AIRG grant application: 2016. Savige

Optimal chaperone therapy to slow the rate of renal failure progression in Alport syndrome: *in vitro* and clinical studies

Titre du project: Optimisation du traitement des chaperons chimiques dans le syndrome d'Alport de retarder l'apparition de l'insuffisance rénale au stade terminal: les études *in vitro* et cliniques

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Please note that we have applied for funding for the clinical trial aspects of this project from the Alport Foundation - Macquarie Pederson - Kidney Foundation of Canada Fund also.

AFFILIATED INSTITUTION RESPONSIBLE FOR THE BUDGET:
The University of Melbourne, Australia
Research domains: proteinuria, renal failure, Alport syndrome, chemical chaperones, ER stress, unfolded protein response, autophagy
Co investigators: Dr Yanyan Wang and Dongmao Wang M Medicine (PhD pending)

RATIONALE:
In both X-linked and autosomal recessive Alport syndrome, missense mutations account for at least 40% of variants, and direct or indirect nonsense changes for 40% (Savige, et al., 2013). With the commoner X-linked disease, 90% of males develop end-stage renal failure by the age of 40, and 15-30% of females by 60. Those with even milder renal impairment (eGFR < 50) still have a greatly increased risk of heart disease. There is currently no cure for Alport syndrome (Savige, 2014). The only known treatment is angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB)(Gross, et al., 2012) which can delay the onset of renal failure by up to 13 years. These agents have their effect through controlling hypertension and reducing proteinuria (which when absorbed by the tubules contributes to tubulointerstitial scarring and renal impairment) and through reducing fibrosis directly through effects on TGFβ.

Missense and nonsense mutations cause disease through different mechanisms. Missense mutations result in misfolded proteins and increased ER retention and stress. The aims of this project are to develop treatments that delay end-stage renal failure by targeting the pathogenetic mechanisms used by missense mutations.

In other genetic diseases caused by missense mutations, treatment with chemical 'chaperones' is helpful. Chaperones have different and often multiple effects, which may be additive. One of the most commonly-used agents is PBA. We have already tested PBA in cell lines from male subjects with X-linked Alport syndrome due to missense mutations, and presented this work at the American Society of Nephrology meeting in San Diego in November 2015. In cell lines the increased ER stress contributed to reduced cell growth of skin fibroblasts, and potentially the

development of proteinuria in glomerular podocytes (the cells that make the collagen a5 chain). We have demonstrated that PBA treatment reduces the amount of cell stress due to the retention of the misfolded mutant protein in the ER in cell culture (PhD theses from R Tan, M Mohamad, V Sivakumar and Dongmao Wang, U Melb).

It is time to test other chaperones in vitro in Alport syndrome since PBA is effective but very expensive, and other chaperones or a combination may be more even effective. It is also time to conduct a clinical trial of a chaperone in Alport syndrome, and we have chosen to use PBA because it is known to be safe. We hope to find a cheaper version of PBA with our proposed in vitro studies. This work is being undertaken independently of any **pharmaceutical company.**

ORIGINALITY AND INNOVATIVE ASPECTS:
We have already demonstrated that treatment with the chaperone PBA in Alport fibroblast cell lines due to missense mutations reduces ER stress more consistently than any effect on collagen IV α5 chain trafficking. The first part of the proposed study is to examine a variety of chaperones in different cell lines from males with X-linked Alport syndrome caused by missense mutations, and to determine whether new chaperones or combinations have an additive effect on the reduction in ER stress. The second part of the study is to use the chaperone PBA in a clinical trial and to confirm that it reduces proteinuria, and ER stress in skin biopsies. The major strength of this project is examining an effect in vitro and in vivo. If proven these agents might be ready to use clinically within only a few years.

FOCUS OF RESEARCH: Therapy with chaperones reduces podocyte ER stress, and proteinuria and hence delays the onset of end-stage renal failure in Alport syndrome

KEYWORDS: chaperones, nonsense-mediated decay, ER stress, proteinuria, collagen IV a5 mRNA expression, podocytes

SPECIFIC AIMS, OBJECTIVES AND HYPOTHESES:
Our first aim is to identify chaperones other than PBA that are just as effective, safe but cheaper. We have a strategy for identifying these that is confidential. These agents will be tested in cell lines derived from males with X-linked Alport syndrome due to missense and other mutations.

