Towards the Integration of Genetic Knowledge into Clinical Practice

Albert C.M. Ong^a Olivier Devuyst^b

^aKidney Genetics Group, Academic Nephrology Unit, The Medical School, University of Sheffield, Sheffield, UK;

^bDivision of Nephrology, Université Catholique de Louvain Medical School, Brussels, Belgium

Key Words

Rare diseases · Disease registries · Orphan drugs · Autosomal dominant polycystic kidney disease

Abstract

The past three decades have seen a revolution in molecular biology and genetics that have changed the way we define disease, diagnose it, understand pathogenesis, initiate new treatments and assess individual responsiveness to intervention. The 'new genetics' has made its biggest impact on monogenic or 'rare' diseases, although its impact is increasingly being felt in the polymorphic so-called 'common' diseases. In this brief review, we summarise the efforts being made to improve the management of rare kidney diseases in Europe through the EUNEFRON network and also the rapid progress being made internationally in translating genetic knowledge for patient benefit in autosomal dominant polycystic kidney disease, the most common inherited kidney disease.

Copyright © 2010 S. Karger AG, Basel

The past three decades have seen a revolution in molecular biology and genetics that have changed the way we define disease, diagnose it, understand pathogenesis, initiate new treatments and assess individual responsive-

ness to intervention. The 'new genetics' has made its biggest impact on monogenic or 'rare' diseases, although its impact is increasingly being felt in the polymorphic so-called 'common' diseases.

The current accepted definition of what constitutes a rare disease varies from country to country. In Europe, this equates to a prevalence of <1:2,000, in the US to 1:1,250 and in Japan to 1:2,500. In an age of economic austerity, it could be argued that money would be best spent on studying and investigating more common diseases. However, even as defined above, the number of diseases involved is over 5,000 (by WHO definitions) [1]. This translates to potentially 30 million affected patients in Europe and another 25 million in North America. The recognition that rare diseases are an important but neglected medical and social problem has given fresh impetus to addressing these unmet needs. Key 'rare disease initiatives' have been started in the USA, Europe and Japan through the lobbying efforts of patient groups, clinicians and researchers and resulted in the establishment of new networks, new legislation and new treatments [2, 3].

In this brief review, we summarise the efforts being made to improve the management of rare kidney diseases in Europe through the European Network for the Study of Orphan Nephropathies (EUNEFRON) network and also the rapid progress being made internationally in

translating genetic knowledge for patient benefit in autosomal dominant polycystic kidney disease (ADPKD), the most common inherited kidney disease.

Impact of Genetic Knowledge for Rare Inherited Disorders of the Kidney

There are at least sixty rare inherited renal diseases which have a large negative impact on the quality of life of patients, often children, and their families [4–6]. The care of patients with rare nephropathies is hampered by major problems. Most of the diseases are chronically debilitating conditions and some are life threatening. Their very rarity and phenotypic variability imply limited knowledge of the underlying disease mechanism(s), natural history, lack of standardisation of diagnostic procedures and fragmentation of clinical and biological data collections, with small cohorts restricting the power of clinical studies [7, 8]. Furthermore, the low prevalence has meant that they have lacked priority for the pharmaceutical industry and public funding.

Despite significant research efforts, our understanding of the natural course and mechanisms of rare inherited diseases of the kidney remains limited. The discovery of the genes involved in these disorders has often led to the characterization of the encoded proteins and the establishment of their role in renal structure and function. However, we know much less about the regulatory pathways or the molecular and cellular mechanisms that cause their malfunction in renal disease. As a result, little progress has been made towards improved diagnosis and therapy for these conditions. Improved coordination between individual areas of expertise is necessary to improve our understanding of such inherited disorders, both at the clinical and mechanistic level, with the aim of developing preventive, diagnostic and therapeutic interventions. In addition to these specific disorders, such efforts could yield more general insights into renal disease progression, blood pressure control, prevention of renal stones, the effects of gender and ageing and multi-system involvement of renal diseases.

There is a growing interest in coordinating research and collecting information pertinent to inherited nephropathies by mobilizing a critical mass of expertise to investigate, on an international scale, the natural history and pathophysiology of such disorders [4, 7, 8]. Genetic knowledge has generated technological opportunities that have been used by international networks and projects, including the European Renal Genome project

funded by the 6th framework program of the European Community. A more recent example is the EUNEFRON network, funded by the FP7 program [4]. The aim of EUNEFRON is to utilise *in vitro* and *in vivo* models to pursue specific objectives in sixteen diseases affecting the glomerulus, the proximal tubule, the thick ascending limb, the distal convoluted tubule, and the collecting duct (fig. 1). These diseases are caused by mutations in twenty genes that encode proteins involved in a wide range of functions, including enzymatic activities, transport mechanisms, structure, and transcriptional regulation. The project also includes initiatives to create European-wide registries and a network of genetic laboratories to foster interactions between physicians and researchers, promote clinical and basic research, and ensure efficient diagnostic procedures. It includes a strategy for dissemination, involving patient organizations, experts in ethics and socioeconomics, as well as specific aspects such as the establishment of biobanks. The integration of genetics, clinical phenotyping and basic sciences will drive the establishment of phenotype-driven screens, the generation of disease models (ranging from transgenic mice to model organisms and cellular systems) and will improve and standardise the clinical expertise necessary to characterise larger patient cohorts. Both are essential to the success of such programs and their ultimate impact on changing clinical practice (fig. 2).

