MCFD2, a second gene defective in combined factor V/factor VIII deficiency, encodes a novel ER protein that interacts with LMAN1 (ERGIC-53) to form a cofactor-specific sorting receptor

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Combined deficiency of coagulation factors V and VIII (F5F8D) is an autosomal recessive disorder that is inherited independently from the co-inheritance of factor V and factor VIII deficiencies. Affected individuals express between 5 and 30% of normal plasma antigen and activity of these necessary clotting cofactors. Approximately 70% of individuals with F5F8D have mutations in LMAN1 (ERGIC-53), a mannose-binding lectin that cycles between the endoplasmic reticulum (ER) and the Golgi apparatus. LMAN1 is proposed to facilitate secretion of factor V and factor VIII by binding to oligosaccharide structures within their B domains. Homozygosity mapping and positional cloning were used to identify defects in a second gene, which we named multiple coagulation factor deficiency 2 (MCFD2), that results in the majority of F5F8D families that do not have mutations in LMAN1. MCFD2 encodes a novel ER-Golgi intermediate compartment (ERGIC) protein with 2 EF hand domains, suggesting a role in calcium binding. Northern blot and RT-PCR analyses indicate that MCFD2 is ubiquitously expressed. In order to study the intermolecular interactions between LMAN1 and MCFD2, these proteins were co-expressed in COS-1 cells and analyzed by [^{35}S]-methionine metabolic pulse-chase labeling. Additionally, we analyzed two point mutations (D129E, I136T) within the EF hand domains that result in F5F8D. In cell extracts, we identified by immunoprecipitation using either anti-LMAN1 or -MCFD2 antibodies both wild-type MCFD2 and LMAN1, suggesting that the two proteins interact. Over-expression of LMAN1 was not required for the observed interaction. Addition of EGTA to cell extracts during immunoprecipitation eliminated the interaction between MCFD2 and LMAN1. Using similar analyses, an interaction between the two MCFD2 point mutants with LMAN1 was not observed, suggesting that the point mutants disrupt the calcium-dependent interaction between MCFD2 and LMAN1. Immunofluorescence demonstrated that wild-type MCFD2 co-localizes with LMAN1 in the ERGIC. In contrast, co-localization with LMAN1 was dramatically reduced for

the two MCFD2 EF hand domain point mutants. These results support that MCFD2 and LMAN1 function as a specific sorting receptor complex for the transport of factor V and factor VIII.

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