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A rare mutation in GCAP1 disrupts the function of the cGMP-signaling protein complex and causes Retinitis Pigmentosa

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1. Main topic

Guanylate cyclase-activating protein 1 (GCAP1) regulates the recovery phase of phototransduction by controlling retinal guanylate cyclase (GC) activity in a Ca^{2+}-dependent manner [1]. At elevated Ca^{2+} concentrations GCAP1 inhibits GC, whereas at low Ca^{2+} levels it activates GC to restore cyclic GMP (cGMP) levels. Pathogenic variants in GUCA1A (encoding GCAP1) or GUCY2D (encoding GC-E) disturb this feedback regulation and are linked to inherited retinal dystrophies, including retinitis pigmentosa (RP) and cone-rod dystrophy (CRD) [1].
Here, we describe the biochemical and functional consequences of a novel GUCA1A missense mutation, c.277C>T (p.Arg93Cys), identified in a patient with autosomal dominant RP. The substitution of the positively charged arginine by a cysteine residue within the EF3-hand motif is predicted to perturb Ca^{2+} coordination and alter protein conformation.

2. Methods and results

Cysteine was introduced at position 93 in human GCAP1 by site-directed mutagenesis. Human wildtype and mutant GCAP1 forms were expressed and purified from E.coli extracts. Human GC-E, the target enzyme of GCAP1, was expressed in HEK293 cells and incubated with purified GCAP1 variants. We investigated the biochemical properties of the cGMP signaling complex by investigating enzyme activity as a function of free Ca^{2+}-concentration and other parameters, similar to the work of Abbas et al. [2].

The R93C mutant showed a decreased Ca^{2+}-sensitivity and a right-shifted IC50, potentially leading to a disruption of phototransduction.
We will discuss potential structural alterations in GCAP1, including conformational changes and dimerization [3], arising from the mutation and compare the functional impact with previously investigated mutations in the GCAP1/GC-E signalling complex.

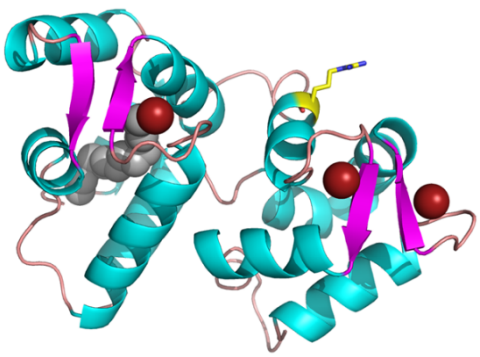

Fig. 1: Three-dimensional model of vertebrate myristoylated GCAP1. The EF-hand motifs are colored in cyan. Ca^{2+}-ions are represented by red spheres and the myristoyl group by gray spheres. The position of the R93C mutation site is highlighted in yellow.

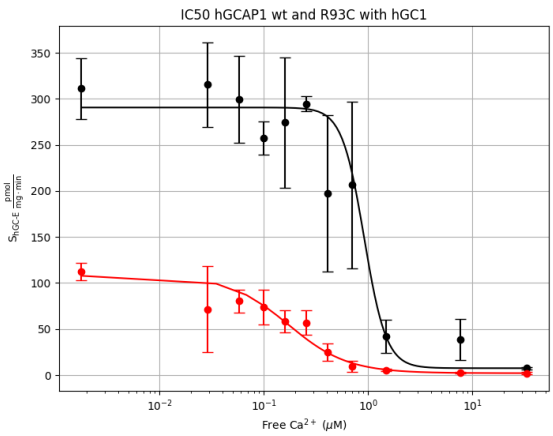

Fig. 2: The activity rate of the GC at varying Ca^{2+} levels is quantified by the cGMP production in the presence of hGCAP wt and R93C mutant

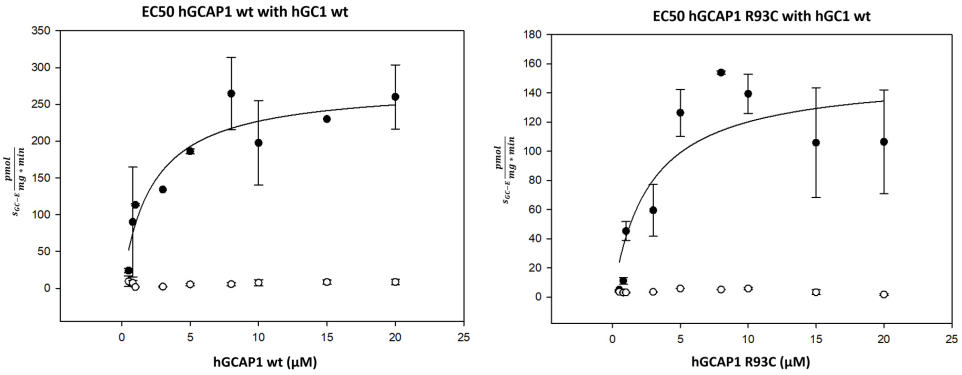

Fig. 3&4: The activity rate of the GC-E at varying Ca^{2+} levels is quantified by the cGMP production in the presence of hGCAP1 wt and R93C mutant.
GC-E and GCAP1 were incubated for 20 min at 30°C, with ~ 10 nM (black dots) or 33 µM (white dots) at different hGCAP wt (**3**) or hGCAP1 R93C (**4**) concentrations.

GC-Assay	IC50	EC50
hGCAP1 wt	0,91 µM	2,17 µM
hGCAP1 R93C	0,17 µM	2,70 µM

Tab.1: The IC50 represents the calcium concentration required to inhibit guanylate cyclase (GC-E) activity by 50%, while the EC50 indicates the GCAP1 concentration needed to activate GC to 50% of its maximum response. The (preliminary) values for IC50 were obtained using a 4-parameter logistic (4PL) curve fit and for EC50 an rectangular hyperbolic 2PL fit.

3. Acknowledgement

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4. References

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