

Antithrombin Oslo: Type Ib Classification of the First Reported Antithrombin-Deficient Family, with a Review of Hereditary Antithrombin Variants

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Key words

Antithrombin III – Familial antithrombin deficiency – Antithrombin antigen

Summary

Patients with classical antithrombin deficiency (Type I) from seven unrelated kindreds were studied by crossed immunoelectrophoresis of plasma in the presence and absence of heparin. The only abnormal pattern was found in the kindred first reported by Egeberg in 1965. An abnormal cathodal peak of antithrombin antigen was found in the presence, but not the absence, of heparin in the first dimension gel. We have named this variant antithrombin Oslo. Such evidence of an abnormal protein, despite equivalent low levels of antithrombin antigen and activity, has been denoted previously by Sas as Type Ib deficiency. In the context of this new report, we review the literature to date on 33 other variants of the Types Ib, II and III subclassifications with a discussion of the value of the classification scheme.

Introduction

In 1965, Egeberg reported the first family with antithrombin III deficiency and associated venous thrombosis, with a decrease in both progressive antithrombin (AT) and heparin cofactor activity to approximately half the normal level (1). In a later publication, Abildgaard et al. reported that this Norwegian kindred had an equally decreased AT antigen level, with a mean of 51% (2). This pattern of AT deficiency, with equally decreased antigen and functional assays with or without heparin, has come to be known as classical, or Type I, deficiency in the classification scheme of Nagy and Losonczy (3). AT variants, with normal antigen levels but reduced functional levels, have been reported in 30 families, and can be classified as Type II, if both progressive AT and heparin cofactor levels were reduced (4–30) or Type III, if only heparin cofactor activity was reduced (31–49). All of the Type III variants (and none of Type II) demonstrated an abnormal extra peak, more cathodal than the normal AT antigen peak, by crossed immunoelectrophoresis (CIE) with heparin present in the first dimension.

Surprisingly, Sas et al. found that a similar peak, representing a fraction of AT that bound poorly to heparin, was present in plasma of affected members of one Hungarian family with classical Type I deficiency (50). They suggested the name Ib for the subtype, while Ia denotes classical deficiency with normal CIE in the presence or absence of heparin. A similar Type Ib

variant, AT Roma (51–52), has been extensively characterized, and another family reported from Johannesburg appeared to have Type Ib variant (53). Another hereditary AT deficiency (AT Utah) was classified as Type I by equivalently low antigen and activity (54). Nevertheless, an abnormal protein was detected by immunoblotting (55), and shown to have a pro → leu⁴⁰¹ substitution (56). We report here the evaluation of 7 kindreds with Type I, classical deficiency (6 from Oslo and 1 from Stony Brook) by CIE in the presence and absence of heparin. Only the Norwegian kindred originally reported by Egeberg (1) showed an abnormal cathodal peak by CIE in the presence of heparin. We propose to call this Type Ib variant “Antithrombin Oslo”. We also review 33 other AT variants we found in the literature, with an analysis of the relative importance of progressive AT vs. heparin cofactor deficiency.

Materials and Methods

CIE of AT antigen was performed as previously reported (57) on a Multiphor apparatus (LKB, Rockville, MD). The first dimension gel was 0.9% agarose (Seakem ME, FMC Biomedical, Rockland, ME) containing on some occasions, 20 U/ml of sodium heparin (Apothekernes Lab., Oslo, or Grade I, porcine intestinal, Sigma, St. Louis, MO). The second