Impact of Genetic Knowledge for ADPKD: From Genes to Treatment

The incidence of ADPKD has been estimated at between 1:500 to 1:1,000, and its prevalence at between 1:500 to 1:4,000 in different populations [9]. This wide variability could be due to differences in methodological assessment and/or ethnic and geographical factors. This makes ADPKD something between a rare and common disease. However, it remains the most prevalent monogenic disease leading to renal failure, accounting for 8–10% of patients on renal replacement therapy.

The Age of Cloning Genes

The history of ADPKD is a good example of the ‘reverse genetics’ approach taken by investigators seeking to pinpoint the genes mutated in single gene disorders (table 1). Until 1985, the ‘ADPKD gene’ was another disease gene waiting to be discovered. Genetic linkage of the major ADPKD locus (subsequently named *PKD1*) to the short arm of chromosome 16 was the major breakthrough

Fig. 1. The sixteen rare inherited nephropathies that are investigated in EUNEFRON, grouped by segment. These diseases are caused by mutations in twenty genes (indicated in italics).

Fig. 2. Pathways of translating genetic knowledge to clinical application. GWAS = Genome-wide association studies.

Table 1. Timeline of the major clinical, genetics and experimental advances in ADPKD

1841	'Cystic degeneration' described pathologically
1888	'Polycystic kidneys' recognised clinically
1899	Dominant transmission documented in some pedigrees
1957	Incidence of 1:1,000 reported
1985	Linkage of PKD1 to chromosome 16
1993	Linkage of PKD2 to chromosome 4
1994	Identification of PKD1
1996	Identification of PKD2
1997	First Pkd1 knockout mice reported
1997	Polycystin-1/polycystin-2 interaction identified
1998	First Pkd2 knockout mice reported
2006	CRISP study
2007	Comprehensive mutation diagnostics for PKD1 and PKD2
2007	First clinical trials started
2009	Revised ultrasound diagnostic criteria for ADPKD

that initiated concerted efforts to clone this gene. Identification of *PKD1* also led to the recognition of a second locus (named *PKD2*) in the 10–15% of families unlinked to chromosome 16. *PKD2* was later linked by several groups to the long arm of chromosome 4. The identification of *PKD1* took another 9 years due in part to the complex nature of the *PKD1* genomic region and genetic duplication of *PKD1*. *PKD2* was identified in 1996.

The identification of both genes had several important consequences [10]. First, the normal functions of the polycystin proteins could be studied directly, and the consequences of mutation on protein function understood. The identification of a whole family of 'polycystin-like' proteins which are likely to mediate other biological functions outside the kidney was an unexpected finding. Second, mice bearing mutations in either gene could be generated directly by gene targeting. These models have proven to be critical for studying the molecular basis of disease and its natural history and serve as models for preclinical testing. Third, the diagnosis of ADPKD could be made with improved accuracy by mutational analysis. This in turn allowed careful genotype-phenotype studies in ADPKD patients to be performed and potentially define subgroups for clinical study and treatment.

Proteins and Mice

The PKD1 protein, named polycystin-1, turned out to be a large, complex, modular integral membrane receptor-like protein, with no known resemblance to previously known proteins. Polycystin-2, the PKD2 protein, on the other hand, had strong similarities in its sequence and

topology to other non-selective calcium channel proteins, and was therefore predicted to function as a channel. We know now that the polycystin proteins likely form a heteromeric complex important in regulating epithelial morphogenesis [10]. In addition, polycystin-2 has been shown to form homomeric complexes and to heterodimerise with other calcium channel subunits (other than polycystin-1) to mediate unexpected biological functions such as the determination of body axis laterality and cutaneous temperature sensation [11, 12]. As expected, expression of both proteins is widespread consistent with the systemic nature of the disease.

