Am J Hum Genet 37:32–41, 1985

Hereditary Thrombosis in a Utah Kindred Is Caused by a Dysfunctional Antithrombin III Gene

SUSAN CLARK BOCK,[1] JOANNA F. HARRIS,[1] CHARLES E. SCHWARTZ,[2] JOHN H. WARD,[2] E. J. HERSHGOLD,[2] AND MARK H. SKOLNICK[2]

SUMMARY

Maximum likelihood analysis of linkage between antithrombin III (ATIII) DNA polymorphisms and ATIII deficiency in a large Utah kindred suggests that thrombotic disease in this family is caused by a dysfunctional ATIII gene. ATIII-deficient family members were identified on the basis of: (1) reduced anticoagulant activity and (2) the presence of an electrophoretically abnormal inhibitor molecule in their plasmas. Affected individuals have two copies of the ATIII structural gene, and both alleles appear normal at the resolution of whole genome Southern blotting. However, family studies revealed statistically significant cosegregation of ATIII-deficiency trait and a particular ATIII DNA polymorphism haplotype (lod = 3.35; θ = 0.0); this result is consistent with the presence of a dysfunctional ATIII gene on a chromosome of the $+,S$ haplotype.

INTRODUCTION

The endogenous anticoagulant protein antithrombin III (ATIII) is essential for maintaining the fluidity of blood. During the past 20 years, more than 50 different kindreds segregating ATIII deficiency have been reported in the medical literature. This genetic disorder is codominantly inherited, and affected heterozygotes suffer recurring episodes of venous thrombosis and pulmonary embolism during adult life. We have cloned the cDNA for human antithrombin III [1] and are utilizing

Received May 17, 1984; revised July 10, 1984.

This work was supported by grants 1-RO1-HL30712 from the National Heart, Lung and Blood Institute and 83-1202 from the American Heart Association (to S. C. B.). J. H. W. is the recipient of a Clinician-Scientist Award from the American Heart Association with funds contributed in part by the Utah Heart Association.

[1] Rockefeller University, 1230 York Avenue, New York, NY 10021.

[2] Departments of Human Genetics and Medicine, University of Utah Medical School, 50 N. Medical Drive, Salt Lake City, UT 84132.

it to investigate the molecular genetics of ATIII deficiencies. Our present study suggests that a dysfunctional ATIII gene is responsible for hereditary thrombosis in a Utah kindred [2]. These results were reported in abstract form [3].

MATERIALS AND METHODS

Peripheral blood samples were obtained from 30 individuals of the Utah kindred. ATIII functional activity was determined both in plasma and in serum using amidolytic (endpoint method of Kabi Diagnostica, Stockholm, Sweden) and clot-inhibition assays [4], respectively. ATIII antigen levels were measured by radial immunodiffusion [5]. Genomic DNA samples were prepared as reported by Bell et al. [6].

DNA polymorphism haplotypes were determined by hybridizing nick-translated ATIII cDNA probe *pAT3c* [7] to Southern blots [8] prepared from 4 μg aliquots of *Pst*I- or *Bam*HI-cleaved genomic DNA.

Electrophoretically abnormal ATIII antigen was detected in plasma samples using an immunoblotting procedure. Samples containing the equivalent of 0.6 μl plasma were applied to a Laemmli gel [9] containing a 5%–10% polyacrylamide gradient. Following electrophoresis, proteins were transferred to nitrocellulose paper [10]. The blot was reacted sequentially with a 1:600 dilution of rabbit antihuman ATIII antiserum (Calbiochem, San Diego, Calif.) and a 1:5,000 dilution of horseradish peroxidase conjugated goat antirabbit IgG antiserum (Cappell, West Chester, Pa.), and developed with hydrogen peroxide and 4-chloro-1-napthol (Sigma, St. Louis, Mo.) [11].

Maximum likelihood analysis of linkage between DNA polymorphisms at the ATIII structural locus and ATIII-deficiency trait was performed using the computer algorithm PAP [12].