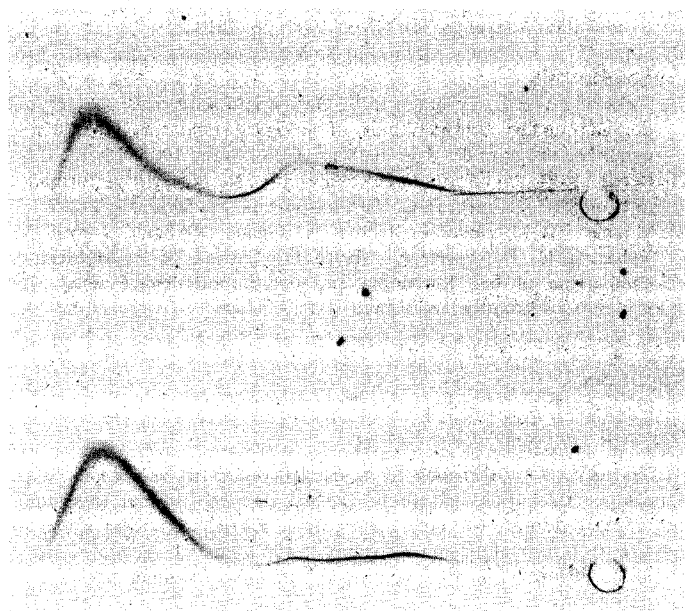


Fig. 1 CIE of AT antigen of pooled normal plasma (bottom) and one affected member of the Oslo kindred with Type Ib AT deficiency (top), with 20 U/ml sodium heparin in the first dimension

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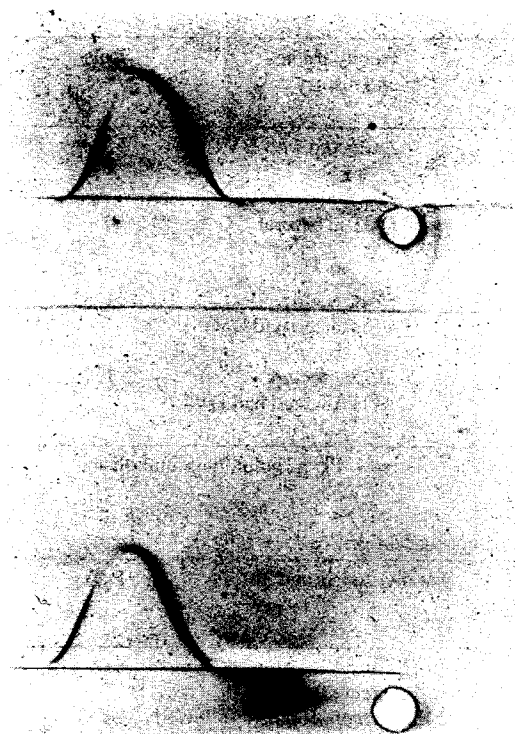


Fig. 2 CIE of AT antigen of pooled normal plasma (bottom) and one affected member of the Oslo kindred with Type Ib AT deficiency (top) (same as in Fig. 1) in the absence of heparin in the first dimension

dimension gel was 1.0% agarose containing 1.5–2.2% rabbit antiserum to human AT (Behringwerke, Marburg, W. Germany). Bromthymol blue was added to one sample in the first dimension, and electrophoresis stopped when the dye marker reached the edge of the plate (generally 2¼ to 2½ h). The second dimension was run 2 h.

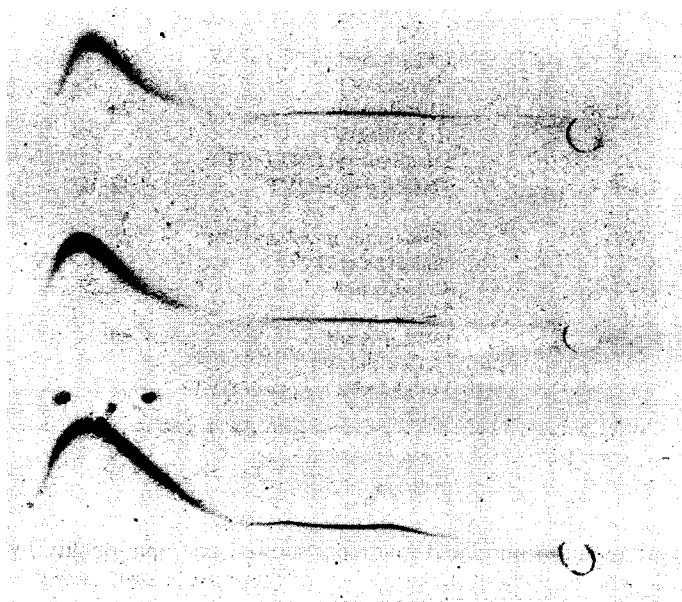


Fig. 3 CIE of AT antigen of pooled normal plasma (bottom) and two affected individuals from separate kindreds with Type I AT deficiency (middle and top), with 20 U/ml sodium heparin in the first dimension

