Molecular approach to the study of inherited kidney diseases: A way to understand the mechanisms of disease

J. P. Grünfeld
Université René Descartes-Paris V. Clinique Néphrologique. Hôpital Nécker. Paris

Progress in DNA technology has allowed the development of molecular genetics. At a rate of about one a week, the gene of an inherited human disorder is located by linkage analysis or identified and cloned, opening to the description of various DNA defects (or mutations) within the gene itself. New Journals have been created to cope with these advances. The questions often raised by clinicians are: What for? What are the clinical consequences?

1. Good clinical observations are decisive for triggering the process of molecular genetics

To promote progress in inherited renal disorders, inventive nephrologists and inventive molecular geneticists should be associated. The first step in genetics is to identify correctly the inherited nature of the disease, and to recognize unusual, potentially important observations. This has been well illustrated in very recent years by the information drawn from chromosomal translocations.

The first example deals with Lowe syndrome, an X-linked recessive disorder which includes congenital cataract, mental retardation and renal tubular dysfunction. This X-linked recessive disease usually affects only males, but a few female patients with Lowe syndrome have been observed. This surprising observation of an X-linked recessive disorder expressed in females is often associated with X; autosomal translocation. Preferential inactivation of the normal X chromosome accounts for full expression of the disease in females. In these affected women, the gene is deleted or disrupted at the translocation breakpoint. This approach has been used successfully to identify the gene of Lowe syndrome. It has also been shown that the gene involved encoded for an enzyme implicated in inositol phosphate metabolism - opening a new field of research [1].

A similar approach has recently been successful in autosomal dominant polycystic kidney disease (ADPKD). A locus (PKD1) had been located in 1985 to the short arm of chromosome 16. In 1994, the PKD1 gene was identified thanks to a family in which both tuberous sclerosis and ADPKD were known. A gene of tuberous sclerosis (TSC2) had been located in 1993 on the short arm of chromosome 16, at close proximity to the PKD1 locus [2]. In this Portuguese family, a translocation between chromosome 16 and chromosome 22 was demonstrated (fig. 1). The patients who inherited the balanced translocation developed ADPKD since the PKD1 gene was disrupted at the breakpoint. The young male patient who inherited unbalanced translocation, and therefore had monosomy, developed tuberous sclerosis (since one TSC2 gene was lost). In addition, he probably also has ADPKD due to disruption of the PKD1 gene by the translocation; several cysts have been detected in both kidneys by ultrasonography. Identification of this family and of this translocation was decisive for finding the PKD1 gene [3]. The association of polycystic kidney disease and tuberous sclerosis has been known for several decades. Translocation involving chromosome 16p is not the only molecular explanation. This association can be found in patients with tuberous sclerosis linked to another locus, TSC1, located on chromosome 9. In addition, it can be seen in TSC2 patients with large *de novo* deletions, involving both TSC2 and PKD1 genes [3].

2. Molecular genetics is a very powerful mean for understanding the mechanisms of diseases - and in the near future to promote adapted curative treatment of some of them

All recent advances in genetics illustrate this statement. The results obtained in cystic fibrosis demonstrate how fast these advances can be made today

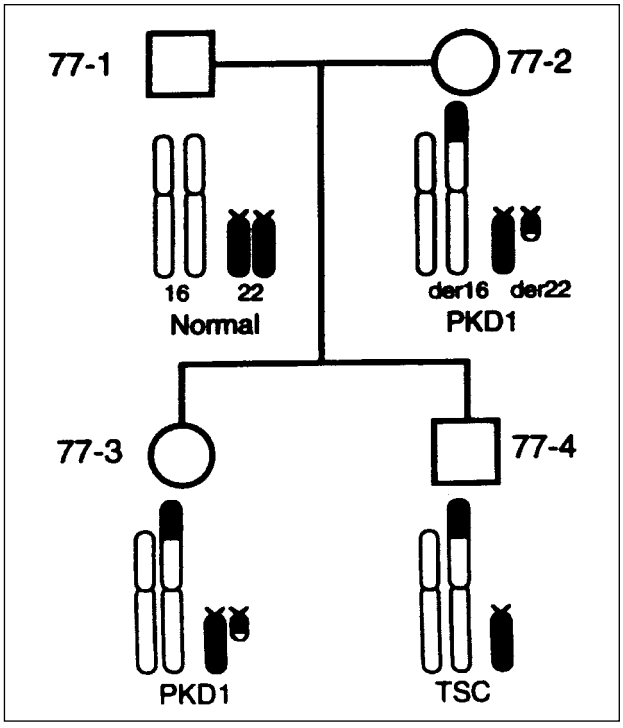

Fig. 1.–A chromosome translocation associated with PKD1 (from «Cell» 1994; 77:881-94). See comments in the text.

with the tools provided by molecular biology. The achievements reached in some renal hereditary disorders in recent years are also encouraging. The recent history of hereditary nephrogenic diabetes insipidus (NDI) is exemplary in this regard. The disease app ears very early in life and is characterised by vasopressin-resistant DI. In most families, it is transmitted as an X-linked recessive trait. Linkage studies had loc ated the NDI locus at the distal end of the long arm of the X chromosome. Pathophysiological investigations pointed to a defect in the V2 vasopressin receptor, involving collecting duct cells (thus explaining DI) and also extrarenal sites, such as endothelial cells. In a few years, the gene encoding for the V2 receptor was located and identified on the Xq chromosome. Subsequently, mutations in this gene were recognized in NDI families[4].

