

PI PITTSBURGH

0026 PI PITTSBURGH [PI, MET358ARG] 'ANTITHROMBIN' PITTSBURGH (Source MEDLINE website)

This structure mutation in the PI gene alters its function such that it becomes an antithrombin and leads to a bleeding disorder. Alpha-1-antitrypsin and antithrombin III (107300) have a similar structure reflecting origin from a common ancestral protein some 500 million years ago. Both are inhibitors of proteolytic enzymes but have different specificities. Alpha-1-antitrypsin protects the body against released elastase, whereas AT III controls coagulation by inhibiting thrombin and other activated coagulation factors. Owen et al. (1983) described a mutation of alpha-1-antitrypsin that converts it to an antithrombin. Whereas synthesis of alpha-1-antitrypsin increases in response to trauma, AT III remains at a constant plasma concentration and requires activation by heparin. The antithrombin activity of the mutant alpha-1-antitrypsin was independent of heparin but its synthesis was stimulated by trauma. The patient was a 14-year-old boy who died in 1981 with a huge hematoma of his leg and abdomen. This was the last of a lifelong series of bleeding episodes occurring after trauma and requiring hospitalization on more than 50 occasions. Lewis et al. (1978) described the clinical picture and identified a variant 'antithrombin' which they called antithrombin Pittsburgh. It had, however, the electrophoretic and antigenic characteristics of a variant alpha-1-antitrypsin. Owen et al. (1983) showed that the variant protein has arginine at position 358, replacing the normal methionine. This finding indicated that the reactive center of alpha-1-antitrypsin is methionine 358, which acts as a 'bait' for elastase, just as the normal reactive center of AT III is arginine-393, which acts as a bait for thrombin. Neutrophils augment tissue proteolysis by the oxidative inactivation of the methionine at the reactive center of alpha-1-antitrypsin. Scott et al. (1986) and Schapira et al. (1986) found that recombinant AAT-Pittsburgh (met358-to-arg) is a potent inhibitor of plasma kallikrein and activated factor XII fragment, although it has lost its anti-elastase activity. They suggested it might have therapeutic potential in hereditary angioedema or septic shock. Vidaud et al. (1992) demonstrated that a G-to-T transition at nucleotide 10038 is responsible for the substitution of arg for met, which converts alpha-1-antitrypsin into an arg-ser protease inhibitor (serpin) that inhibits thrombin and factor Xa more effectively than antithrombin III. They observed a 15-year-old boy who surprisingly had no bleeding history. They suggested that a large decrease in protein C concentration may account for the mild or absent bleeding tendency. The deficiency of protein C in turn was attributed to deleterious effect of the abnormal inhibitor on both intracellular processing and catabolism of protein C. In later studies, Emmerich et al. (1995) suggested that strong affinity of the mutant AAT for protein C leads in the patient of Vidaud et al. (1992) to an increased turnover and thus to a low circulating level of protein C. They proposed that in the presence of the Pittsburgh mutant protein C can be activated and is abnormally rapidly cleared. The resultant relative lack of protein C anticoagulant function may ameliorate the bleeding diathesis expected to be associated with the Pittsburgh mutation. Wilkie (1994) discussed the molecular basis of genetic dominance and provided a useful table. He indicated altered substrate specificity as one mechanism and antithrombin Pittsburgh as a specific example.

N Engl J Med. 1983 Sep 22;309(12):694-8.

Mutation of antitrypsin to antithrombin. alpha 1-antitrypsin Pittsburgh (358 Met leads to Arg), a fatal bleeding disorder.

Owen MC, Brennan SO, Lewis JH, Carrell RW.

Our previous studies predicted a functional relationship between the plasma proteins alpha 1-antitrypsin and antithrombin III. To elucidate this relationship we investigated the plasma of a 14-year-old boy who had died from an episodic bleeding disorder. A variant alpha 1-antitrypsin was identified in which the methionine at position 358 had been replaced by an arginine. This had converted the alpha 1-antitrypsin from its normal function as an inhibitor of elastase to that of an inhibitor of thrombin. This finding indicates that the reactive center of alpha 1-antitrypsin is methionine 358, which acts as a bait for elastase, just as the normal reactive center of antithrombin III is arginine 393, which acts as a bait for thrombin. The

independence of the new thrombin inhibitor from heparin control explains the bleeding disorder; it also indicates that heparin normally acts directly on antithrombin III, revealing its inherent inhibitory activity. The episodic nature of the bleeding was a consequence of the mutant protein's being an acute-phase reactant, the level of which increased several-fold after trauma.

Brantly, et.al., "Molecular Basis of Alpha-1 Antitrypsin Deficiency, THE AMERICAN JOURNAL OF MEDICINE, June 24, 1988, VOL. 84 (Suppl 6A)

Only one dysfunctional A1AT variant has been identified, the A1AT Pittsburgh protein. This rare form of A1AT is associated with a bleeding diathesis and reduced anti-elastase capacity. The A1AT serum levels of A1AT Pittsburgh were in the normal range and the protein migrated in isoelectric focusing gels in a position cathodal to the normal M variants.

Although A1AT Pittsburgh has not been sequenced in its entirety, it clearly results from a mutation in the active inhibitory site of A1AT at residue 358 (normal Met358 to Arg358 in A1AT Pittsburgh). This causes the A1AT to no longer act as an excellent inhibitor of neutrophil elastase but instead to act similar to antithrombin III, i.e., as an inhibitor of thrombin. As such, the molecule has profound effects on the clotting system, resulting in the bleeding diathesis, even though it was inherited only in the heterozygous state.

Crystal RG, et.al, The Alpha-1 antitrypsin gene and its mutations—Clinical consequences and strategies for therapy, CHEST 95:1; Jan 1989, 196-208

"Alpha-1 AAT_{pittsburgh}", the only known example of a dysfunctional AAT variant, was found in an individual with a bleeding disorder. This form of AAT was partially sequenced and shown to have a single amino acid substitution at the AAT active inhibitory site (Met³⁵⁸→Arg). Interestingly, this gives the active site of the AAT molecule a remarkable homology with antithrombin III, the natural inhibitor of thrombin, and the affected individual died from hemorrhage following trauma. Evaluation of the AAT_{pittsburgh} protein has demonstrated it is an inhibitor of thrombin and a [poor inhibitor of neutrophil elastase](#).

References

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