The 2014 International Workshop on Alport Syndrome

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Alport syndrome, historically referred to as hereditary glomerulonephritis with sensorineural deafness and anterior lenticonus, is a genetic disease of collagen α3α4α5(IV) resulting in renal failure. The collagen α3α4α5(IV) heterotrimer forms a network that is a major component of the kidney glomerular basement membrane (GBM) and basement membranes in the cochlea and eye. Alport syndrome, estimated to affect 1 in 5000–10,000 individuals, is caused by mutations in any one of the three genes that encode the α chain components of the collagen α3α4α5(IV) heterotrimer: *COL4A3*, *COL4A4*, and *COL4A5*. Although angiotensin-converting enzyme inhibition is effective in Alport syndrome patients for slowing progression to end-stage renal disease, it is neither a cure nor an adequate long-term protector. The 2014 International Workshop on Alport Syndrome, held in Oxford, UK, from January 3–5, was organized by individuals and families living with Alport syndrome, in concert with international experts in the clinical, genetic, and basic science aspects of the disease. Stakeholders from diverse communities—patient families, physicians, geneticists, researchers, Pharma, and funding organizations—were brought together so that they could meet and learn from each other and establish strategies and collaborations for the future, with the overall aim of discovering much needed new treatments to prolong kidney function.

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Alport syndrome is a hereditary glomerular disease leading almost inevitably to end-stage renal disease. The syndrome is usually associated with sensorineural hearing loss and distinct ocular abnormalities.[1] In the early 1990s Alport syndrome was shown to be caused by defects in collagen α3α4α5(IV), one of the three network-forming isoforms of type IV collagen.[2] This isoform is the major collagen IV component of the kidney glomerular basement membrane (GBM).[3] Although there have been recent improvements in patient management, there is no cure for Alport syndrome.

The 2014 International Workshop on Alport Syndrome, 'Shining a Light on Alport Syndrome', was held at the Said Business School in Oxford, UK, from January 3–5. This meeting was organized through the concerted efforts of patient advocacy groups from around the world. It brought together an internationally diverse group of physicians, geneticists, and scientists from academia and Pharma, many of whom were not specifically Alport syndrome experts, to learn about and discuss the latest findings regarding diagnosis, treatment, and molecular mechanisms of disease progression. The Workshop had four major areas of focus: Genetics/Diagnosis, Basic Science, Treatment, and Patient Registries/Clinical Trials. An important aspect of the Workshop was the ability of scientists working in laboratories to meet individuals and families affected by Alport syndrome and to hear firsthand their perspective about what it is like to live with the disease. Although most of the attendees typically

focused on the kidney disease features of Alport syndrome, eye and hearing defects were also discussed as being very important diagnostic and quality-of-life aspects that need to be considered.

As reported in the Genetics/Diagnosis session and at a special pre-meeting focused on the same topics, mutation screening of the relevant *COL4* genes is now widely available.[4] Because of this, although relatively expensive, increasing numbers of affected individuals have had their mutation(s) identified. There are currently six databases for variants in *COL4A5*, the gene affected in the X-linked, most common form of Alport syndrome. The consortium of genetic-testing laboratories for Alport syndrome has chosen to use the Leiden Open Variant Database system.[5] This freely accessible database (http://www.lovd.nl/3.0/home) includes clinical features, multiple examples of the same variant from unrelated individuals, and normal variants. The value of internationally accessible, regularly updated variant databases is clear. Recently, members of the Alport Variant Consortium added 500 variants to the *COL4A5* database, bringing the total number to 1900, with 1100 unique changes. The variants include 40% missense mutations and approximately 50% nonsense mutations. Glycine substitutions occur four times as often as substitutions of other amino acids in the Gly-X-Y triplet repeat collagenous segments. This is consistent with the necessity of a Gly at every third residue to form and stabilize the triple helical structure of the collagenous domain.