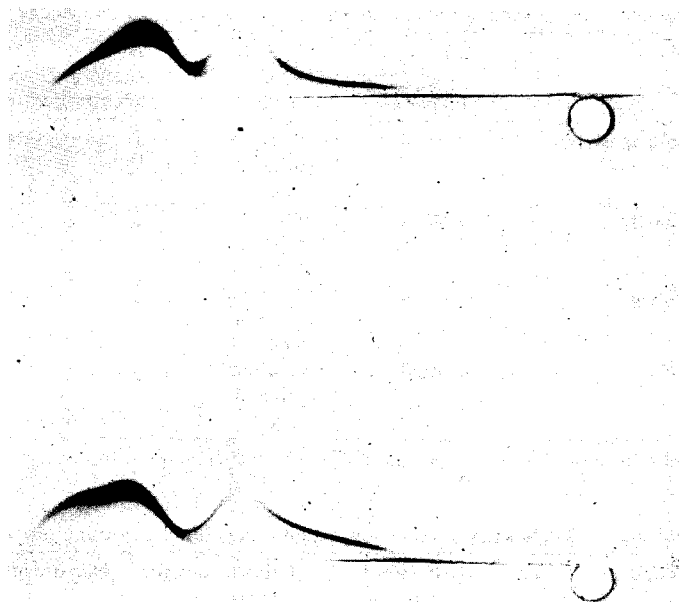


Fig. 4 CIE of AT antigen of two affected members of the Oslo kindred with Type Ib AT deficiency (bottom, same individual as in Fig. 1), with 20 U/ml sodium heparin in the first dimension

Results

During the course of other studies on abnormalities of AT demonstrated by CIE (58), we were stimulated to examine the CIE patterns of patients with Type I familial AT deficiency, because of preliminary studies (McKay and Abildgaard, unpublished) that had demonstrated an abnormal cathodal peak in CIE (with heparin) of two affected members of the kindred reported by Egeberg. We have now confirmed, on fresh samples taken from these same individuals, that a consistently reproducible cathodal peak is found by CIE with heparin in the first dimension (Figs. 1, 4) but not in its absence (Fig. 2). This pattern is clearly

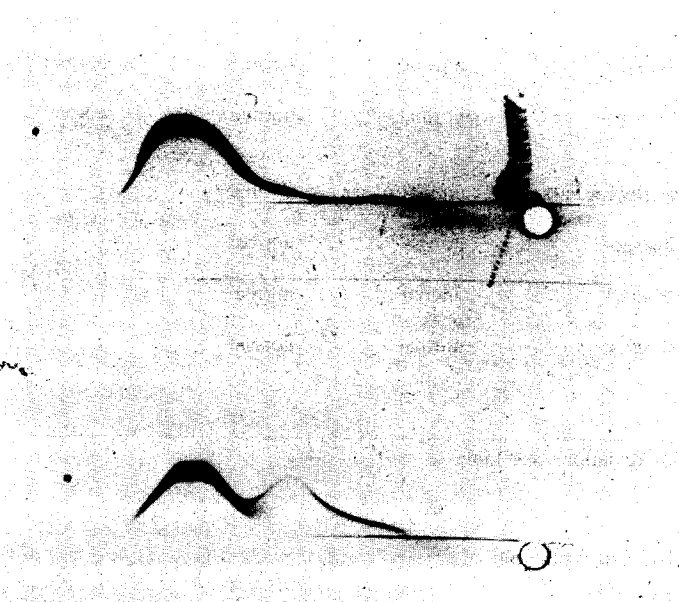


Fig. 5 CIE of AT antigen of an unaffected member of the Oslo kindred (top) compared to an affected member (bottom), with 20 U/ml sodium heparin in the first dimension

Table 1 AT deficiency Type Ib: low activity and antigen, with evidence of abnormal AT

