



Hyperhomocysteinemia and Thrombosis: Studies of Genetic Implications

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This PhD thesis is based on four published, original articles – and on work carried out in 1995-1999 at the Department of Clinical Biochemistry, Aarhus University Hospital, Skejby, in collaboration with the Centre for Haemophilia and Thrombosis at Aarhus University Hospital.

Hyperhomocysteinemia is an independent risk factor in arterial and venous thrombosis. Deficiencies in folate, vitamin B6 and vitamin B12, either alone or in combination with genetic factors, may cause hyperhomocysteinemia. Mutations in genes encoding enzymes involved in the metabolism of homocysteine, e.g. cystathionine β -synthase (CBS) and methylenetetrahydrofolate reductase (MTHFR), may account for reduced enzyme activity and elevated plasma homocysteine levels (tHcy).

To determine some of the underlying genetic causes of hyperhomocysteinemia in thrombosis patients, we established a novel mutation scanning method to detect mutations in the CBS gene based on amplification and direct sequencing of genomic DNA. This method allows the detection of mutations in the entire coding region of the gene, including splice site mutations and mutations resulting in a reduced amount of transcript. For this purpose, we first had to elucidate the intron-exon boundaries of the human CBS gene. Furthermore, an analysis to detect a common polymorphism in the MTHFR gene (677C \rightarrow T) was established.

These methods were used to characterise thrombosis patients referred for thrombophilia investigation at the Centre for Haemophilia and Thrombosis, Skejby Sygehus. During this study, we identified seven patients with CBS deficiency, the most common inborn error of sulphur amino acid metabolism. The disease causing mutations in these patients and in other two CBS deficient patients was detected. The majority of the patients (six out of nine) were compound heterozygotes. In these patients, six novel mutations were detected; four

missense mutations, a splice mutation, and a 22-bp deletion. The four missense mutations were investigated in an *E.coli* expression system to determine their effect at protein level.

In a cohort of 28 consecutive thrombosis patients with intermediate or severe hyper-homocysteinemia ($30\mu\text{mol/l} < \text{tHcy} \leq 100\mu\text{mol/l}$, and $\text{tHcy} > 100\mu\text{mol/l}$, respectively), we determined the prevalence of MTHFR 677C→T polymorphism and of mutations in the CBS gene. Using this approach, three of the above-mentioned patients with CBS deficiency were identified. Furthermore, the MTHFR 677T/T genotype was shown to be present in 73.9% of patients with intermediate hyperhomocysteinemia, thus being the most significant genetic determinant in these cases. We have previously shown that the MTHFR T/T genotype was present in 8.3% of the general Danish population, and in 37.8% of thrombosis patients with mild hyperhomocysteinemia (elevated $\text{tHcy} < 30\mu\text{mol/l}$). This study also showed that the co-existence of the MTHFR T/T genotype and a common 68-bp insertion variant (844ins68) in the CBS gene apparently is a risk factor in thrombosis, being present in 10.7% of the patients (compared with 1.2% of the general population).

To estimate the prevalence of CBS deficiency in Denmark, we analysed the frequency of the most commonly detected CBS mutation world-wide (833T→C) in a cohort of new-born babies ($n = 500$). This showed a surprisingly high prevalence of the mutation at 1.4%, indicating that 1 in 20,500 is homozygous for the mutation. This prevalence is approximately 10 times higher than prevalences previously estimated or determined by new-born screening in other countries. Actually, three of the above-mentioned Danish CBS deficient patients were homozygous for the CBS 833T→C mutation. These patients had a mild phenotype, thromboembolisms apart. Considering the number of patients estimated to have this genotype, our results emphasise the importance of measuring plasma homocysteine levels routinely in thrombosis patients and in patients presenting other sequelae of CBS deficiency (mental retardation and neuropsychiatric symptoms, ectopia lentis, marfanoid features and osteoporosis). The identification of these patients is highly relevant because the development of severe clinical manifestations or the deterioration of pre-existing sequelae can be prohibited by lowering the level of tHcy in plasma by vitamin supplementation.