Genetics of hemolytic uremic syndrome

CHAPTER 9

Summary

Hemolytic uremic syndrome (HUS) is a rare disease of microangiopathic hemolytic anemia, low platelet count and renal impairment due to platelet thrombi occluding the renal circulation. In children, the disease is most commonly triggered by certain strains of *E. coli* that produce powerful exotoxins, the Shiga-like toxins (Stx1 and Stx2) and manifests with diarrhea, often bloody. Non-Shiga-toxin-associated HUS (non-Stx-HUS) comprises a heterogeneous group of patients in whom an infection by Stx-producing bacteria could be excluded as a cause of the disease and can be sporadic or familial. Collectively, non-Stx-HUS have a poor outcome, with death or permanent renal dysfunction being the final outcome in the large majority of cases. Since early seventies an underlying genetically determined condition predisposing to these forms of HUS has been hypothesized. Finding of depressed complement C3 levels and increased concentrations of C3 breakdown products in patient blood suggested an inherited defect causing hyperactivation of the complement cascade.

In this thesis a number of studies were designed and performed with the aims to provide more information as for the incidence and pathogenetic role of complement disregulation in non-Stx-HUS and to investigate the genetic basis of such abnormalities. Sporadic observations in literature provided evidence that activation of complement occurs also in patients with thrombotic thrombocytopenic purpura (TTP). The latter syndrome has in common with HUS microangiopathic hemolytic anemia, consumption thrombocytopenia and microvascular thrombosis, but differs because it manifests mainly with central nervous system symptom, whereas predominant renal involvement characterizes HUS. Examination of very large series of patients with HUS and TTP has pointed out that a neat clinical

distinction between the two syndromes is difficult. Thus, search for complement activation markers and of genetic abnormalities of complement regulatory genes was also undertaken in patients with diagnosis of TTP.

In **chapter 2**, in a case-control study by multivariate analysis, we correlated putative predisposing conditions, including low C3 serum levels, with history of disease in 15 cases reporting one or more episodes of familial HUS and TTP, in 25 age- and gender-matched healthy controls and in 63 case-relatives and 56 control-relatives, respectively. The relationship between history of disease, low C3, and factor H (CFH) abnormalities was investigated in all affected families and in 17 controls. Seventy-three percent of cases compared with 16% of controls ($P < 0.001$), and 24% of case-relatives compared with 5% of control-relatives ($P = 0.005$) had decreased C3 serum levels. At multivariate analysis, C3 serum level was the only parameter associated with the disease within affected families and in the overall study population. Thus, subjects with decreased C3 serum levels had a relative risk of HUS or TTP of 16.56 (95% confidence interval [CI], 1.66 to 162.39) within families and of 27.77 (95% CI, 2.44 to 314.19) in the overall population, compared to subjects with normal serum levels. CFH abnormalities were found in four of the cases, compared with three of the healthy family members ($P = 0.02$) and none of the controls ($P = 0.04$) and, within families, CFH abnormalities were correlated with C3 reduction ($P < 0.05$). These results indicate that reduced C3 clusters in familial HUS and TTP is likely related to a genetically determined deficiency in CFH and may predispose to the disease.

Studies of **chapters 3 and 4** were aimed at clarifying whether *CFH* mutations were

involved in genetic predisposition to non-Stx-HUS, by performing linkage and mutation studies in patients referred to the International Registry for Recurrent and Familial HUS/TTP. In **chapter 4**, five mutations in the *CFH* gene were identified. Three mutations, identified in two families and in a sporadic case, are heterozygous point mutations causing amino acid exchange within the most C-terminal short consensus repeat 20 (SCR20) of CFH, resulting in single amino acid substitutions. The other two mutations introduce premature stop codons that interrupt the translation of CFH. A heterozygous nonsense mutation was identified in SCR8 in one family, and a homozygous 24-bp deletion within SCR20 was identified in a Bedouin family with a recessive mode of inheritance. Reverse transcription-PCR analysis of cDNA from peripheral blood leukocytes from the Bedouin family showed that the deletion lowered CFH mRNA levels. Although heterozygous mutations were associated with normal CFH levels and incomplete penetrance of the disease, the homozygous mutation in the Bedouin family resulted in severe reduction of CFH levels accompanied by very early disease onset.

