NEW GENETIC DIAGNOSTIC TOOLS FOR PATIENTS WITH CYSTIC KIDNEY DISEASES - AN INTEGRATED APPROACH WITH A CLINICAL NEPHROGENETIC CONSULTATION

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During the last 10 years, improvement of our genetic knowledge has permitted to offer genetic diagnosis for a number of diseases (Hildebrandt, 2010). This has led to the identification of diseases and of important genes implicated in nephrogenesis and in the mechanism inducing branching and nephron formation. Among them, genes involved in cystic diseases, which belong to a group of genetic disease named ciliopathies, are the most frequent genetic cause of end-stage kidney disease and kidney transplantation in the first 3 decades of life and are increasingly recognized in older children and adults. In pediatrics, hereditary nephropathies participate to a large proportion of causes of chronic renal insufficiency (Schwab et al., 2003; Potter et al., 2010). In adults, autosomal dominant polycystic disease is highly prevalent and other types of cystic diseases, such as in medullary cystic diseases or HNF1beta are increasingly diagnosed in children and young adults.

Development of accurate imagery techniques during pregnancy, childhood and adulthood contributes to a better classification of diseases but genetic diagnosis only in many cases can determine the final diagnosis due to overlapping presentations of diseases.

A better knowledge of a genetic disease and the mutation also paves the way to therapeutic improvements. In the case of autosomal dominant polycystic disease, new therapeutic options such as somatostatin (Hogan et al., 2010) and V2 receptor antagonist (Torres et al., 2004) are promising among others. This is also true in pediatric nephrology as cysts may already be visible on kidney ultrasound during pregnancy, or infancy leading to a pediatric follow-up and questioning on which therapeutic intervention could be proposed to delay disease progress already in these patients (Shamshirsaz et al., 2005). In other genetic disease, replacement therapy is or will be available soon. It is very likely that in polycystic disease as well as in other genetic disease, response to treatment may depend on the specific type of mutation involved. With regards to living donor transplantation, the demonstration of the absence of kidney disease disease-causing mutations is also crucial in relative of a patient.

Developing a genetic diagnostic tool for cystic kidney disease will permit to provide accurate and early diagnosis, counseling regarding prognosis and plan methods of renal replacement and therapy tailored to each patient (Hildebrandt, 2010). Setting up a multidisciplinary approach is also mandatory to improve patient counseling. In addition, we believe that the ability to perform genotyping locally will increase our awareness for genetic diseases and simplify the procedure for patients, thereby improving their care.
MULTIDISCIPLINARY CLINICAL APPROACH

Setting up a multidisciplinary consultation for patient and their family with genetic renal disease will be an essential aspect of pre-genetic testing and disclosure of the genetic analysis results. Appropriate family counseling will also be available for other family members, and for discussing risks and advances in therapeutic interventions and if desires in reproductive planning. We are aware that the revelation of genetic diseases already in childhood could increase anxiety in the child and modify the integration of this child in the family and this particular aspect will be evaluated in priority (Levy et al., 2001). The genetic testing will be proposed to a child and his family only, and this is a relevant point, if the testing proposed could improve his care or if there is a need to clarify the renal disease in situations of unclear clinical presentation.

CONCERNED PATIENTS

The screening in the selected genes will be carried out in the patient’s DNAs, extracted from peripheral blood (3-5ml of blood). These patients will be referred for genetic analysis after at least one preliminary multi-disciplinary consultation, with both nephrologist and geneticist presents. The analysis will be proposed only when there is a relevant clinical question, and only if the patient gives a fully informed consent. Such consent will be signed by the patient and the physician (document from the Swiss society of Medical Genetics will be used for that purpose). We will neither accept self-prescriptions nor perform the analysis when the index-case has no medical reasons to undergo such procedure. Pre-natal analysis will not be performed during this period (at least one-year long) of development of the project and validation of our analysis setup.

TECHNICAL DEVELOPMENTS AND ANALYSES

DNA sequencing of the human genes has become indispensable for basic researchers studying biological processes, as well as in clinical application such as diagnosis by mutation detection. DNA sequencing has significantly accelerated biological research and discovery of the genetic basis of numerous human diseases (Voelkerding et al., 2009). The high speed/high throughput sequencing could now be reached with cutting-edge DNA sequencing technologies such as next generation sequencing. These novel and recently-made available technologies (Shendure and Ji, 2008) permits to increase the power of the sequencing process, producing thousands or millions of sequences at once. The application of these approaches to the field of medical genetics makes possible the sequencing of hundreds of genes to thousands of genes in a single experiment. Practically, this means that genetic disorders can be investigated in a much shorter period of time, for an affordable cost. While yesterday years were needed to screen genes for mutation, now in a week, tens of genes can be analyzed in a large number of patients.

We thus propose to analyze the recruited patient DNA for a selection of genes that are known or reaso-
nable candidates for nephropathies, mainly those that are within the group of cystic kidney diseases. Our laboratory has already a wide experience in screening a large number of genes using novel technologies including next-generation sequencing on selected genes by DNA capture. Currently it is possible to use either microarray or in liquid-phase DNA capture that are mainly offered by 2 manufacturers (Nimblegen, Agilent). Agilent has been chosen for microarray design of our selected genes. This method has already been successfully tested in several issues by our laboratory. For the microarray design: the coordinates of each exon of all selected genes has been generated using the Galaxy tool (http://main.g2.bx.psu.edu/) from human genome database (i.e. RefSeq database). These coordinates will be submitted to manufacturer in the near future, in order to prepare the design of oligonucleotide probes to cover all the selected coding regions. In that process all highly repeated regions will monitored in order to try including those that are less likely to bias the selection. We have chosen, within the context of this project, to design such a microarray, in which the following genes are present: Autosomal Dominant PKD, Autosomal Recessive PKD, Tuberous Sclerosis, Nephrophtisis, Bardet- Biedl syndrome, Meckel syndrome, Ellis-van Crevel syndrome, Joubert Syndrome, SDCCAG8, HNF1B.

Practically, for the candidate genes, sequencing will be performed in our laboratory using our Illumina GA-IIx high throughput sequencing instrument. This sequencing machine is already in use for other purposes in our laboratory and we already have the know-how to perform the above-described techniques.

To date, in Switzerland, no genetic center is able to offer the molecular diagnosis of these genetic kidney diseases.

**EXPECTED RESULTS**

Our recruitment will first be targeted on kidney cystic diseases patients from the area of Geneva and Lausanne (collaboration with the nephrology ward).

We expect to provide to patients the possibility of a genetic diagnosis of some clinically-relevant cystic kidney diseases and therefore be able to better target their follow-up, treatment, and improve their family counseling and treatment plan.

**FUTURE DEVELOPMENTS**

Our recruitment would then be extended to all Switzerland and other countries, on demand. After a research funded period, during which we will design and validate our diagnostic method, we will try to have the genetic diagnosis reimbursed by the patient’s insurance, or by the patient, or by a combination of clinical research further funding and patient’s participation.

We plan to generate a mutation database and establish genotype-phenotype correlations, as well as determinate if there is genotype-response to treatment correlations.
REFERENCES


Set-up phase
Sequencing reagents, DNA extraction kits, enzymes: 10’000
Chemicals, plastics, disposables 5’000

Validation of Analysis phase
10 patients to be screened for mutations 15’000
(estimate cost for this validation phase: 1’500 CHF/patient)

TOTAL 30’000 CHF