RESULTS

Affected Individuals in the Utah Kindred Produce a Qualitatively and Quantitatively Abnormal ATIII Molecule

Antithrombin III deficiency displays autosomal codominant inheritance in the Utah family under investigation (fig. 1). In an earlier study of this kindred, Cosgriff et al. [2] identified ATIII-deficient individuals on the basis of several criteria including evidence of: (1) symptomatic thrombosis, (2) decreased ATIII anticoagulant activity, and (3) decreased antigen levels. In 1983, at the beginning of our present study, peripheral blood samples were obtained from 30 family members. Each sample was split into several aliquots for determination of antithrombin functional activity and antigen levels, and preparation of genomic DNA. ATIII functional activity was assayed both in plasma and in serum; antigen levels were determined in plasma only. Assay values obtained for individuals involved both in this and the previous study [2] correlated well with those determined 5–7 years earlier. In general, plasma antigen levels were directly related to functional activity levels measured in plasma (fig. 2*A*) or serum (fig. 2*B*). The data define two distinct phenotypes in the kindred: unaffecteds who have ATIII levels within normal ranges and are homozygous for the wild-type ATIII gene, and affecteds who are heterozygous for the abnormal ATIII gene and whose ATIII levels are subnormal. However, as in the earlier study, assignment of individual II-11 to the affected or unaffected category was difficult due to assay values that fell between the ranges of those observed for normals and those obtained from individuals who were unambiguously deficient.