In **chapter 4**, we analyzed the complete *CFH* gene in 101 patients with non-Stx-HUS, in 32 with TTP and in 106 controls to evaluate the frequency of *CFH* mutations, the clinical outcome in mutation and non-mutation carriers and the role of *CFH* polymorphisms in the predisposition to HUS. We found 17 *CFH* mutations (16 heterozygous, one homozygous) in 33 HUS patients. Thirteen mutations were located in exons XXII and XXIII. The disease manifested earlier and the mortality rate was higher in mutation carriers than in non-carriers. Kidney transplants invariably failed for disease recurrences in patients with *CFH* mutations,

while in non-mutated patients half of the grafts were functioning after 1 year. Three *CFH* polymorphic variants were strongly associated with non-Stx-HUS: -257T (promoter region), 2089G (exon XIV, silent) and 2881T (963Asp, SCR16). The association was stronger in patients without *CFH* mutations. Two or three disease-associated variants led to a higher risk of HUS than a single one. Analysis of available relatives of mutated patients revealed a penetrance of 50%. In 5/9 families the proband inherited the mutation from one parent and two disease-associated variants from the other, while unaffected carriers inherited the protective variants. In conclusion *CFH* mutations are frequent in patients with non-Stx-HUS (24%). Common polymorphisms of *CFH* may contribute to non-Stx-HUS manifestation in subjects with and without *CFH* mutations.

These data provide compelling molecular evidence that genetically determined deficiencies in CFH are involved in both autosomal-dominant and autosomal-recessive HUS and identify SCR20 as a hot spot for mutations in the disease.

Of interest, in studies of **chapter 4**, none of the 32 patients with a diagnosis of TTP and no renal impairment carried *CFH* mutations, which indicates that CFH abnormalities are specifically associated with renal manifestations of the thrombotic microangiopathy.

Evidence is now available that most cases of TTP are triggered by deficiency of ADAMTS13, a plasma metalloprotease that cleaves von Willebrand factor multimers. ADAMTS13 is usually normal in HUS, however there are rare unequivocal cases of HUS characterized by low or undetectable ADAMTS13 activity. In **chapter 5** we investigated the genetic basis of phenotype heterogeneity in patients with ADAMTS13 deficiency in two

sisters within one family. The patients had ADAMTS13 deficiency as a result of two heterozygous mutations (causing V88M and G1239V changes). In addition, a heterozygous mutation (causing an S890I change) in *CFH* was found in the patient who developed chronic renal failure but not in her sister, who presented with exclusive neurologic symptoms. These data indicate that in the above patient CFH haploinsufficency had a role in determining the renal complication and the HUS phenotype that superimposed on the systemic disease caused by ADAMTS13 deficiency.

Two third of patients with non-Stx-HUS have no *CFH* mutations, despite decreased serum concentrations of C3. The aim of **chapter 6**, therefore, was to assess whether genetic abnormalities in other complement regulatory proteins are involved. We screened genes that encode the complement regulatory proteins- i.e. factor H related-5, complement receptor 1, and membrane cofactor protein (MCP)-by PCR-single-strand conformation polymorphism (PCR-SSCP) and by direct sequencing, in 25 consecutive patients with non-Stx-HUS, an abnormal complement profile, and no *CFH* mutation, from our International Registry of Recurrent and Familial HUS/TTP. We identified a heterozygous mutation in MCP, a surface-bound complement regulator, in two patients with a familial history of HUS. The mutation causes a change in three aminoacids at position 233-35 and insertion of a premature stop-codon, which results in loss of the transmembrane domain of the protein and severely reduced cell-surface expression of MCP. Findings of an MCP mutation in two related patients suggest that impaired regulation of complement activation might be a factor in the pathogenesis of genetic forms of HUS. MCP could be a second

putative candidate gene for non-Stx-HUS. The protein is highly expressed in the kidney and plays a major part in regulation of glomerular C3 activation. We propose, therefore, that reduced expression of MCP in response to complement-activating stimuli could prevent restriction of complement deposition on glomerular endothelial cells, leading to microvascular cell damage and tissue injury.

Finally the review **chapter 7**, was aimed at summarize the more recent advances from our and form other groups on the pathogenesis, the genetics and treatment of different forms of HUS.