Genotype and renal outcomes in Alport Syndrome: A retrospective cohort study using National Registry of Rare Kidney Diseases (RaDaR) data

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Background

Alport syndrome (AS) results from pathogenic variants in *COL4A3*, *COL4A4 & COL4A5* genes. The clinical course of Alport Syndrome can be highly variable. Previous genotype-phenotype correlation studies have shown protein length altering variants are associated with a more severe phenotype in males with *COL4A5* variants[1], and individuals with 1 or 2 *COL4A3* and *COL4A4* variants[2]. In males with *COL4A5* missense variants affecting glycine (Gly) residues, certain molecular characteristics including distance from the carboxyl terminus, proximity to non-collagenous domains and substituting residue have been associated with differing clinical severity[3].

This study investigated the renal outcomes associated with pathogenic variant types in a UK AS cohort, using National Registry of Rare Kidney Diseases (RaDaR) data.

Methods

RaDaR is linked with the UK Renal Registry for Renal Replacement Therapy (RRT) initiation and kidney transplantation data, and Regional Genetics hubs for clinical genetic reports. Variants classified as "Pathogenic" or "Likely Pathogenic" were included. Variants were classified into 1) Protein Length Altering (splice site, stop gain, frameshift, deletions/insertions, exon deletion/duplications) 2) Non Protein Length Altering (missense Gly and non Gly substitutions).

Molecular characteristics

Missense Gly substitutions were further classified by 1) Exon position (1-20 or 21-carboxyl terminus) 2) being adjacent to a non-collagenous region 3) degree of predicted instability caused by the replacing residue (Mildly destabilising: Ala, Ser, Cys, Highly destabilising: Arg, Val, Glu, Asp, Trp)

Statistical Analyses

Kaplan-Meier analysis and the log rank statistic were used to compare survival curves, stratified by variant type and molecular characteristic, for a) age at RRT start b) time between last eGFR ≥90 and last eGFR ≥30 ("therapeutic window") c) kidney transplant survival, censored for death. Analyses were run comparing variant type and molecular features for 1)*COL4A5* males 2)*COL4A5* females 3)Heterozygous *COL4A3* or *COL4A4* 4) Homozygous or 2 *COL4A3* or *COL4A4* variants. Where two pathogenic variants were present, the variant predicted to be least damaging was used for analyses.

Results

Genetic report data were available for 343/934 (37%) AS patients recruited to RaDaR. 294/343 (86%) had a detected pathogenic mutation, 28/343 (8%) had no mutation detected and 21/343 (6%) had variants of uncertain significance. Of those with pathogenic variants, 140 patients had protein length altering mutations (17 exon deletion/duplication, 39 frameshift, 21 insertion/deletions, 44 splice site and 30 stop gain mutations), and 154 non-protein length altering mutations (134 Gly substitutions, 20 non-Gly substitutions) (Table 1).

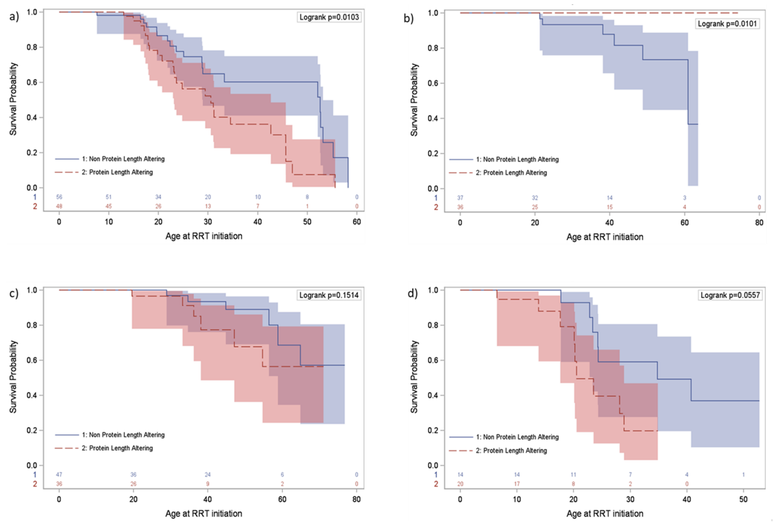
Protein length altering vs. non protein length altering variants

