/> Face dysmorphism | <input type="checkbox"/> Skeletal deformity |
| <input type="checkbox"/> Microcephaly | <input type="checkbox"/> Polydactyly/syndactyly |
| <input type="checkbox"/> Mental retardation | <input type="checkbox"/> Pulmonary hypoplasia |
| <input type="checkbox"/> Deafness | <input type="checkbox"/> Heart anomalies |
| <input type="checkbox"/> Blindness | <input type="checkbox"/> Allergy |
| <input type="checkbox"/> Growth retardation | <input type="checkbox"/> Other: _____ |

IV. Remarks

*Thank you very much for your assistance.
Please provide us with the following information in order to facilitate further correspondence.*

Name: _____	Phone: _____
Address: _____	Fax: _____
Address: _____	eMail: _____

BLOOD SAMPLE COLLECTION FOR MUTATIONAL ANALYSIS

- 1. Please call us** or send us an email to the below mentioned address before, or at the time when shipping the samples, so we can be certain to receive them within 2 days or otherwise trace them.
- 2. Venipuncture:** Draw 5 ml **EDTA-blood** or **Na-Heparin** under **sterile** conditions (wear gloves, do not touch rim of tubes); **immediately invert tubes several times** to prevent coagulation. If syringes and tubes are being used **rinse syringe with Na-Heparin**.
- 3. Storage: Always keep blood samples at room temperature! (Never chill, never freeze!)**
- 4. Transport:** Send samples and filled-out forms: consent and clinical questionnaire (inside shipping envelope), customs forms (outside shipping envelope or package). Immediately address to the name below by the fastest route possible, e.g. **2-day Express Air Mail, Federal Express, DHL Worldwide Express**. Get a guarantee from the carrier to deliver samples to our destination **within 1-2 days** (regular air mail is much too slow for blood samples). Protect samples from the cold by wrapping them in gauze or packaging them in Styrofoam. Don't forget to contact us!

If you like, you can use one of our personal courier accounts. For information on the account numbers please contact Virginia Vega-Warner at vvegaw@umich.edu or Professor Friedhelm Hildebrandt at fhilde@umich.edu.

Thank you for your cooperation!

Send samples to:

Prof. Dr. med. F. Hildebrandt
University of Michigan, Department of Pediatrics
1150 West Medical Center Dr, 8220C MSRB 3
Ann Arbor, Michigan 48109-5646, USA
Fax: 734-615-1386
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Invoice

Shipper:

Consignee:

Prof. Dr. med. F. Hildebrandt
University of Michigan, Department of Pediatrics
1150 West Medical Center Dr, 8220C MSRB 3
Ann Arbor, Michigan 48109-5646, USA

Content:

1 Parcel containing:
Documents and human blood or DNA, **non-hazardous, non-toxic, non-infectious**, sample for laboratory research use only, no commercial value.

\$ 1 value for customs purposes only.

Date / Signature