Commentary

Genetic Testing and FOX News

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Genetic testing is transforming kidney care, arguably with similar impact as the adoption of kidney biopsies in the 1950s. It is incumbent on nephrologists to teach the genetics of kidney disease and the accessibility of genetic testing. Proper usage of genetic testing can avoid "diagnostic odysseys" with multiple nonspecific investigations, some of them invasive like a kidney biopsy and repeated consultations with multiple different specialists [1].

The Evolution of Genetic Testing

Initial genetic testing was typically on a single gene ba- sis, but with the advent of massively parallel sequencing, hundreds or thousands of genes or even the entire genome can now be sequenced easily and at relatively low cost. As always, such technological advances have advantages and downsides: given the phenotypic heterogeneity of many kidney disorders, unbiased testing such as whole- exome sequencing or whole-genome sequencing (WGS) can provide a higher diagnostic yield, as they allow analysis of more than just the top candidate gene(s). Yet, each genome contains approximately 4-5 million variants from the reference sequence, including about 12,000 peptide changing variants, 150–200 predicted loss-of-function variants (nonsense or splice-site variants), and 25–30 variants annotated as disease-causing in ClinVar [2]. Thus, the more we sequence, the more variants we find, and it becomes increasingly difficult to sort the disease- relevant variants from all the others, risking false-positive findings. We therefore prioritize variants based on our knowledge of gene-disease association, allele frequency, and disease biology. This highlights the critical importance of close communication between genetic scientist and clinician and specificity of the clinical phenotype for the respective gene is one of the criteria for variant annotation. In fact, the first and most important question is always: does the gene fit the phenotype?

Genetic Testing in Kidney Diseases

With the evolution of genetic testing, its use in the diagnosis of kidney diseases is changing, as well: from specific testing for individual inherited disorders to panels of genes for related disorders, to larger panels or even whole- exome sequencing or WGS to encompass all kidney disease-relevant genes. A substantial portion of subjects with kidney disease is found to have

causative genetic variants. For instance, in chronic kidney disease, close to 10% of adults are reported to have an identifiable genetic cause and obviously, the odds of finding a genetic cause increase, if there is a family history of nephropathy or younger age of onset [3].

In order to avoid the problem of excessive variant identification and the ethical dilemma of unexpected secondary findings e.g., in cancer genes, a common approach currently is to perform genetic testing using a fixed panel of genes, selected for their relevance to the disorder in question. In this issue of the journal, Bleyer et al. [4] report on their experience with just over 1,000 subjects using a commercially available panel for kidney diseases, containing 382 genes.

A Few Important Genes

Overall, the authors report a diagnostic yield of just over 20%. This is consistent with referral bias typical for studies from clinical genetic testing laboratories: clinicians are much more likely to order a genetic test if they suspect an underlying inherited disorder. But the interesting part is that only 48 of the 382 genes in the panel contributed to the diagnostic yield and the vast majority (~70%) of diagnostic hits was in only 6 genes. Some of these are expected, for example, *PKD1* and *PKD2*, which accounted for ~44% of positive results. But perhaps surprising was the frequency of hits in *COL4A3*, *COL4A4*, and *COL4A5*, which together accounted for almost a quarter of all positive findings. Unfortunately, no data are presented about the clinical phenotype of these patients, even though this is critical to assess true pathogenicity. Heterozygous variants in the *COL4A* genes are not necessarily associated with kidney failure. Many carriers maintain lifelong normal kidney function and their diagnosis would have previously been called "benign familial haematuria." Indeed, predicted pathogenic variants in *COL4A3/4* are seen in more than 1% of the general population [5]. Again, the key question is: does the gene fit the phenotype? Identification of a *COL4A* variant is much more likely to be causative in someone with proteinuric kidney disease than cystic dysplasia. This highlights the importance of close communication between clinician and genetic scientists and the difficulties of many genetic laboratories, who often are given only limited clinical information. Yet, just as the pathologist will want to have detailed clinical information when assessing a biopsy, so does the genetic scientist when performing variant annotation.

Susceptibility Genes: "We Report, You Decide"

This uncertainty is even more problematic with some other genes included in the panel, that are not directly causative for kidney disease but associated with an in-creased susceptibility, such as *APOL1*, *HBB* (associated with hemoglobinopathies, such as sickle cell disease), *TRR* (associated with amyloidosis), or *ADCY10* (associated with absorptive hypercalciuria).

The genetic variants in *APOL1* present a fascinating story: *APOL1* encodes apolipoprotein-1, which is involved in the defense against African sleeping sickness caused by *Trypanosoma brucei rhodesiense* and *gambiense*. Two variants (termed G1 and G2) were identified that have enhanced lytic activity against *trypanosoma* in vitro. These variants are found in >50% of some African populations and the variant frequency correlates with the prevalence of endemic sleeping sickness. This is consistent with evolutionary selection, as the variants provide protection against the frequently lethal disease [6]. Unfortunately, biallelic presence (G1/G1, G1/G2, or G2/G2) is strongly associated with increased susceptibility to chronic kidney disease and more rapid decline in kidney function [6].