Table 1: Genetic variant type of individuals recruited to RaDaR, stratified by gene

Cohort	Protein length altering n (%)	Non protein length altering n(%)
***COL4A5* Male**	48 (34)	56 (36)
***COL4A5* Female**	36 (26)	37 (24)
***COL4A3/4* Heterozygous**	36 (26)	47 (31)
COL4A3/4* Homozygous or 2x variants	20 (14)	14 (9)
Total	140	154

*11 confirmed biallelic

Figure 1: Kaplan Meier Survival analyses comparing age at RRT start for protein length altering vs non-protein length altering variants for a) *COL4A5* males b) *COL4A5* females c) *COL4A3* or *COL4A4* heterozygous d) 2x *COL4A3* or *COL4A4* variants



Males with protein length altering *COL4A5* variants were younger at RRT start (log-rank p=0.013) compared with non-protein length altering variants (median age 30.6 vs 52.6 years). Conversely 0/36 females with protein length altering *COL4A5* variants had started RRT, compared to 6/37 with non protein length altering variants (p=0.01) (Figure 1).
76/294 (26%) underwent kidney transplantation. Variant type only affected graft survival for individuals with 2x *COLA3* or *COL4A4* variants, where 6/9 with protein length altering variants experienced graft failure at 10 years, compared to 0/6 with non protein length variants (p=0.034)
Time in therapeutic window was not significantly different in any group.

Missense Glycine Substitutions

Figure 2: Molecular characteristics of missense Gly substitutions, stratified by gene

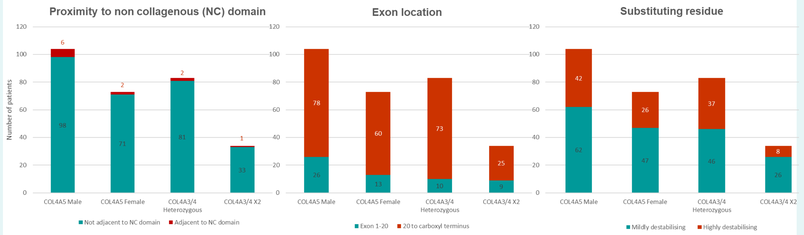
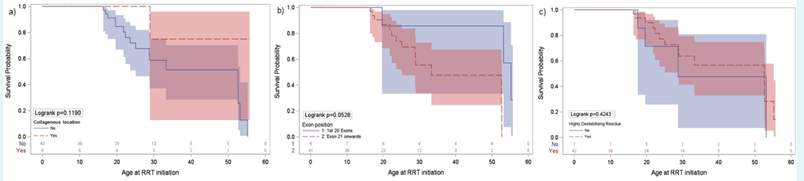


Figure 3: Kaplan Meier Survival analyses comparing age at KRT start for COL4A5 males with missense glycine substitutions stratified by a) collagenous location b) exon location c) substituting residue



The molecular characteristics of variants in the RaDaR database are summarised in Figure 2, stratified by gene.

Age at kidney failure in males with COL4A5 missense Gly substitutions did not differ by proximity to non-collagenous domain or replacement residue (Figure 3). Those with Gly substitutions in Exons 21-53 reached kidney failure earlier compared to Exons 1-20 (median age at RRT start 33.3 years vs 55.2 years), but this did not reach significance (log rank p=0.053).

Molecular characteristics had no observed effect on age at kidney failure, graft survival or time in therapeutic window for any group.

Conclusion

- Males with protein length altering *COL4A5* variants were younger at RRT initiation than those with non length altering variants.
- Females with protein length altering *COL4A5* variants had better kidney survival than those with non length altering variants; however the overall number of RRT events in this group was small
- No significant differences in renal outcomes were observed when stratifying by molecular characteristics in this AS cohort
- Linkage of the RaDaR AS cohort with genetic report data is ongoing; further correlations may be observed with larger numbers

References:
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2. Lee JM et al. Features of Autosomal Recessive Alport Syndrome: A Systematic Review. J Clin Med. 2019 Feb 3;8(2):178.
2. Gibson JT et al. Genotype-phenotype correlations for COL4A3-COL4A5 variants resulting in Gly substitutions in Alport syndrome. Sci Rep. 2022 Feb 17;12(1):2722.

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