Name	CIE without heparin on plasma	CIE with heparin on plasma	Heparin cofactor activity	Progressive AT activity	Propositus: Type of thrombosis	Family studies and history	Refs
Johannesburg	normal	abnormal cathodal peak	↓	N.R.	DVT	3 affected with DVT	53
Budapest 2	normal	abnormal cathodal peak	↓	↓	DVT	DVT in several	50
Roma	normal	abnormal cathodal peak	↓	N.R.	DVT, PE	5/8 affected; 4/5 affected had DVT	51, 52
Oslo	normal	abnormal cathodal peak	↓	↓	DVT	11/23 affected 9/11 affected had DVT	1, 2

Abbreviations: N.R. = not reported, DVT = deep venous thrombosis, CIE = crossed immunoelectrophoresis, PE = pulmonary embolism.

Table 2 AT deficiency Type II: low activity (heparin cofactor and progressive antithrombin) and normal antigen

Name	CIE without heparin on plasma	CIE with heparin on plasma	Heparin affinity	Propositus: Type of thrombosis	Family studies and history	Refs
Budapest	normal	abnormal cathodal peak	↓	DVT	10/14 affected; 5/10 affected had DVT	4-6
Malmö	normal	abnormal cathodal peak	↓	DVT	2 children of propositus affected but without DVT	24
Charleville	normal	abnormal cathodal peak	↓	DVT	2/5 affected; probable PE in propositus father	25
Tokyo	N.R.	abnormal cathodal peak	↓	DVT	N.R.	18
Aalborg	normal	normal	normal	DVT	7/20 affected; 4/7 affected had DVT	6, 7
Vicenza	normal	normal	normal	DVT	5/9 affected; 2/5 had DVT; 2 others died of PE	8-10
Hamilton	normal	normal	N.R.	DVT	4/7 affected; 2/4 had DVT	27, 28
Denver	normal	normal	normal	DVT	2/6 affected; 0/2 DVT	16, 17
Hvidovre	normal	normal	normal	DVT	3 affected with DVT	20
Trento	anodal peak	slightly abnormal	normal	DVT	5/11 affected; 1 DVT	11
Aalborg 2	N.R.	normal	N.R.	DVT	6/9 affected; 6 DVT	19
Milano 2	normal	normal	normal	PE	3/13 affected; 1/3 had DVT	26
Pescara	normal	normal	normal	DVT, PE	5/10 affected; 1/5 with DVT; 5 deaths from DVT or PE	29
Northwick Park	anodal peak	normal	↑	DVT	3 affected with DVT	12-15
Chicago	normal	normal	↑	DVT	12/46 family members have clinical hx of DVT	23
Milano 1	anodal peak	normal	↑	DVT	8/14 affected; 2 DVT	21, 22
Glasgow	normal	normal	↑	DVT	13/19 affected, 4/13 with DVT; 3 others with history of DVT or PE	30

Abbreviations: see Table 1.

different from that of pooled normal plasma (Fig. 1) and has not been detected in any normal individuals examined in our laboratory (data not shown). Since the plasma was obtained by careful venipuncture and rapid separation by centrifugation and stored frozen at -40 to 70 °C, it is very unlikely to be an ex vivo artifact. None of our other 6 patients with Type I AT deficiency,

representing 6 unrelated kindreds, showed any abnormality by CIE with or without heparin. Two of these are shown in Fig. 3. Furthermore, an unaffected member of the Oslo kindred did not show an abnormal cathodal peak by CIE with heparin (Fig. 5). Minor variations in the shape of both the normal and abnormal peaks are seen when electrophoretic conditions varied slightly

Table 3 AT deficiency Type III: low heparin cofactor activity, normal progressive AT activity, normal antigen

