The 2014 International Workshop on Alport Syndrome

Jeffrey H. Miner[1], Colin Baigent[2], Frances Flinter[3], Oliver Gross[4], Parminder Judge[2], Clifford E. Kashtan[5], Sharon Lagas[6], Judith Savige[7], Dave Blatt[8], Jie Ding[9], Daniel P. Gale[10], Julian P. Midgley[11], Sue Povey[12], Marco Prunotto[13], Daniel Renault[14], Jules Skelding[15], A. Neil Turner[16] and Susie Gear[15]

[1]Renal Division, Washington University School of Medicine, St Louis, Missouri, USA; [2]Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, UK; [3]Department of Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, London, UK; [4]Clinic of Nephrology and Rheumatology, University Medicine Goettingen, Goettingen, Germany; [5]Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota, USA; [6]Alport Syndrome Foundation, Phoenix, Arizona, USA; [7]Melbourne Health, The University of Melbourne, Parkville, Victoria, Australia; [8]Alport Foundation of Australia, Valentine, New South Wales, Australia; [9]Pediatric Department, Peking University First Hospital, Beijing, China; [10]University College London-Center for Nephrology, London, UK; [11]Department of Pediatrics, Alberta Children's Hospital, Calgary, Alberta, Canada; [12]Department of Genetics, Evolution and Environment, University College London, London, UK; [13]Discovery Technology Department, Pharma Research and Early Development, Basel, Switzerland; [14]Association for Information and Research on Genetic Renal Diseases (AIRG)-France, Paris, France; [15]Alport UK, Tetbury, UK and [16]Renal Medicine, Royal Infirmary, University of Edinburgh, Edinburgh, UK

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.

Correspondence: *Jeffrey H. Miner, Renal Division, Washington University School of Medicine, 660 S Euclid Avenue, St Louis, Missouri 63110, USA. E-mail: minerj@wustl.edu or Susie Gear, Alport UK, Tetbury, GL8 0AW, UK. E-mail: research@alport.info*

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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 $\alpha3\alpha4\alpha5$(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