As summarized by Frances Flinter, there are several molecular approaches for identifying variants. Although Sanger sequencing is the gold standard, custom next-generation sequencing panels, described by Michael Yau (GSTS Pathology, Guy's & St Thomas' Hospital, London, UK), plus whole-exome and whole-genome sequencing, are rapidly being validated and introduced into clinical practice. The challenges associated with the interpretation of variants were explained by Helen Storey (GSTS Pathology), as many mutations are novel and specific to individual families.[6] There are clearly founder mutations in some populations, however—e.g. in Britain, and in Cyprus, where Constantinos Deltas (University of Cyprus, Nicosia, Cyprus) has been studying a large number of families in which significant renal impairment has been noted in association with a single autosomal *COL4* mutation in some families.[7] Mutation detection within *COL4* genes remains incomplete partly because of missed rearrangements and cryptic splice site mutations. In some cases, RNA analysis is necessary in order to establish the potential pathogenicity of a variant. The tantalizing prospect of extracting RNA from podocytes in urine needs further exploration, as excreted podocytes could be a readily available source of RNA.

The unexpected clinical variation among affected individuals in some families was discussed by Daniel Gale and Jie Ding and could reflect variable control of hypertension and other environmental influences; variable inheritance of mutations or copy number variants in modifier genes could also be important. The introduction of more comprehensive screening technologies such as next-generation sequencing and exome sequencing allows simultaneous screens for mutations in other potentially relevant genes. Moin Saleem (University of Bristol, Bristol, UK) presented a gene panel for proteinuria potentially containing up to 37 genes for Alport syndrome and focal segmental glomerulosclerosis, including *NPHS1*, *NPHS2*, *MYH9*, and complement pathway genes.

It was noted that a clear genotype–phenotype correlation has emerged for X-linked Alport syndrome.[8,9] Large deletions and rearrangements, nonsense mutations, and missense mutations toward the carboxy terminus all result in more severe disease. In addition, some amino-acid substitutions (Arg, Glu, Asp) for Gly in the Gly-X-Y repeat collagenous regions are more damaging.

Clifford Kashtan described the natural history and considerable variations in the presentation of Alport syndrome. Indeed, a lively debate is underway regarding the autosomal genes and the appropriate nomenclature for individuals with a heterozygous *COL4A3* or *COL4A4* mutation. Some such individuals have hematuria and may develop renal impairment later in life vs. patients with classic Alport syndrome, but they do not manifest any extrarenal features. Some experts regard them as carriers of autosomal recessive Alport syndrome, acknowledging that this genetic status is associated with thin basement membrane nephropathy and an increased risk of hypertension and renal impairment, whereas others describe them as having autosomal dominant Alport syndrome, although many of these patients do not fulfill the standard clinical diagnostic criteria.[10]

Why is this issue so important? It is highly likely that the current EARLY PRO-TECT Alport trial of early ACE inhibition (www.clinicaltrials.gov; identifier NCT01485978) will be followed by other trials, as candidate therapies emerge from basic science research or from other clinical trials. It is thus essential that all patients who enroll in these trials have their diagnosis confirmed at the DNA level so that any possible genetic factors that may influence response to therapy are identified. Judith Savige noted that another diagnostic test that may be useful when genetic testing is not available is a retinal photograph that can show the characteristic fleck retinopathy or an optical coherence tomography scan that often shows temporal retinal thinning. It was also noted that the lens capsule removed during the surgery for lenticonus can be a good source of abnormal collagen α3α4α5(IV) for research, although this would depend upon the nature of the mutation.

The Basic Science aspects of the workshop focused initially on collagen IV structure, biochemistry, and assembly of heterotrimeric collagen IV building blocks into networks. Billy Hudson (Vanderbilt University Medical Center, Nashville, Tennessee, USA) spoke about the involvement of the enzyme peroxidasin in catalyzing the formation of the novel sulfilimine bond.[11,12] This bond, which was explained as important for strengthening the collagen IV network, links α chains in one heterotrimer to those in an adjacent heterotrimer via conserved Met and Lys residues. A phylogenetic

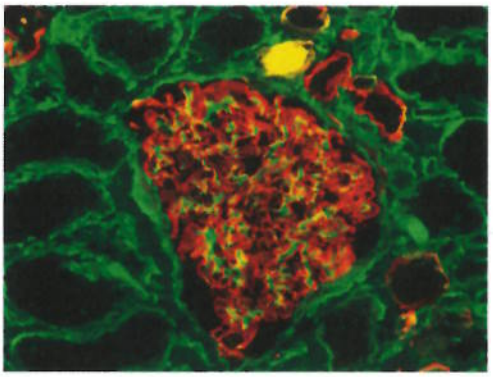

Figure 1 | The normal human kidney glomerular basement membrane (GBM) contains primarily collagen α3α4α5(IV), which is defective or missing in Alport syndrome. A frozen human kidney section was stained in red for COL4A4, representing the α3α4α5(IV) network, and in green for collagen α1α1α2(IV). The GBM and a few tubular basement membranes are enriched in collagen α3α4α5(IV). The α1α1α2(IV) network is detected primarily in the mesangial matrix and in tubular basement membranes.