Our second hypothesis is that the oral administration of PBA to subjects with X-linked Alport syndrome will reduce ER stress and proteinuria, and hence delay the onset of end-stage renal failure. We believe that this effect will be additive to the use of ACE inhibitors. PBA is widely available, safe and has few side effects. However it is expensive because of a licensing agreement.

Our **Specific Aims** and **Objectives** are:
1. To identify chemical chaperones other than PBA that also reduce ER stress in subjects with missense mutations. To combine chaperones in our in vitro system to determine whether the effect is additive. To determine any effect of chemical chaperones in subjects with other types of mutations such as nonsense or complex mutations.

2. To demonstrate that PBA reduces the amount of proteinuria in human disease in subjects with X-linked Alport syndrome due to missense mutations in a single centre, randomised, cross-over study. This is a study of 8 subjects with proven missense mutations and proteinuria > 0.5 g/day who will be observed for 2 weeks; and then given PBA for 2 weeks; and no PBA for a further 2 weeks. Proteinuria will be measured by a 24 hour urinary albumin excretion rate. Study subjects levels of proteinuria will be compared with their own values pre-treatment.

Subjects will also undergo a skin biopsy (4 mm from the lower back) before treatment and immediately after treatment to compare the reduction in proteinuria with any change in ER stress marker mRNA levels.

BACKGROUND AND SIGNIFICANCE

X-linked Alport syndrome is an inherited kidney disease that affects at least one in 10,000 individuals and invariably leads to end-stage renal failure requiring dialysis or transplantation (Hasstedt and Atkin, 1983; Persson, et al., 2005). Most families (85%) have X-linked disease with mutations in the *COL4A5* gene (Barker, et al., 1990; Cosgrove, 2011; Feingold, et al., 1985; Hudson, et al., 2003a; Mochizuki, et al., 1994), and most of the others have autosomal recessive disease with homozygous or compound heterozygous mutations in the *COL4A3* or *COL4A4* genes. The *COL4A1* – *COL4A6* genes code for the collagen IV a1–a6 chains.

Affected males with X-linked Alport syndrome present with haematuria at about the age of 6, proteinuria soon after, and end-stage renal failure by a median of 21 years (Mochizuki, et al., 1994). Hearing loss is common, lens abnormalities occur in 30%, and retinal flecks are found in more than half (Hudson, et al., 2003a). These clinical features are explained by the distribution of the collagen IV a3a4a5 network in the glomerular basement membrane in the kidney, cochlea (in the ear), and the lens capsule and retina (in the eye). About 15% of all females develop end-stage renal failure by the age of 60 (Dagher, et al., 2001), because they also have a normal X chromosome.

Clinically, Alport syndrome is the most significant inherited disease affecting the glomerular basement membrane. The GBM lies between the endothelial and epithelial cells of the kidney glomerular filtration barrier. It comprises mainly type IV collagen, which is a heterotrimer of 3 of the possible 6 chains, a1–a6. The heterotrimers produce 3 distinct networks, a1a1a2, a3a4a5 and a5a5a6. The a1a1a2 network is found mainly in vascular basement membranes. It is also present in the embryo and infant kidney but is completely replaced by the a3a4a5 network by ~6 years of age in the kidney, ear and eye (Hudson, et al., 2003b). The a5a5a6network is found in the kidney Bowman's capsule and the epidermal basement membrane.

Mutations in X-linked Alport syndrome: How *COL4A5* mutations cause disease. About 1200 unique pathogenic variants have been described in X-linked Alport syndrome (Hertz, et al., 2011). They comprise rearrangements, insertions and deletions, splicing mutations, missense mutations and nonsense mutations (Hertz, et al., 2011). Overall, about 40% variants result in a change in the amino acid ('missense mutation'), typically a glycine substitution in the collagenous domain (Hertz, et al., 2011). Clinical features and severity depend on the location and nature of mutations, and carboxy terminal missense mutations typically result in early onset renal failure, hearing loss, and ocular abnormalities (Tan, et al., 2010), whereas amino terminal missense mutations are often associated with late onset renal failure with fewer extra-renal disease features.

