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Congenital afibrinogenemia is a rare bleeding disorder characterized by a complete absence of fibrinogen in circulation. Synthesized in hepatocytes, fibrinogen is a 340 kDa glycoprotein composed of two sets of three homologous polypeptide chains (alpha, beta and gamma) which assemble to form a hexamer. Each polypeptide is encoded by a distinct gene (FGA, FGB, and FGG) on chromosome 4q28-31.

Since our identification of the disease locus and the first mutation for congenital afibrinogenemia (an 11 kb deletion in FGA; Neerman-Arbez et al., 1999), numerous other mutations have been described in FGA, FGG and FGB. Apart from four missense mutations in FGB (L383R, G430D, G444S Y447G; Duga et al, 2000 and 2001; Vu et al., 2003), all mutations described so far are null and are predicted to cause a complete lack of the corresponding fibrinogen chain. The missense mutations all lie in the C-terminal portion of FGB, which is highly conserved amongst

Investigation of the molecular mechanisms regulating fibrinogen secretion

vertebrates. In addition, two nonsense mutations in the same region were recently characterized. The first, W470X, was identified in heterozygosity in an asymptomatic patient (Homer et al, 2002). The second nonsense mutation, W467X, three codons upstream, was identified in our laboratory in homozygosity in two Palestinian sisters with afibrinogenemia and results in a fibrinogen-beta chain which lacks only the last 25 amino acid residues (Neerman-Arbez et al, 2003).

Expression studies in transfected cells performed for four of these FGB C-terminal mutations both in our laboratory (G444S, W467X) and by others (L383R, G430D, Duga et al) demonstrated that an intact FGB C-terminal domain is necessary for hexamer secretion into the circulation, although assembly inside the cell appears unimpaired. Interestingly, this property of the fibrinogen beta-chain differs from the C-terminus of the fibrinogen gamma chain which has been shown to be necessary for intracellular hexamer assembly (Okumura et al, 2002).

The aim of this special project proposal is to determine the amino acid sequences and/or tridimensional structures in the FGB C-terminus implicated in hexamer secretion. To this goal we propose to construct serial deletions from the C-terminus by site-directed mutagenesis of the FGB wild-type cDNA construct as previously described (Neerman-Arbez et al, 2003; Vu et al, 2003). As a first step towards the identification of sequences involved in hexamer secretion, four to five amino acids

will be deleted at a time by introduction of a premature stop codon. The methodology used to assay intracellular assembly and secretion will be as previously described.

If this first experiment is successful, and the constructs allow us to pinpoint a specific amino acid sequence necessary for hexamer assembly, further deletion constructs, amino acid by amino acid will be made in order to further refine the region of interest. Computer modelling of the various mutant molecules will be performed in order to determine the underlying three-dimensional structure responsible for hexamer secretion. Results from this study may allow us to gain insight into secretion mechanisms of other coagulation proteins.

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