The generation of knockout mice for PKD1 and PKD2 has given us many useful models for studying the molecular basis of cyst formation as well as for testing potential therapies. The generation of an unstable Pkd2 allele susceptible to somatic recombination (ws25) led to experimental confirmation of a 'two-hit' model of cyst formation [13]. Nonetheless, other hypomorphic mouse alleles indicate that a dosage-dependent (or haploinsufficient) mechanism can also give rise to cysts and specific phenotypes. Early models were limited in their usefulness for preclinical testing by embryonic lethality. However, this has been overcome through the generation of new conditionally inactivated alleles which can be controlled in time (foetal or adult) and space (tissue or segment selective inactivation).

New Treatments

Building on previous evidence of a central role for cyclic AMP in cyst growth (through effects on cell proliferation and fluid secretion), the effects of a small molecule vasopressin type 2 receptor antagonist were tested in a Pkd2 mouse model (and other non-orthologous models) and found to dramatically reduce cystic disease [14]. This is currently being tested in a multicentre phase 3 clinical trial (TEMPO). Two other classes of compounds, mTOR inhibitors (everolimus, sirolimus) and long-acting somatostatin analogues (octreotide) are also undergoing clinical trials, the former having showed efficacy in ADPKD rodent models. Since loss of GFR occurs over decades in human ADPKD, it had been important to identify other surrogate markers for disease progression both to identify patients at higher risk and to monitor the effects of treatment. Results of the Consortium of Radiological Imaging Studies of Polycystic Kidney Disease study suggested that the rate of change in renal volume (assessed by MRI) could be a useful marker for the rate of GFR decline even when the latter was relatively well preserved (measured GFR >70 ml/min) [15].

Diagnosis and Prognosis

The widely accepted ultrasound criteria used for diagnosing PKD1 patients have been recently revised to encompass PKD2 [16]. Nonetheless, making a positive diagnosis in the absence of a positive family history or disease exclusion in those with a positive family history but equivocal scans remains difficult in a significant number. Advances in DNA sequencing have, however, made mutational analysis of *PKD1* and *PKD2* feasible and routine with diagnostic rates of up to 90% [17].

Several studies have established clear correlations between particular *PKD1* genotypes and specific disease phenotypes (age of ESRD, vascular complications and early-onset disease). Of interest, several hypomorphic *PKD1* alleles with no apparent phenotype in the heterozygous state have recently been reported with an early-onset presentation when transmitted either as homozygous or compound heterozygous mutations (with null alleles) [18]. Similarly, individuals trans-heterozygous for mutations of *PKD1* and *PKD2* have been described. The ability to identify individual patient *PKD1* and *PKD2* mutations will be important in defining the role of individual alleles in determining prognosis in the context of non-allelic genetic modifiers and environmental factors, as well as for individual responses to different treatments [19].

Genetic Knowledge: Basis for Registries and Networking

Most inherited diseases show a significant phenotypic and genetic heterogeneity, which hampers their clinical characterization and the initiation of therapeutic trials. The collaboration of expert clinical centres will facilitate the centralisation of clinical and biological information based on a large recruitment of patients through the creation of international registries and networks of genetic laboratories [7, 8]. The registry is a critical tool to improve our knowledge of the natural course of the disease, standardise diagnostic criteria and disease outcomes, facilitate the dissemination of information, promote basic and clinical investigation and improve clinical care and follow-up. For instance, extension of the UK Cystinosis Registry to other European centres will allow the role of gender, age and the effect of cysteamine and additive therapies to be assessed more widely. Similarly, the creation of a network of genetic laboratories should improve the procedures for genetic diagnosis, simplify access to genotyping and facilitate the search for new genes.

Genetic Knowledge: Engagement with Patient Groups

For ADPKD, the role of the PKD Foundation (USA) has been pivotal in engaging patients, raising funds for research and lobbying for it to be a priority for federal support. Many other patient groups have similarly engaged in this process [20]. In the USA, more than 1,200 patient organisations are linked to one of two networks, the National Organisation for Rare Disorders or the Genetic Alliance. In Europe, the European Organisation for Rare Diseases through the Orphanet database lists around 1,700 organisations. These networks have helped to establish and disseminate reliable disease information, improved access to diagnostic testing and stimulated co-operation with other stakeholders in determining the national and transnational agenda in terms of research funding, public policy and new legislation. A unique feature of the Japanese National Programme on Rare and Intractable Diseases is that it allows patients in one of 45 specified diseases to self-enrol in a national register with the benefits of government subsidies for treatment [2].

Genetic Knowledge: Engagement with Industry

Finally, there is a need to engage with pharmaceutical companies to drive drug development for rare diseases. These developments could be further incentivised above the commercial advantages already available through the Orphan Drug Act in the USA (1983) and the European Commission Committee for Orphan Medicinal Products (1999) [21]. Many of the new treatments are truly innovative, at the cutting edge of drug discovery and may have applicability to the future treatment of common diseases.

Acknowledgements

O.D. acknowledges the EUNEFron (FP7, GA No. 201590) projects of the European Community. A.C.M.O. acknowledges funding from the Wellcome Trust and the PKD Foundation (USA).