The unfolded protein response. Specific point mutations that produce proteins that fail to achieve their folded state and do not move from the ER to their appropriate subcellular location are responsible for many forms of inherited disease. Cell processes such as trafficking, secretion and regulation of the cell cycle depend on folding and unfolding events. Failure of proteins to fold correctly, or to remain correctly folded, results in dysfunctional proteins disease (Dobson, 2003). 'Chaperones' within the cells have a critical role in protein folding and trafficking.

The stress response from the accumulation of misfolded proteins retained in the ER elicits a coping mechanism known as the 'unfolded protein response' (Patil and Walter, 2001). This results in the cessation of translation initiation, translation of a selection of adaptive proteins, and activation of transcription of a number of genes. ER stress also triggers apoptosis, with associated upregulation of proapoptotic factors and downregulation of antiapoptotic factors.

Chemical chaperones are low molecular weight compounds that have a similar effect to naturally occurring molecular chaperones. By preventing protein aggregation and assisting in the refolding of misfolded proteins to their wild-type configuration, they reverse the intracellular retention of misfolded proteins and rescue function (Hartl, 1996). The most effective chemical chaperones in experimental studies include glycerol, trimethylamine-N-oxide (TMAO), dimethylsulphoxide (DMSO) and phenylbutyric acid (PBA). These all enhance the folding of chemical chaperones and non-specifically stabilise mutant proteins and promote protein folding in the ER that enable their transport. PBA has been studied in a number of inherited diseases to examine it effect on clinical features (www.Clinical trials.gov).

New treatment options for Alport patients. There are limited treatment options available to prevent or slow the development of renal failure in Alport syndrome in humans. It is likely that a cocktail of treatments will be required. These will include ACE inhibitors, chaperones and possibly stem cells in an effort to delay the onset of end stage renal failure and the need for dialysis. ACE inhibitors, including ramipril, reduce proteinuria in children with X-linked disease (Proesmans and Van Dyck, 2004), and delay the onset of end-stage renal failure and improve life-expectancy in adult males and females even when begun before the onset of proteinuria (Gross, et al., 2011). ACE inhibitors work through blood pressure control, with antiproteinuric and antifibrogenic effects, and by interfering with the local podocyte renin-angiotensin system (Liebau, et al., 2006). Angiotensin receptor blockers and aldosterone inhibitors have a further beneficial effect (Kaito, et al., 2006).

Experimental treatments, such as bone marrow transplantation and bone marrow-derived stem cell therapy have more risks than benefits for patients (Gross and Kashtan, 2009). Chemical chaperones hold hope. We have demonstrated in in vitro studies that chemical chaperone treatment with PBA reduces ER retention of the abnormal collagen IV chain and ER stress and hence potentially proteinuria (Inagi 2010). Treatment with a combination of chaperones may be even more useful in subjects with Alport syndrome.

Sodium phenylbutyrate (PBA) is a chaperone already in clinical practice. It is a non-toxic pharmacological compound that is FDA-approved for use in urea cycle disorders as an ammonia scavenger (Brusilow and Maestri, 1996). However PBA has multiple biological activities. It also acts as a chemical chaperone through activating transcription of a variety of genes including those encoding the heat shock proteins needed for protein folding (Rubenstein and Lyons, 2001). It has been used in protein misfolding diseases such as cystic fibrosis (Iannitti and Palmieri, 2011). In general the effect on the misfolded protein is small but there may be a more significant effect on reducing ER stress. PBA treatment has been used in cystic fibrosis (Chanoux and Rubenstein, 2012), sickle cell disease (Dover, et al., 1994); Huntington disease, and juvenile Parkinson's disease (Iannitti and Palmieri, 2011).

PBA is an orphan drug marketed under the trade name Buphenyl by Hyperion or Ammonul or other names. It has been registered by the FDA for use in human disease. It is given orally as a tablet or powder and tastes salty and bitter. Treatment for urea cycle disorders was approved by the FDA in 1996. In adults it is given as 12 g/day in equally divided doses with meals. Patients

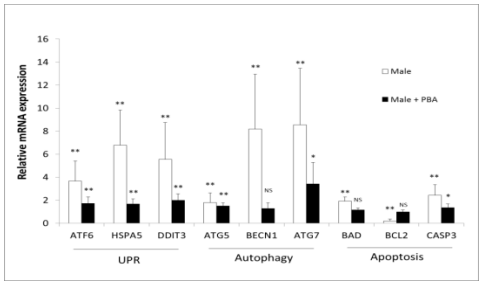
have to take a large number of tablets and compliance can be an issue. Adverse effects include menstrual irregularities; loss of appetite, body odour and unpleasant taste occur in 10% of patients; and gastrointestinal side effects. It must not be used in pregnancy.

