Towards the Integration of Genetic Knowledge into Clinical Practice

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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.

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) \cite{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 \cite{2,3}.

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.

Key Words
Rare diseases \quad Disease registries \quad Orphan drugs \quad Autosomal dominant polycystic kidney disease
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.
Towards the Integration of Genetic Knowledge into Clinical Practice

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

\textbf{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].

\textbf{Proteins and Mice}

The \textit{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 \textit{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 \textit{PKD1} and \textit{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).

\begin{table}
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\caption{Timeline of the major clinical, genetics and experimental advances in ADPKD}
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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 \\
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 \\
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**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 cooperation 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.

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