Name	CIE without heparin on plasma	CIE with heparin on plasma	Propositus: Type of thrombosis	Family studies and history	Refs
Ann Arbor	normal	abnormal	none	no DVT in family	31
Basel	normal	abnormal	superficial-left leg	mother affected; no history of thrombosis stated	32–34
Paris 1	normal	abnormal	DVT	6/9 affected; no DVT	35
Padua 1	normal	abnormal	none	4/15 affected; no DVT	36
Padua 2	normal	abnormal	none	4/11 affected; no DVT	37
Toyama	normal	abnormal	DVT and cerebral thrombosis (homozygous)	6/8 affected; heterozygotes-no DVT; consanguinity present	38, 39
Paris 2	normal	abnormal	DVT and arterial thromboemboli	1/2 affected; no DVT	40
Tours	normal	abnormal	none	several affected; no DVT	41, 42
Alger	normal	abnormal	arterial thromboemboli	8/14 affected; heterozygotes no DVT; consanguinity present	43–45
Fontainebleau	normal	abnormal	intra-cardiac thrombosis (homozygous)	3 affected heterozygotes asymptomatic; consanguinity present	46
Geneva	normal	abnormal	PE	7/11 affected; no DVT	47
Clichy	normal	abnormal	none	4/8 affected; no DVT	48
Rouen	N. R.	N. R.*	none	1/2 affected; no DVT	49

Abbreviations: see Table 1.

* propositus plasma contained an AT fraction with decreased heparin binding affinity.

(compare Fig. 1 and Fig. 4 run in Oslo and Stony Brook respectively). The abnormal cathodal peak is quite faint, suggesting the possibility that it is antigenically weak. This may in part explain the failure to detect an excess of AT antigen (compared to activity) by radial immunodiffusion or Laurell assay.

In our review of 33 detailed reports of Type Ib, II or III variants, we have found that the clinical significance of the classification of variants suggested by Nagy et al. (3) separating Type II from Type III, is supported by the accumulated data (Tables 1–3). We were unable to include a few reported variants because the classification of the pedigrees was unclear. In the 33 pedigrees reviewed, all the propoiti of Type Ib and II families, as well as one or more members of most of these families, had suffered from deep venous thrombosis and/or pulmonary emboli (Tables 1–2). There is no clear cut difference in the severity or frequency of thrombosis in these families, all of whom have decreased progressive AT activity, whether the variant shows decreased heparin binding, normal heparin binding or increased heparin binding. In contrast, only 6 of 13 propoiti of Type III families, and no other members of any of these well-studied families, were reported to have deep venous thrombosis (4 propoiti) and/or arterial thromboemboli (4 propoiti) (Table 3). Interestingly, one of these symptomatic Type III propoiti (AT Toyama) was homozygous for the AT variant and suffered both arterial and venous thrombosis (38). The propoiti of the AT Alger (44) and the AT Fontainebleau (46) families were also homozygous (<5% heparin cofactor) and suffered only arterial thromboemboli, associated with mitral valve insufficiency (44), or tricuspid valve dysfunction with intracardiac thrombosis (46).

Discussion

We have shown that AT Oslo meets the criteria for Type Ib AT deficiency. The abnormal CIE peak in AT Oslo most likely reflects an underlying qualitative abnormality in AT structure and/or metabolism. Studies of the exact genetic defect in hereditary AT deficiency have been reported for 8 variants, 3 showing defects close to the active site arg³⁹³ (Denver, Hamilton, Utah)

(16, 27, 48) and five showing substitutions in the heparin-binding region: arg → cys⁴⁷ (Toyama, Tours and Algiers) (39, 42, 45), arg → his⁴⁷ (Rouen) (49), and pro → leu⁴¹ (Basel) (34). Similar studies of other AT variant pedigrees could add to our understanding of the important functional domains of AT. Such studies on the AT Oslo variant are in progress.

Our review of AT variants supports the conclusion that a moderate heparin binding defect [Type III, or type 2c in the Sas classification (59)] by itself is not clinically as deleterious as an equivalent moderate reduction in progressive AT activity (Type II), that is, the ability to neutralize thrombin in the absence of heparin. However, the arterial thromboemboli seen in patients with <5% heparin cofactor activity suggest that binding of AT to heparin-like substances in the arterial vascular or valve surfaces may be part of a normal protective mechanism in the arterial circulation.

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Note added in proof

Two other recent communications reached similar conclusions regarding the importance of the classification of hereditary AT deficiency (60, 61).

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