In some NDI kindreds, however, the disease is inherited as an autosomal recessive trait and endothelial function abnormalities are lacking. A defect in the V2 receptor cannot therefore be implicated. Meanwhile, the water channel aquaporin family has been identified. Aquaporin 2 was found to be the water channel responsible for water movement in collecting duct cells. The gene encoding for aquaporin 2 has been cloned and located on chromosome 13. Mutations of this gene have been detected in autosomal recessive NDI families[5]. Molecular genetics have been very helpful for understanding the molecular mechanisms of NDI. In turn, the defective V2 receptors or aquaporins will shed some light on the structure-function relationships of these molecules.

Similar progress has been achieved recently in a rare autosomal dominant disease called familial benign hypercalcemia with hypocalciuria. The disease is benign since hypercalcemia does not lead to visceral complications, such as nephrolithiasis. However, severe neonatal hyperparathyroidism has been observed in a few cases in families affected by familial benign hypercalcemia. The pathogenesis of the disease was clarified by the finding by Brown et al of a calcium-sensing receptor located at the cell membrane. The gene encoding for this mechanism has been cloned and mutations have been found in families with familial benign hypercalcemia. The patients are heterozygous, only one copy of the gene on one chromosome being mutated in this dominant disease. Homozygous patients develop severe neonatal hyperparathyroidism[6, 7]. The defective calcium-sensing mechanism on parathyroid cells and on renal tubular epithelial cells explains why PTH secretion is not suppressed and urinary calcium excretion is not increased by hypercalcemia, and finally why hypercalcemia develops in these patients.

Similar advances have been obtained in the understanding of two forms of secondary hypertension: glucocorticoid-remediable aldosteronism (which is determined by a chimeric gene containing DNA fragments encoding for both aldosterone syntethase and 11-OHase) and Liddle syndrome, which is an intrinsic renal disease due to a defect in the gene encoding for the amiloride-sensitive Na channel in the apical membrane of renal tubular cells.

3. Most inherited disorders are heterogeneous: genetic heterogeneity means diversity of the molecular mechanisms, and thus, new classification of the diseases

Heterogeneity has been highlighted in the two most frequent inherited kidney disorders, autosomal dominant polycystic kidney and Alport syndrome.

Regarding ADPKD, the PKD1 form is the most prevalent and represents approximately 85 to 90 percent of the cases. A second locus was, however, subsequently recognized on chromosome 4. This PKD2 form probably has slower development of the renal cysts; the age at ESRD is 10 to 15 years more than in the PKD1 form[8-11]. Lastly, a few families have been

identified in which no linkage to either the PKD1 or PKD2 locus has been documented, opening the possibility that a third form of ADPKD exists. The challenge for the future will be to identify the gene products of these three diseases, to know whether cyst formation is dependent on similar mechanisms, and to understand what accounts for the rates of progression.

In Alport syndrome, most gene products are known. It was first established that the primary defect affects a main component of the glomerular basement membrane (GBM), type IV collagen. Each type IV collagen molecule is formed of 3 α chains. Six different α chains have been identified: α1 and α2, whose genes are located on chromosome 13, α3 and α4, whose genes are on chromosome 2, and α5 and α6 chains, whose encoding genes are localized on the X chromosome. Genetic heterogeneity of Alport syndrome has been documented for many years: in 80 percent of the families, the disorder is transmitted as an X-linked dominant trait, whereas in the remaining kindreds, it is inherited as an autosomal trait, either dominant or recessive (in the latter case, a consanguineous marriage predisposes to the appearance of the diseased, homozygous state in the offspring, both parents being heterozygotes).

The classical X-linked Alport syndrome is characterized by mutations involving the gene encoding for α5(IV). More than 150 mutations have been identified so far. Most of them are «private», being found in only a single family, and differing from one family to another. In a few families, nephritis and hearing loss are associated with diffuse leiomyomatosis. The DNA lesion is different from that found in classical Alport syndrome: it is characterized by a DNA defect involving both α5 and α6(IV) genes [12].

The existence of autosomal recessive forms of Alport syndrome had been strongly suggested several years ago by analysis of pedigrees (Feingold et al). Molecular genetics have definitively established this mode of inheritance. The mutations affect α3 and α4(IV) genes [13]. As expected, the disease is uniformly distributed among males and females. These patients have an early progression to ESRD. Progression to ESRD in a girl at 15 years of age or less should be highly suggestive of this type of disease.

Thus, Alport syndrome encompasses several diseases, the biochemical and molecular defects of which have been in part characterized. These advances will lead to a new classification based on these defects. However, a more complete characterization is still awaited; for example, the defect(s) involved in the autosomal dominant forms, including that with macrothrombocytopenia, are unknown. In an Irish autosomal dominant Alport family, linkage to α3 and α4(IV) has been recently observed [14].

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