However the rights to PBA have been bought by a pharmaceutical company and while it is inexpensive to produce it costs about $200,000 annually for pharmaceutical grade medication for a single patient.

Thus we have chosen to examine other chemical chaperones related to PBA for an effect on the ER as well as confirming that reducing ER stress minimises proteinuria in individuals with X-linked Alport syndrome due to missense mutations. We have a strategy to identify further chaperones that are likely to be effective with missense mutations in X-linked Alport syndrome. We will also investigate whether there is ER stress in subjects with Alport syndrome due to nonsense or other complex mutations.

RESEARCH DESIGN AND METHODS

Part 1: Development of new chaperones for the treatment of Alport syndrome. We have already demonstrate that PBA treatment of fibroblast cell lines from individuals with X-linked Alport syndrome due to missense mutations has its major effect through reducing ER stress rather than by improving cell trafficking. However we also know that reducing ER stress reduces proteinuria, and that the rate of progression to renal failure depends on the level of proteinuria. (Urinary protein leaks through the glomerular filter and is mainly reabsorbed through the tubule-interstitium; excessive urinary protein increases TGFb levels, and sets up an inflammatory response and fibrosis). We are interested to see if PBA and similar chaperones are also effective with complex mutations and nonsense mutations. We will examine this in vitro in the first instance. If this is demonstrated to be true then all affected individuals might be helped with inexpensive chaperone therapy rather than necessarily having to determine the mutations first.



This figure demonstrates an increased unfolded response (UPR, ATF6, HSPA5 and DDIT3) and also increased autophagy (ATG5, BECN1 and ATG7) in males (compared with values of 1.0 for normal males) and that these are all reduced after PBA treated in cell lines in vitro.

We already have fibroblast cell lines from males with known *COL4A5* mutations but would like to obtain at least 5 more. We will then establish cell lines and test the effect of PBA and other related chaperones on ER stress in these cell lines. The other chaperones are related to PBA produced in its development. However we will also check further chaperones in clinical use. These are all likely to work through mechanisms that are independent of ACE inhibitors. We will be aiming for chaperones that are not patented and so can be cheap, and safe.

Cell culture and treatment. Primary dermal fibroblast cultures will be established from 4 mm skin punch biopsies from each subject. The tissues are teased out, incubated with Dulbecco's modified Eagles' medium (DMEM, Invitrogen) with 10% foetal bovine serum (Invitrogen), 1% penicillin/

streptomycin/l and 2mM L-glutamine (Invitrogen) in 5% CO_2, and fibroblasts grown to confluence at 37^{o}C. Ascorbic acid (0.25 mM, Sigma) is added to the medium for 48 hours to increase and stabilize collagen expression (Geesin, et al., 1988).

One flask of cells is left untreated, and the others are incubated with 10 mM PBA (Sigma) of other chaperone for 48 hours at 37^{o}C, and the cells harvested. In general, data are obtained in duplicate from three independent experiments for each cell line.

Quantitation of the unfolded protein response, cell stress, apoptosis and autophagy pathways. Total RNA is extracted from cells using an RNA Isolation Kit (Zymo Research), and concentrations determined spectrophotometrically (Nanodrop Technologies). The samples are subjected to DNase-treatment using a DNA-free kit (Ambion), and one mcg from each sample reverse transcribed using oligo dT and SuperScript III First Strand Synthesis System Kit (Invitrogen). Levels of mRNA corresponding to markers of the unfolded protein response (cell stress), pro- and antiapoptotic pathways, and autophagy, are quantitated using purchased primers as follows. (For apoptosis: ***CASP3, BAD, Bcl2;*** unfolded protein response: ***HSPA5 (BiP), DDIT3 (CHOP), ATF6*** and autophagy: ***ATG6 (Beclin1), ATG5, ATG7***)).

