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Letter to the Editor

Genotype–phenotype correlation in X-linked Alport syndrome patients carrying missense mutations in the collagenous domain of *COL4A5*

To the Editor:

Alport syndrome (AS) is a hereditary progressive glomerulopathy in which kidney function drops progressively and it can finally lead to end-stage kidney disease (ESKD).

We herewith attempted a genotype–phenotype correlation of missense mutations within the collagenous domain of X-linked-*COL4A5*. We searched the public human gene mutation database (1) to locate papers that reported such missense mutations in males. We performed linear regression and Spearman correlation using mutations for which an exact age-at-onset of ESKD was given (72 [Correction added after online publication on 28 February 2012. The correct number of mutations should be 72, not 772 as was originally stated.] mutations). Recurrent mutations, reported more than once, were counted once, although the clinical severity was based on the information provided for all patients. Analysis was performed with GraphPad Prism (version 5.00-Windows, GraphPad Software, San Diego, CA: www.graphpad.com). Statistical significance was for p-value <0.05.

For odds ratio analysis, subjects were split amongst two groups: severe phenotype (ESKD $\leq$31 years old) and mild phenotype (ESKD $\geq$35 years old). The grey area in between, i.e. 32–34 years, was treated as severe if there was hearing loss, ocular changes, or characteristic glomerular basement membrane findings, along with their ESKD. If ESKD was the only sign amongst these patients, they were classified as 'mild'.

Table 1 illustrates results from the analysis that did not reach significance.

Nonparametric correlation examined whether the difference in the number of side-chain carbon atoms introduced by the mutation correlated to disease phenotype. A correlation was detected with a Spearman r-value of -0.3545 (95% confidence interval: -0.5492 to -0.1234) and a p-value of 0.0026. In addition, linear regression showed statistically significant correlation with age-at-onset of ESKD decreasing with increasing number of side-chain carbon atoms in the substituting residue (r^2: 0.1362; p: 0.0017) (Fig. 1).

Jais et al. (2) found that missense mutations tend to give phenotypes that are milder compared with others, in terms of both age-at-onset of ESKD and probability of developing hearing loss. Gross et al. (3) found that glycine substitutions in the first 20 exons resulted in a milder phenotype than glycine substitutions in exons 21–47. Bekheirnia et al. (4), however, failed to show an association between missense mutation position and age at ESKD.

In addition, according to Persikov et al. (5) the identity of the amino acid that substitutes glycine in AS and other connective tissue diseases is not random. They report that the distribution of substituting residues is different than that predicted by gene-based mutation rates. One reason could be that only certain amino acid substitutions cause a severe enough phenotype, which, however, is compatible with life and therefore capable of being detected. This apparent non-random substitution was replicated in our results (data not shown).

Here we provide evidence that the larger the side chain of the amino acid substituting a glycine, the more severe the disease is. Our results agree with Beck et al. (6) concerning patients with Osteogenesis Imperfecta who showed that the identity of the residue replacing glycine is important in determining the phenotype. Interestingly, linear regression of distance of mutation from closest interruption against age at ESKD gave a p-value of 0.2532. Although this is statistically insignificant, it contradicts recent evidence from our group that mutations close to certain interruptions might give a milder phenotype (7). Therefore, one could presume that different interruptions in the collagenous domains have different functions and biological significance. It is not appropriate to predict prognosis solely based on our single finding; however, Gross et al. (3) showed that glycine substitutions in exons 1–20 associate with milder phenotype, while Bekheirnia et al. (4) showed the same when all kinds of mutations were taken into consideration. Our data, without reaching significance, showed a trend in the same direction (Table 1). Therefore, a reasonable concluding algorithm could be that glycine substitutions with bulkier residues closer to the 7S domain might be associated with worst prognosis.

Table 1. Results from the analysis that did not reach significance

Analysis	Mutation inclusion criteria (number of mutations analysed)	Analysis performed	Results		
			Comments	Analysis value	Significance
Location of mutation along the length of the *COL4A5* gene	Missense mutations with a known age at ESKD (72)	Linear regression	Analysis showed that mutations further away from the 7S domain result in more severe phenotypes	r^2: 0.002998	p-value: 0.6478
Distance of mutation from closest interruption	Missense mutations with a known age at ESKD and lying between the first and last interruptions (66)	Linear regression	Analysis showed that mutations further away from the closest interruptions result in mild phenotypes	r^2: 0.02035	p-value: 0.2532
Proportion of mutations found within and outside of interruptions	Missense mutations in collagenous domain (134)	Odds ratio	The odds ratio of having a mutation in interruptions was not statistically significant	Odds ratio: 0.6983	95% CI: 0.3849–1.267
Amino acid charge changes and correlation to disease phenotype	Missense mutations that could be classified as giving a mild or severe phenotype (86)	Odds ratio	The odds ratio of having a severe phenotype was not statistically significant	Odds ratio: 1.467	95% CI: 0.5365–4.009
Hydrophobicity index change and correlation with disease phenotype	Missense mutations that could be classified as giving a mild or severe phenotype (86)	Odds ratio	Odds ratio of having a severe phenotype if the mutation produced a change in polarity was not statistically significant	Odds ratio: 0.4706	95% CI: 0.1230–1.801

CI, confidence interval; ESKD, end-stage kidney disease.

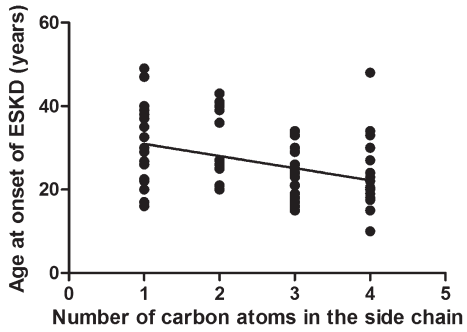

Fig. 1. Genotype–phenotype correlation examining the effect of the difference in the number of side-chain carbon atoms carried by the substituting amino acid (X-axis) with age-at-onset of end-stage kidney disease (Y-axis). r^2-value: 0.1362; p-value: 0.0017; number of mutations analysed: 70.

To conclude, we showed the importance of the number of carbon atoms carried on the side chain of the mutant amino acid that substitutes a glycine in *COL4A5*. This implies a mechanism involving the spatial area around glycines and the steric hindrance imposed by bulkier residues on the structure and function of the mature heterotrimer. A recognized limitation of this meta-analysis is that some of the data used were derived from individuals of the same family.

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