Testing for these variants creates challenges for result interpretation, since these *APOL1* variants do not constitute pathogenic variants in the usual way. For instance, if a variant in *PKD1* is considered pathogenic, then it segregates with the disease: virtually everybody who carries that variant will be affected with polycystic kidney disease and the variant will not be

identified in unaffected controls. Yet, the G1 and G2 alleles are very common in African controls. Thus, if these variants are identified in a patient with unexplained kidney disease, are they the explanation? Or just an incidental finding? The cohort reported by Bleyer et al. [6] included 171 individuals of African ancestry and the variants were found in 57 (33%), similar to control populations. Thus, did these variants "cause" the kidney disease in any of these individuals? Unfortunately, the authors do not investigate this aspect at all, but it does raise the issue of how useful it is to include susceptibility genes in a diagnostic panel. While there is no doubt that they contribute to kidney disease on a population level, it gets much more difficult when assigning attribution on an individual level. Imagine, you are caring for a 40-year-old gentleman of African ancestry with proteinuric kidney failure and a histological picture of FSGS. One of the key questions in individuals with FSGS is whether the disease will recur after transplantation. As patients with an identified genetic cause are very unlikely to have post-transplant recurrence, you performed genetic testing in him, using the panel reported by Bleyer et al. [6] and the only positive finding is the presence of a high-risk *APOL1* allele. What do you tell the patient? Has the result shed any light on disease cause and recurrence risk? Is it the cause of the disease? Or a contributing factor? If the latter, how much did it contribute and to what underlying process?

Interestingly, some of the tested subjects were unaffected by kidney disease and testing was performed to establish suitability for kidney donation. Four of these were found to carry a high-risk *APOL1* genotype. The utility of testing for *APOL1* prior to transplantation is still controversial and for precisely the reason discussed above: the effect of high-risk alleles on kidney disease is clearly important on a population level, but difficult to quantify on an individual level. So, did the identification of the high- risk genotypes affect decisions on donation? Unfortunately, the authors again do not discuss this at all. They and the makers of the panel seem to adopt the motto of FOX News: "we report, you decide." This also applies to the other susceptibility genes in the panel. Interpretation is easier if the respective gene is associated with a specific phenotype. For instance, identification of a disease-associated variant in *TRR* is likely to be causative in a patient with amyloidosis, but not in one with cystic kidney disease. But how would the finding of sickle cell trait help in counselling our 40-year-old gentleman with FSGS and kidney failure?

The Future of Genetic Testing

These examples highlight the difficulties in interpreting genetic testing results, but also in establishing a useful diagnostic panel: as detailed above, most causative variants are present in a relatively small number of genes. Thus, increasing the number of genes in the panel only marginally increases the diagnostic yield. Yet, as with any testing: the more tests are performed, the higher the likelihood of getting false-positive results. Another obvious problem of fixed testing panels is that they get dated easily: identification of newly discovered disease genes renders the panel outdated. If a patient had been tested previously without a positive finding, the whole testing needs to be repeated on a revised panel. Thus, fixed testing panels suffer from the paradox of having too many genes on the panel and therefore increasing the likelihood of false- positive findings and not enough genes, increasing the chance of false negative results.

Genetic Testing

The future, in our opinion, is WGS, as recently reported in a large national project in the UK [7]. Indeed, in the UK, genetic testing, which is part of the National Health Service, is currently in the process of transitioning all sequencing tests to WGS. This technique can detect causative variants not identifiable by traditional methods that focus on the coding regions of the genome, such as deep intronic variants that generate new splice sites, or large inversions with intronic breakpoints. Moreover, it is essentially "future proof": if a new relevant disease gene is identified, the testing laboratory only needs to go back to the existing sequencing data and

assess for variants in the relevant gene. It also has the advantage of dramatically simplifying the work process in the genetics laboratory, as only the same single test is performed for almost all sequencing applications. Of course, the risk of false-positive findings is dramatically increased when sequencing 3 billion base pairs. But, this is mitigated by using "virtual panels": only the sequencing data from genes relevant to the patient's phenotype are analyzed. Phenotype-specific panels are available, e.g., via "panelapp" (https://pan- elapp.genomicsengland.co.uk), which is crowdsourced (by registered users, similar to Wikipedia), peer-reviewed, and regularly updated.

Yet, until the widespread adoption of WGS, fixed panels, such as reported by Bleyer et al. [6], do a satisfactory job at identifying causative genetic variants, but great care and skill are needed in the interpretation of individual results, a skill that we will all need to master if we want to be "future proof" nephrologists, because, in the end, the lab reports, we decide.

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