Samples are then assayed for transcripts**,** using the fluorescent intercalating agent SYBR Green 1 (Qiagen) and the ABI 7500 real time PCR System (Applied Biosystems). Individual reactions comprise 5 µl of 2x Quanti Tect SYBR Green RT-PCR Master Mix (Qiagen), 0.7 µl of each 20 ng/µl sense and antisense primer and 2 µl of 100 ng/µl cDNA template, in a total volume of 10 µl. The Ct value is calculated at the end of each run using GAPDH as the internal control, and software provided by the manufacturer. Primer sets for the transcripts are purchased from Life Technologies, Australia. Each sample is examined in duplicate and the assays performed in triplicate. The results are compared with the expression in normal fibroblasts, and untreated cells.

ER size and co-localisation experiments. ER size is examined in the primary dermal fibroblasts using two methods: electron microscopy and immunocytochemistry. For electron microscopy, fibroblast cell pellets are fixed in 1.5% chilled glutaraldehyde (Sigma), post-fixed in 2% OsO4 (Sigma), and prepared routinely. The grids are examined and images captured with a transmission electron microscope (Phillips CM 120BioTWIN, The Netherlands). ER size is measured using Image J (NIH freeware, http://rsb.info.nih.gov/nih-image) of coded images. The ImageJ program quantifies the pixels within a defined area containing 4 – 7 cells to determine average pixel number per cell. The ER size in 5 consecutive fields are measured and averaged. For immunostaining, fibroblasts are grown on cover slips and processed as for immunohistochemistry, but with antiHSP47 (1: 400, Enzo Life Sciences). ER volume from 50 consecutive fields are quantified using Image J software (Image j.nih.gov/ij/).

Statistical analyses. Data are described as mean $\pm$ SD, and statistical analyses performed using the unpaired or paired student t test. Differential mRNA expression was analysed by ANOVA. Statistical analyses are performed using Graph Pad Prism, version 5. A p value < 0.05 is considered significant.

Part 2: Chaperone therapy *in vivo* to determine whether chaperones reduce proteinuria in individuals with missense mutations whether or not they are already treated with ACE inhibitors.

Recruitment of patients with X-linked Alport syndrome due to missense mutations and proteinuria. We have a cohort of about 60 families with X-linked Alport syndrome of whom at

least 20 have missense mutations. We can identify affected subjects from this cohort with missense mutations and proteinuria. We have chosen a reduction in proteinuria as the endpoint because we understand that ER stress is a major contributor and itself it is a factor in renal failure progression.

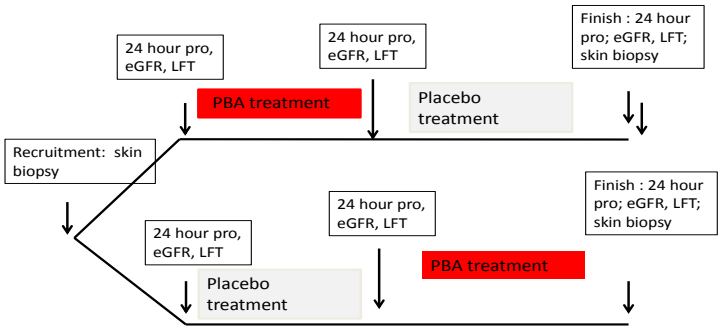
Study subjects with proteinuria > 500 mg/ day will be treated with PBA for 2 weeks to see if this reduces proteinuria. This is a single centre double blind randomised placebo-controlled cross-over trial of PBA in the treatment of X-linked Alport syndrome due to missense mutations. Subjects will be randomly assigned to receive either PBA or placebo for 2 weeks, and then crossed over to receive the other treatment for 2 weeks. PBA dose is maximal at 10 pills four times a day with meals.

Inclusion criteria: Age more than 18 years; Confirmed diagnosis of X-linked Alport syndrome due to missense mutation; Proteinuria > 500 mg/day on 2 occasions; eGFR > 30 ml/min /1.73 m^2; Participants must have a history of compliance to treatment; must be capable of completing study procedures
Exclusion criteria: Inclusion in another study; Not pregnant at study commencement, and using acceptable contraception methods throughout; No known hypersensitivity to phenylacetate or phenylbutyrate

Planned sample size: 8 subjects with X-linked Alport syndrome due to known missense mutations and proteinuria > 500 mg/day. Adverse events will be recorded and likely causal relationship determined. Study duration is 4 weeks.