investigation of this finding revealed that the sulfilimine bond is an ancient evolutionary adaptation first apparent in a subset of Cnidarians that was accompanied by the appearance of the peroxidasin gene.[13] Peroxidasin's enzymatic function was shown to involve the production of a bleach-like chemical that could theoretically be detrimental to the basement membrane if overproduced; this is one potential novel mechanism that could explain the GBM splitting that invariably occurs in Alport syndrome.

Three important aspects of normal GBM (Figure 1) and Alport GBM composition were addressed. Rachel Lennon (University of Manchester, Manchester, UK) described a brute-force high-resolution mass spectrometric analysis of isolated total glomerular extracellular matrix that revealed the presence of many proteins previously unknown to be resident in glomeruli.[14] Some of these will certainly be localized to the GBM (rather than mesangium) and could be secondarily involved in Alport GBM pathology. One particular standout localized to GBM is type VI collagen, which is known to be important in skeletal muscle but could act in the GBM to modify collagen IV assembly or function. In fact, collagen VI has already been shown to be increased in human Alport GBM.[15]

Jeffrey Miner presented rescue studies of *Col4a3* −/− Alport mice carrying a doxycycline-inducible Col4a3 transgene. This work provided a proof-of-principle demonstration that conversion of an abnormal Alport GBM containing only the collagen α1α1α2(IV) network to one also containing the missing collagen α3α4α5(IV) network is possible.[16] Restoration of the missing collagen IV network at various ages, as late as 7.5 weeks, was shown to slow down kidney disease progression and extend the life span. It was reported that, the earlier the induction with doxycycline, the longer the life span.

As presented by Dominic Cosgrove (Boys Town National Research Hospital, Omaha, Nebraska, USA), the presence of ectopic laminin α2 in the Alport GBM, secreted by mesangial cell processes that invade the peripheral capillary wall,[17] activates phosphorylation of focal adhesion kinase in podocytes, which causes detrimental foot process changes. This identifies focal adhesion kinase phosphorylation as a potential drug target for slowing down Alport syndrome. The involvement of integrin-mediated signals and the pathogenic effects of hypertension suggest that increased biomechanical strain[18] due to the defective GBM is a major factor in promoting podocyte injury.

Concepts thought by the collective basic science group to be important for the future included the following: defining targets for therapy; developing additional animal models to better model human mutations; and deriving cell lines from Alport mice and/or patients to gain a better understanding of how changes in the GBM cause changes to the overlying podocytes, which appear to be key to disease progression.

There were two major targets discussed for developing new treatments. The first was the defect in the GBM itself. Although most agreed that gene repair, gene replacement, protein replacement, or podocyte replacement therapies should be effective if applied early enough during the course of disease (again demonstrating the importance of early diagnosis), the technical hurdles are significant, as found previously in stem and blood cell infusion studies,[19] gene transfer studies with pigs[20] and, as mentioned by Karl Tryggvason (Karolinska Institute, Stockholm, Sweden and Duke NUS, Singapore City, Singapore), in a canine Alport model.

The second target discussed at length consisted of the collective 'molecular and cellular symptoms' that result from the primary defect in the GBM. Inhibition of focal adhesion kinase phosphorylation was suggested by Dominic Cosgrove as a therapy to protect podocytes, as was inhibition of collagen receptors DDR1 and integrin α2β1, the activation of which exacerbates Alport disease progression in mice,[21,22] as reported by the group of Oliver Gross. Studies on the effect of attenuating interstitial fibrosis on survival of Alport mice have yielded mixed results, with some studies showing improved survival and others showing no improvement.[23] It was agreed that efforts aimed at slowing down disease progression should focus on both the glomerular and the tubulointerstitial compartments, with protection of podocytes as a high priority. There was frequent mention during the Workshop of findings in mice showing that the same mutation can result in highly variable rates of progression to kidney failure, reinforcing the concept that genetic background has a significant role.[24,25] As mentioned above, next-generation sequencing could be beneficial in identifying the modifier genes responsible and hopefully reveal additional pharmacological targets. Infusion of amniotic fluid–derived stem cells into Alport mice was reported by Laura Perin (University of Southern California, Los Angeles, California, USA) to dampen angiotensin II receptor activation and attenuate fibrosis, but without any effect on GBM composition.[26] The targeting of the pro-fibrotic microRNA-21 was also discussed by Oliver Gross as a

very promising new approach to treatment.[27] Although most of the attendees were focused on mouse models, the existing dog models of Alport syndrome should also be considered for the testing of new therapies, given their larger size and longer life span compared with mice.

