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The macular degeneration-linked C1QTNF5 (S163) mutation causes higher-order structural rearrangements

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ABSTRACT

The C1q-tumor necrosis factor 5 (C1QTNF5) protein plays a significant role in retinal pigmented epithelium (RPE) cellular adhesion. The *C1QTNF5* gene is co-transcribed with the frizzled-related protein (*MFRP*) gene. A Ser-to-Arg mutation at site 163 (S163R) in C1QTNF5 is known to cause late-onset retinal macular degeneration (L-ORMD). Here we also found that C1QTNF5 monomers can multimerize into a bouquet-like octadecamer. We found that a novel intermolecular hydrogen-bond network of S163 that glues adjacent globular heads of C1QTNF5 together was weakened or abolished by the R163 pathogenic mutation. These findings could underlie the structural basis of this protein's adhesive function and relate to the pathogenesis of its S163R mutation. Additionally, the fact that C1QTNF5 immobilized to a resin selectively enriched detergent extracted membrane-bound MFRP, further confirmed their interaction, implying functions other than cellular adhesion for C1QTNF5.

1. Introduction

L-ORMD is a fully penetrant autosomal dominant progressive eye disorder caused by a single mutation c.686C>G (p.S163R) in the *C1QTNF5* protein (Ayyagari et al., 2005; Hayward et al., 2003). Affected patients start to experience visual difficulties in their 40s and eventually lose their sight after their 60s. The exact prevalence of this condition is unknown, in part because it is easily misdiagnosed, mostly as AMD in its early stage and as retinitis pigmentosa subsequently (Borooah et al., 2009a). L-ORMD consistently exhibits thick extracellular deposits of lipid-rich material between the retinal pigmented epithelium (RPE) and Bruch's membrane (Borooah et al., 2009b; Shu et al., 2006a). Understanding the molecular pathogenesis of L-ORMD is important for the design of appropriate therapeutics. However, it is still unclear how C1QTNF5 functions in RPE cellular adhesion and how the pathogenic S163R mutation modifies its molecular function.

The human *C1QTNF5* gene encodes a 25 kDa secretory and membrane-associated protein containing three domains: a signal peptide (sp domain, residues 1–15), a collagen domain (residues 30–98 composed of 23 uninterrupted Gly-X-Y repeats) and a globular C1q domain (gC1q domain, residues 103–243) (Hayward et al., 2003; Mandal et al., 2006b; Tu and Palczewski, 2012) (Fig. 1A). C1QTNF5 is a novel member of the C1q family that includes C1q, adiponectin, collagens, and other structurally related proteins. C1q family proteins share the common feature of trimerization (Shapiro and Scherer, 1998). Their collagen domains intertwine into a stalk-like triple helix and their gC1q domains trimerize into flower-like globular heads (Fig. 1A) (Tu and Palczewski, 2012). Although studies of complement protein C1q and adiponectin have shown that six trimers can further multimerize into a bouquet-like octadecameric assembly through their collagen triple helices (Fig. 1A), there is no direct evidence that C1QTNF5 also adopts similar architecture.

The S163R pathogenic mutation is located within the gC1q domain of C1QTNF5. This mutant is not secreted and displays a significant reduction in implementing cellular adhesion (Mandal et al., 2006b; Shu et al., 2006a). It also is unstable and prone to aggregation *in vitro* (Hayward et al., 2003; Shu et al., 2006b). Our previous study has revealed the structural mechanism underlying this instability resulted from aggregation of the S163R mutant (Tu and Palczewski, 2012) but it is still unclear how C1QTNF5 functions in RPE adhesion. Moreover, it is notable that all examined patients with this mutation are heterozygous, meaning that wild-type C1QTNF5 and its S163R mutant co-exist within secreted oligomer(s) of this protein. Thus, the structural effects of the S163R mutation which cause reduction or loss of function of the heterozygous C1QTNF5 oligomers leading to L-ORMD need to be investigated.

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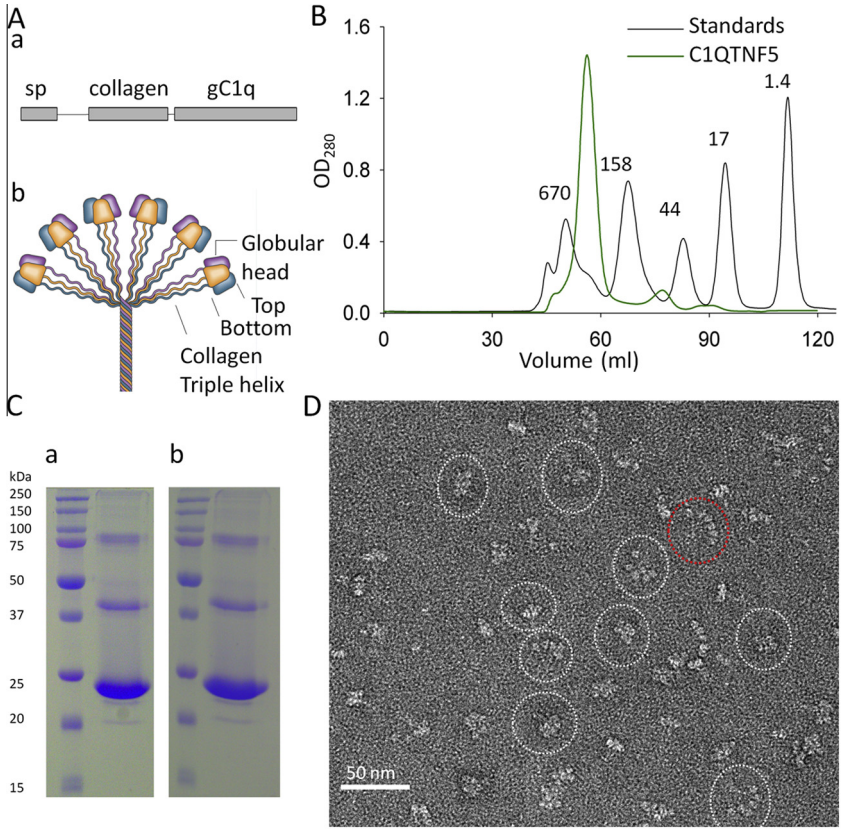