References

- Schieppati A, Henter JI, Daina E, Aperia A: Why rare diseases are an important medical and social issue. *Lancet* 2008;371:2039–2041.
- Hayashi S, Umeda T: 35 years of Japanese policy on rare diseases. *Lancet* 2008;372:889–890.
- Remuzzi G, Garattini S: Rare diseases: what's next? *Lancet* 2008;371:1978–1979.
- Devuyst O, Meij I, Jeunemaitre X, Ronco P, Antignac C, Christensen EI, Knoers NV, Levtschenko EN, Deen PM, Muller D, Wagner CA, Rampoldi L, van't Hoff WG: EUNEFron, the European Network for the Study of Orphan Nephropathies. *Nephrol Dial Transplant* 2009;24:2011–2015.
- Grunfeld JP: Monogenic renal diseases: a clinical introduction. *J Nephrol* 2002; 15(suppl 6):S43–S46.
- Scheinman SJ, Guay-Woodford LM, Thakker RV, Warnock DG: Genetic disorders of renal electrolyte transport. *N Engl J Med* 1999;340:1177–1187.
- Ayme S, Schmidtke J: Networking for rare diseases: a necessity for Europe. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2007;50:1477–1483.
- Watson MS, Epstein C, Howell RR, Jones MC, Korf BR, McCabe ER, Simpson JL: Developing a national collaborative study system for rare genetic diseases. *Genet Med* 2008;10:325–329.
- Levy M, Feingold J: Estimating prevalence in single-gene kidney diseases progressing to renal failure. *Kidney Int* 2000;58:925–943.
- Ong AC, Harris PC: Molecular pathogenesis of ADPKD: the polycystin complex gets complex. *Kidney Int* 2005;67:1234–1247.
- Kottgen M, Buchholz B, Garcia-Gonzalez MA, Kotsis F, Fu X, Doerken M, Boehlke C, Steffl D, Tauber R, Wegierski T, Nitschke R, Suzuki M, Kramer-Zucker A, Germino GG, Watnick T, Prenen J, Nilius B, Kuehn EW, Walz G: TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol* 2008;182:437–447.
- Giamarchi A, Feng S, Rodat-Despoix L, Xu Y, Bubenshchikova E, Newby LJ, Hao J, Gaudio C, Crest M, Lupas AN, Honore E, Williamson MP, Obara T, Ong AC, Delmas P: A polycystin-2 (TRPP2) dimerization domain essential for the function of heteromeric polycystin complexes. *EMBO J* 2010;29: 1176–1191.
- Wu G, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, Maeda Y, Le TC, Hou H Jr, Kucherlapati R, Edelmann W, Somlo S: Somatic inactivation of PKD2 results in polycystic kidney disease. *Cell* 1998;93:177–188.
- Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH 2nd: Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med* 2004;10:363–364.
- Grantham JJ, Torres VE, Chapman AB, Guay-Woodford LM, Bae KT, King BF Jr, Wetzel LH, Baumgarten DA, Kenney PJ, Harris PC, Klahr S, Bennett WM, Hirschman GN, Meyers CM, Zhang X, Zhu F, Miller JP: Volume progression in polycystic kidney disease. *N Engl J Med* 2006;354:2122–2130.
- Pei Y, Obaji J, Dupuis A, Paterson AD, Magistroni R, Dicks E, Parfrey P, Cramer B, Coto E, Torra R, San Millan JL, Gibson R, Breuning M, Peters D, Ravine D: Unified criteria for ultrasonographic diagnosis of ADPKD. *J Am Soc Nephrol* 2009;20:205–212.
- Rossetti S, Consugar MB, Chapman AB, Torres VE, Guay-Woodford LM, Grantham JJ, Bennett WM, Meyers CM, Walker DL, Bae K, Zhang QJ, Thompson PA, Miller JP, Harris PC: Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 2007;18: 2143–2160.
- Rossetti S, Kubly VJ, Consugar MB, Hopp K, Roy S, Horsley SW, Chauveau D, Rees L, Barratt TM, van't Hoff WG, Niaudet WP, Torres VE, Harris PC: Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. *Kidney Int* 2009;75:848–855.
- Paterson AD, Magistroni R, He N, Wang K, Johnson A, Fain PR, Dicks E, Parfrey P, St George-Hyslop P, Pei Y: Progressive loss of renal function is an age-dependent heritable trait in type 1 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 2005; 16:755–762.
- Ayme S, Kole A, Groot S: Empowerment of patients: lessons from the rare diseases community. *Lancet* 2008;371:2048–2051.
- Haffner ME, Torrent-Farnell J, Maher PD: Does orphan drug legislation really answer the needs of patients? *Lancet* 2008;371:2041–2044.