Nevertheless, the generation of new mouse models to better mimic human Alport syndrome was identified as a short-term need. This is because most mouse models are autosomal *Col4a3* or *Col4a4* gene knockouts, whereas most patients have missense mutations in the X-linked *COL4A5* gene, and many of these mutations are Gly substitutions that likely impair trimer assembly, secretion, network formation, or all three processes. Given the improved technologies for manipulating the mouse genome, as presented by Paul Potter (MRC Harwell, Oxfordshire, UK), generation of a few additional animal models was considered to be a reasonable goal for the near future. Two new mouse models were presented at the Workshop: one, generated by Constantinos Deltas (University of Cyprus, Nicosia, Cyprus), has a targeted Gly substitution in COL4A3 that is analogous to a human mutation (G1334E) common in Cyprus;[28] and one, isolated at The Jackson Laboratory, has a spontaneous splice site mutation in *Col4a4* that results in exon skipping and production of defective α3α4α5(IV) trimers.[29]

Data presented by Constantinos Deltas suggest that podocyte production of defective collagen IV chains can cause endoplasmic reticulum stress and perhaps exacerbate injury;[28] chaperones that promote protein folding could therefore be therapeutic in some settings.[27] Developing the appropriate tools for high-throughput testing of these or other classes of compounds in either cell or zebra fish models was thought to be an important goal for the future. Finally, the establishment of a 'toolbox' of reagents, including well-characterized collagen IV antibodies and cDNAs, for scientists working on Alport syndrome was proposed as an excellent mechanism to facilitate research efforts.

In the Registries and Clinical Trials Session it became clear that in Alport syndrome the main therapeutic priority is for treatments that are able to delay the progression of chronic kidney disease. For most patients, this progression occurs during childhood or adolescence; therefore, future trials of novel drugs are likely to be conducted chiefly in the pediatric and young adult setting. The vulnerability of this target population and the need for prolonged treatment suggest that evidence of safety for new drugs will be particularly important. Colin Baigent described experiences in other areas of medicine, indicating that most drug treatments have at best moderate effects (e.g., a 15–20% reduction) on major clinical outcomes[30] (e.g., progression to end-stage renal disease), implying the need for substantial sample sizes in new trials. For these reasons—that is, the need for large trials to provide robust evidence on both safety and efficacy—the Alport research community needs to establish appropriate infrastructure to facilitate such studies. At present, it is difficult to identify large numbers of eligible patients, as exemplified by the experience of the ongoing EARLY PRO-TECT Alport trial[31] (presented by Oliver Gross) assessing the effects of ramipril among children with no or minimal proteinuria. Current clinical practice recommendations focusing on early identification of proteinuria and treatment with angiotensin blockade[10,32] were supported by the Workshop participants.

Infrastructure to support trials implies an ability to identify and invite potentially eligible patients, and this in turn requires that patients are registered, phenotyped, genotyped, and actively followed up in national registries. As insufficient numbers of patients are available in any single country, a further requirement is that national registries are linked via a coordinating center, allowing simultaneous study of patients in multiple countries. Currently, there are registries of patients with Alport Syndrome in the United States (Alport Syndrome Treatments and Outcomes Registry; http://www.alportregistry.org), the United Kingdom (Renal Rare Disease Registry; https://www.renalradar.org), China (China Alport Syndrome Treatments and Outcomes Registry), the European Alport Registry, Italy (the Italian Alport National Registry and Biobank), and France (the French National Rare Disease Registry). In total, these registries include around 3000 individuals.