Fig.1. C1QTNF5 adopts a bouquet-like assembly without N-terminal disulfide bonding. (A) Primary structural features of the gC1q domain (a) and a bouquet-like assembly of C1q family proteins (b). sp, signal peptide; gC1q, globular C1q domain. (B) Appearance of C1QTNF5 after gel filtration chromatography. The column was calibrated by gel filtration molecular weight standards, namely bovine thyroglobulin (670 kDa), bovine γ-globulin (158 kDa), chicken ovalbumin (44 kDa), horse myoglobin (17 kDa) and vitamin B12 (1.4 kDa). (C) Non-reducing SDS–PAGE (a) and reducing SDS–PAGE (b) of the predominant peak fraction (~450 kDa) of C1QTNF5 obtained by gel filtration chromatography. (D) Appearance of C1QTNF5 expressed in bacteria after negative staining electron microscopy. Shapes of particles can be divided into two major categories: bouquet-shaped and fan-shaped. Representative images are outlined with white and red dotted ovals, respectively.

The *C1QTNF5* gene is located entirely within the 3′-UTR of the *MFRP* gene and is co-transcribed with *MFRP* (Hayward et al., 2003). C1QTNF5 can co-immunoprecipitate with MFRP, and *vice versa* (Mandal et al., 2006a). Moreover, a study has shown that C1QTNF5 can pull down GST-tagged CUB domains of MFRP expressed in bacteria (Shu et al., 2006a), indicating that they directly interact with each other. Though native CUB domains contain two disulfide bonds, heterologously expressed CUB domains lack disulfide bonds so their interactions with C1QTNF5 could be artificial and caused by the partially folded CUB domain. Thus, this pull-down study needs to be further confirmed with MFRP expressed in mammalian cells that allow formation of disulfide bonds. Their interaction would imply that C1QTNF5 could have functions other than cellular adhesion.

Thus, in this study, we aimed to examine whether C1QTNF5 could form a bouquet-like octadecameric assembly, understand how C1QTNF5 expedites RPE cellular adhesion, gain insights into the molecular pathogenesis of the S163R mutant in attenuating RPE cell adhesion and provide further proof that C1QTNF5 can interact with MFRP.

2. Results and discussion

2.1. Expression, purification and electron microscopic (EM) imaging of C1QTNF5

His-tagged C1QTNF5 was expressed in the cytosol of *Escherichia coli* Rossetta (DE3) and purified by metal affinity in tandem with gel filtration chromatography. C1QTNF5 eluted as a predominant peak with an apparent mass of ~450 kDa on gel filtration chromatography (Fig. 1B). There were two minor peaks with apparent masses corresponding to the trimer (72 kDa) and monomer (24 kDa). One explanation for this phenomenon is that overexpression of C1QTNF5 in bacteria results in a small fraction of incompletely folded protein that could not be assembled into the octadecamer. Another possibility is that there exists an equilibrium among octadecamer, trimer and monomer. We then performed non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) on the purified ~450 kDa peak fraction and found that it migrated as a major band with an estimated molecular weight of ~24 kDa, close to the theoretical molecular weight (23.5 kDa) of a single C1QTNF5 molecule (Fig. 1Ca). This result indicates that monomeric C1QTNF5 can form a high-molecular-weight multimer. All C1q family protein multimers have a basic oligomerization unit of a trimer. A trimer of C1QTNF5 has a mass of ~72 kDa, so an apparent mass of ~450 kDa indicates that this multimer is possibly composed of 6 trimers, making it an octadecamer.

C1QTNF5 contains three Cys residues; two (C28 and C98) are localized in the N-terminal region and one (C145) is in the gC1q domain (Wong et al., 2008). With respect to the two visible minor oligomeric bands noted on non-reducing SDS–PAGE, it is known that Rossetta (DE3) bacteria have a highly reduced cytosolic environment which prevents formation of disulfide bonds. We thus performed reducing SDS–PAGE by adding 10 mM fresh DTT to the sample loading buffer to rule out possible disulfide-bond formation during the purification process. However, the reducing