Statistical analyses: Each study subject is tested three times, at recruitment, two weeks later and two weeks after that. They are randomized to receive PBA or placebo in first or second two week block. Data will be analysed using ANOVA or a Student's *t* test (paired). Statistical analysis is performed using GraphPad Prism. A *P* value <0.05 is considered statistically significant.

Study design: Individuals are screened, and those with missense mutations and proteinuria > 500 mg/day will be recruited. They will undergo a skin biopsy in which ER stress markers are measured (unfolded protein response (ATF6, HSPA5, DDIT3), autophagy (ATG5,BECN1,ATG7) and apoptosis (BAD, BCL2,CASP3) mRNA) by real time PCR. They will be seen two weeks later and start PBA in thrice daily dose of 12 gm total each day, and followed up two weeks later. Proteinuria will be measured with a timed overnight urinary albumin excretion rate. The skin biopsy will be repeated after 8 weeks.



DETAILED BUDGET

This study includes a clinical trial so we are asking for the full amount of 150 euros. Our budget is provided in USD because we buy most of our reagents from the US. The main expenses are staff but also medication for tests, and for analysis of the skin biopsies. We are also exploring alternatives for PBA but the study proposed here is important because it will demonstrate that chaperones work in vivo.

Budget Categories as listed: (in USD)

Part 1: Identification of new chaperones

Scientist (8 months) ($67,000)
Tissue culture reagents, qPCR reagents,
primers, antibodies, ($20,000) $87,000

Part 2: Clinical trial of chaperones

Study Coordinator (equivalent to
4 months, incl on costs) ($33,800)

Scientist (2 months full time incl on costs) ($18,000)

Pharmacy costs ($1000, preparing placebo etc)

SUBTOTAL $52,800

Equipment and Supplies (listed by category):

Full dose PBA x 8 patients x 14 days each (Hyperion, = $140K pa) $43,080

Urinary alb excretion rate x $25 eGFR; and LFT x$25
Skin biopsy – dressing packs and biopsy needles x 16 $1000

SUBTOTAL

Patient Care (itemized by type of expense):

SUBTOTAL **N/A**

Other (itemized by type of expense):

Immunohistochemistry of skin biopsies – antibodies, slides = $2500
mRNA extraction kits and Real time PCR, tissue culture media = $2500

SUBTOTAL $5,000

TOTAL BUDGET $USD188,800

FIVE SIGNIFICANT RECENT PUBLICATIONS:

1.Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of Alport syndrome and TBMN. JASN 2013; 24: 364-75.

2.Savige J, Sheth S, Leys A, Nicholson A, Mack HG, Colville D. Ocular features in Alport syndrome: pathogenesis and clinical significance. C JASN 2015: 10: 703-9.

3.Storey H, **Savige J,** Sivakumar V, Flinter FA. Clinical and molecular features in 40 unrelated individuals with autosomal recessive Alport syndrome. JASN 2013: 24: 1945-54.

4.Savige J. Alport syndrome: its effects on the glomerular filtration barrier and implications for future treatment. J Physiol 2014: 86:679-84.

5. Wang D, Ricardo S, **Savige J.** Chaperone therapy in stem cells derived from fibroblasts with missense mutations in X-linked Alport syndrome (Abstract ASN 2015).

REFERENCES:

Barker DF, Hostikka SL, Zhou J, Chow LT, Oliphant AR, Gerken SC, Gregory MC, Skolnick MH, Atkin CL, Tryggvason K. 1990. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. Science 248(4960):1224-7.

Brusilow SW, Maestri NE. 1996. Urea cycle disorders: diagnosis, pathophysiology, and therapy. Adv Pediatr 43:127-70.

Chanoux RA, Rubenstein RC. 2012. Molecular Chaperones as Targets to Circumvent the CFTR Defect in Cystic Fibrosis. Front Pharmacol 3:137.

Cosgrove D. 2011. Glomerular pathology in Alport syndrome: a molecular perspective. Pediatric nephrology.