A review of these registries, as summarized by Parminder Judge, indicated that there is substantial overlap in the information collected in them, but that a major limitation if they are to be utilized to support trials (and other studies) is that they do not typically seek regular follow-up. An exception is the UK RaDaR registry, which links to 'Renal Patient View' (RPV; https://www.patientview.org). RPV incorporates laboratory results from participating UK renal clinics; thus, linkage between RaDaR and RPV allows phenotypic and genotypic information to be linked with renal functional data longitudinally.

The Alport syndrome research community present at the meeting agreed that there is a need to: (i) expand coverage of existing national registries (as only a fraction of registrants are likely to be eligible and willing to enter new trials); (ii) improve data collection in existing registries (especially with regard to arranging for regular follow-up information to be obtained); (iii) identify new countries where collections of Alport syndrome patients exist that could be incorporated into registries; (iv) build the infrastructure necessary to allow collaboration between registries in future trials and epidemiological studies; and (v) seek cooperation with companies that have promising agents for the treatment of Alport syndrome and explore practical technologies to allow international studies in conjunction with them.

Representatives from National Patient Organizations were in attendance from Australia, China, France, Germany, Italy, The Netherlands, Spain, United Kingdom, and the United States. The primary objective of these organizations was to discuss ways of working together to support research that will result in an improved quality-of-life for individuals and families living with Alport Syndrome. Susie Gear from Alport UK (http://www.alportuk.org), Daniel Renault from

AIRG-France (http://www.airg-france.fr), and Sharon Lagas from the Alport Syndrome Foundation (USA; http://www.alportsyndrome.org) presented personal perspectives on how this disease affects their lives and those of their constituencies by afflicting multiple family members in every generation with kidney failure and hearing loss. Research perspectives and strategies were also presented for each organization and included the need for patient input into research priorities, the use of seed funding to facilitate progress, and development of strategies to promote collaborations on larger funding initiatives.

Patient organizations have a pivotal role in educating their lay communities about current research and the value of patient involvement. At the Workshop, patient representatives learned about current research, listened to experts discuss and debate future strategies and priorities, and interacted with researchers to obtain answers to questions of interest to the patient community. Collaboration between the patient and research communities is critical for research on a rare disease such as Alport syndrome. This Workshop served to both strengthen existing collaborations and expand collaborations on an international scale. The development of an international alliance for Alport syndrome involving both the patient and research communities is a potential outcome of the Workshop.

Patient organizations recognize they can support research by recruiting patients and communicating the essential role of registries and clinical trials to foster more patient participation. In addition, patient organizations can communicate to the research community those patient concerns that are not being addressed for development of future research priorities. For example, when surveyed about their priorities for research, the majority of patients identified hearing and psychological/social support as important, but these are areas often overlooked in Alport syndrome research. Innovative workshops such as this that combine patient, clinical, scientific, and industry aims serve to foster collaboration and strategic planning that is critical to advance research. Future objectives to build upon the success of this Workshop include:

- Strengthening the patient communities in the respective countries by recruiting more patients and working with local clinicians, geneticists, and registries (or starting a new or joining an existing registry)
- Expanding patient organizations to additional countries
- Organizing at an international level to work strategically to support collaboration between national patient registries and research initiatives
- Developing national meetings with patients, clinicians, and scientists to foster collaboration, communication, and education
- Supporting future international workshops that bring together all stakeholders in the Alport syndrome community (patients, clinicians, geneticists, scientists, and Pharma).

In conclusion, the 2014 International Workshop on Alport Syndrome was not only able to 'shine a light on Alport syndrome', but the interactions among the various stakeholder groups were very effective at 'motivating, energizing, and inspiring' (these were actual responses on the Workshop feedback forms). Closing remarks by Karl Tryggvason on the history of Alport syndrome research and the prospects for future therapies and by Ted Bianco (Acting Director of the Wellcome Trust, London, UK) on prospects for funding international collaborations were especially encouraging. The participants agreed to try to translate the enthusiasm generated at the Workshop into an international program of collaborative research and funding proposals in the near future. This will involve both young and senior scientists and clinicians combining their efforts to find new strategies to protect the injured kidney, ear, and eye from additional damage and to eventually find a cure to prevent or reverse the disease. Several promising new therapies, all with different targets and mechanisms of action, were discussed as having potentially additive benefits to existing therapies and perhaps to each other. This provides hope that Alport syndrome can be prevented or attenuated in most patients by such a multimodal therapy that will likely continue to include ACE inhibition as the basic standard of care.

DISCLOSURE

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