

ALLELE-SPECIFIC ANALYSIS OF COMPLEMENT FACTOR H PROTEIN IN ATYPICAL HAEMOLITIC UREMIC SYNDROME

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Introduction

Atypical haemolytic uremic syndrome (aHUS) is caused by abnormalities of the complement alternative pathway (AP) regulation. Factor H (FH) is an important regulatory protein of the AP, which plays a role in the inactivation of the active C3b molecule as the cofactor of factor I. FH (*CFH*). Mutations and polymorphisms in *CFH* may affect the functional characteristics or quantities of the protein. A new ELISA method specific for a single amino-acid difference of Factor H at position 402 (tyrosine or histidine) was recently described. Using this allele-specific method it becomes possible to determine the level of Y402 or H402 factor H proteins (and their ratio), which are produced from the different alleles in Y402H heterozygous subjects.

Based on the literature the *CFH*-H3 haplotype is associated with the development of aHUS, but the background of the connection is still unexplored. We hypothesize that the H3 haplotype acts through reduced factor H concentration as a risk factor for aHUS. Since the Y402H polymorphism is a constituent of the H3 haplotype, allele-specific protein measurement may help investigate the above hypothesis.

Methods

PCR-RFLP technique and allele-specific ELISA was used in the group of 79 healthy control subjects and 39 aHUS patients or their family members to determine the genotype of Y402H polymorphism and Y402 or H402 factor H antigen levels.

In addition to the Y402H, additional polymorphisms of factor H (-332:T (promoter region), V62I:G, Q672Q:G, E936D:T) were genotyped with PCR-RFLP and allelic discrimination real time PCR.

Results

In case of patients carrying a mutation with a known damaging effect (Arg1149X, Ser722X, Cys959Ser) reduced protein level was observed according to the mutation carrier allele.

Moreover, we detected decreased factor H concentrations in case of some mutation carrier patients (Val609Asp and Thr1216Del), who's variations have not been characterized functionally previously.

The ratio of Y402/H402 factor H protein levels allowed to determine that the protein according to the H3 haplotype (tyrosine at position 402) was produced in smaller quantities than the non-H3 protein (histidine at position 402).

Conclusion

We optimized an Y402H allele-specific ELISA method, which allows to measure the factor H concentrations produced from the two alleles of *CFH*. Currently the method is appropriate to detect the effect of the mutations or polymorphisms onto protein expression in Y402H heterozygous subjects. Based on our observations the H3 haplotype negatively affects the amount of the produced factor H protein novel, and using this ELISA mutations can be characterized functionally.