Dagher H, Buzza M, Colville D, Jones C, Powell H, Fassett R, Wilson D, Agar J, Savige J. 2001. A comparison of the clinical, histopathologic, and ultrastructural phenotypes in carriers of X-linked and autosomal recessive Alport's syndrome. American journal of kidney diseases : the official journal of the National Kidney Foundation 38(6):1217-28.

Dobson CM. 2003. Protein folding and misfolding. Nature 426(6968):884-90.

Dover GJ, Brusilow S, Charache S. 1994. Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. Blood 84(1):339-43.

Feingold J, Bois E, Chompret A, Broyer M, Gubler MC, Grunfeld JP. 1985. Genetic heterogeneity of Alport syndrome. Kidney Int 27(4):672-7.

Geesin JC, Darr D, Kaufman R, Murad S, Pinnell SR. 1988. Ascorbic acid specifically increases type I and type III procollagen messenger RNA levels in human skin fibroblast. J Invest Dermatol 90(4):420-4.

Gross O, Kashtan CE. 2009. Treatment of Alport syndrome: beyond animal models. Kidney international 76(6):599-603.

Gross O, Licht C, Anders HJ, Hoppe B, Beck B, Tonshoff B, Hocker B, Wygoda S, Ehrich JH, Pape L and others. 2011. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. Kidney Int.

Gross O, Licht C, Anders HJ, Hoppe B, Beck B, Tonshoff B, Hocker B, Wygoda S, Ehrich JH, Pape L and others. 2012. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. Kidney Int 81(5):494-501.

Hartl FU. 1996. Molecular chaperones in cellular protein folding. Nature 381(6583):571-9.

Hasstedt SJ, Atkin CL. 1983. X-linked inheritance of Alport syndrome: family P revisited. Am J Hum Genet 35(6):1241-51.

Hertz JM, Thomassen M, Storey H, Flinter F. 2011. Clinical utility gene card for: Alport syndrome. Eur J Hum Genet.

Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. 2003a. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. The New England journal of medicine 348(25):2543-56.
Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. 2003b. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med 348(25):2543-56.
Iannitti T, Palmieri B. 2011. Clinical and experimental applications of sodium phenylbutyrate. Drugs R D 11(3):227-49.
Kaito H, Nozu K, Iijima K, Nakanishi K, Yoshiya K, Kanda K, Przybyslaw Krol R, Yoshikawa N, Matsuo M. 2006. The effect of aldosterone blockade in patients with Alport syndrome. Pediatr Nephrol 21(12):1824-9.
Liebau MC, Lang D, Bohm J, Endlich N, Bek MJ, Witherden I, Mathieson PW, Saleem MA, Pavenstadt H, Fischer KG. 2006. Functional expression of the renin-angiotensin system in human podocytes. American journal of physiology. Renal physiology 290(3):F710-9.
Mochizuki T, Lemmink HH, Mariyama M, Antignac C, Gubler MC, Pirson Y, Verellen-Dumoulin C, Chan B, Schroder CH, Smeets HJ and others. 1994. Identification of mutations in the alpha 3(IV) and alpha 4(IV) collagen genes in autosomal recessive Alport syndrome. Nat Genet 8(1):77-81.
Patil C, Walter P. 2001. Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. Curr Opin Cell Biol 13(3):349-55.
Persson U, Hertz JM, Wieslander J, Segelmark M. 2005. Alport syndrome in southern Sweden. Clin Nephrol 64(2):85-90.
Proesmans W, Van Dyck M. 2004. Enalapril in children with Alport syndrome. Pediatr Nephrol 19(3):271-5.
Rubenstein RC, Lyons BM. 2001. Sodium 4-phenylbutyrate downregulates HSC70 expression by facilitating mRNA degradation. Am J Physiol Lung Cell Mol Physiol 281(1):L43-51.
Savige J. 2014. Alport syndrome: its effects on the glomerular filtration barrier and implications for future treatment. J Physiol 592(18):4013-23.
Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. 2013. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. J Am Soc Nephrol 24(3):364-75.
Tan R, Colville D, Wang YY, Rigby L, Savige J. 2010. Alport retinopathy results from "severe" COL4A5 mutations and predicts early renal failure. Clin J Am Soc Nephrol 5(1):34-8.