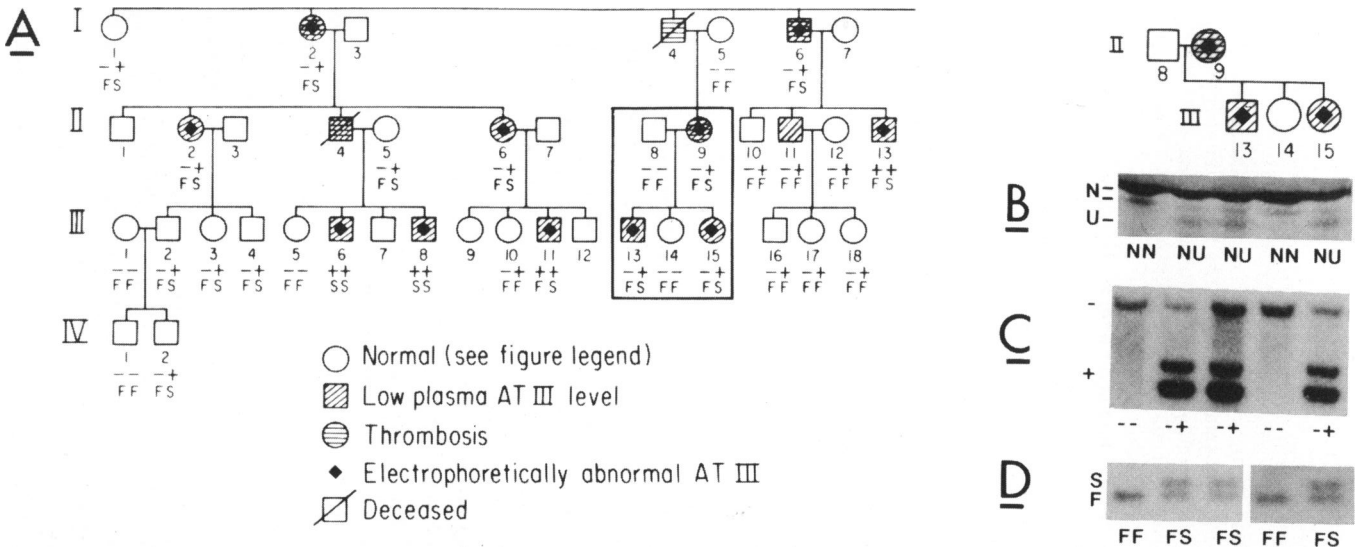

FIG. 1.—*A*, Pedigree showing segregation of decreased plasma ATIII levels, electrophoretically abnormal ATIII protein species, and two ATIII DNA polymorphisms in the Utah kindred. Presence of symptomatic thromboses and/or decreased plasma ATIII levels were scored in generations I and II on the basis of published information [2] and data from figure 2. No individuals in generations III and IV have suffered thrombotic episodes yet; clinical symptoms of hereditary thrombosis are usually not manifest until after the second decade. This pedigree was drawn by birth order. DNA polymorphism genotypes were determined by Southern blot hybridization of *Pst*I- and *Bam*HI-cleaved genomic DNA samples with nick-translated insert of cDNA clone *pAT3c* (for examples, see *C* and *D*). (+) indicates the *Pst*I-sensitive allele of the gln-305 sequence polymorphism, and (−), the *Pst*I-resistant allele [13]. *F* is the fast-mobility allele of the 5′ length polymorphism (32-bp variable segment), and *S* is the corresponding slow-mobility allele (108-bp variable segment) [7]. Representative immunoblot and Southern-blot experiments for the nuclear family consisting of individuals II-8 and II-9 and their offspring (boxed in *A*) are shown in *B*, *C*, and *D*. *B*, ATIII immunoreactive protein species in the plasma of selected Utah kindred members; *genotypes are indicated below the immunoblot*. *NN* indicates the presence of a quantitatively and qualitatively normal plasma ATIII pattern; *NU* indicates that the major and minor ATIII bands observed in normal plasma are present at reduced levels and that an electrophoretically abnormal ATIII species is also observed. *C* and *D*, Southern blot analysis of ATIII DNA polymorphism genotypes for the same five individuals. Four-μg aliquots of genomic DNA isolated from peripheral blood samples were digested with *Pst*I (*C*) or *Bam*HI (*D*). Southern blots prepared from these digests were hybridized to ^{32}P-labeled insert of ATIII cDNA clone *pAT3c*. +, −, *F*, and *S* designate ATIII DNA polymorphism alleles as indicated in *A*.

In an attempt to clarify this situation, we developed an alternative to the conventional biological assays for ATIII deficiency. This technique uses an immunoblotting procedure to detect electrophoretically abnormal ATIII antigen in plasma samples separated on SDS-polyacrylamide gels. When plasma samples from 30 members of the Utah kindred were analyzed using the immunoblotting technique, we found that plasma from the 11 individuals with unambiguously reduced activity and antigen levels produced a pattern that was qualitatively and quantitatively different from that observed for normals (for examples, see fig. 1*B*). This abnormal pattern is characterized by a decrease in the intensity of the major and minor ATIII immunoreactive bands observed in normal plasmas, and the appearance of a new immunoreactive species of slightly greater mobility. The new species is observed in plasma from affected individuals of the Utah family, but not in plasmas from several other ATIII-deficient families we have examined (S. C. B., unpublished observations, 1983). Plasma from individual II-11 produced a quantitatively and qualitatively *normal* ATIII immunoreactive pattern in this immunoblotting assay, and he was therefore classified as unaffected by ATIII deficiency in the maximum likelihood linkage study described below, although his anticoagulant and antigen levels are borderline as indicated in figure 2.

ATIII Deficiency in the Utah Kindred Is Tightly Linked to the ATIII Structural Gene

A maximum likelihood linkage study was used to show that ATIII deficiency in the Utah kindred is due to a mutation in or very near to the anticoagulant structural gene. Two DNA polymorphisms detected by hybridization of ATIII cDNA clone *pAT3c* to genomic DNA blots served as linkage markers for the ATIII structural gene. The molecular bases of these polymorphisms are a sequence heterogeneity [13] and an unusual length heterogeneity [7], and each has two common alleles ("$-$" and "$+$", and "*F*" and "*S*", respectively) whose frequencies are indicated in table 1. The alleles of both polymorphisms appear to be in Hardy-Weinberg equilibrium; however, analysis of Southern blot data on unrelated individuals from the Utah genealogy [14] revealed substantial disequilibrium ($D = -.137 \pm 0.013$) with respect to haplotype formation. Calculated haplotype frequencies were .11 for the $+,F$ haplotype; .22 for the $+,S$; .64 for the $-,F$; and .03 for the $-,S$. A disproportionate number of $-,F$ and $+,S$ haplotype chromosomes were observed. Calculation of the overall polymorphism information content [15] for the four haplotypes generated by these two two-allele polymorphisms yielded a value of .45, indicating that not more than 2 dozen segregating individuals would be required to prove or disprove tight linkage ($\theta = 0$) between the structural gene and the deficiency trait [16].

The linkage relationship of ATIII deficiency and the ATIII structural gene was determined by comparing segregation patterns of antithrombin deficiency and ATIII gene DNA polymorphisms in the Utah kindred. DNA polymorphism haplotypes were determined by hybridizing an ATIII cDNA probe to Southern blots prepared from genomic DNA of 30 individuals in the family. The genotype of each person with respect to plasma antithrombin levels, the presence of an electrophoretically abnormal ATIII plasma protein species, and the two ATIII DNA

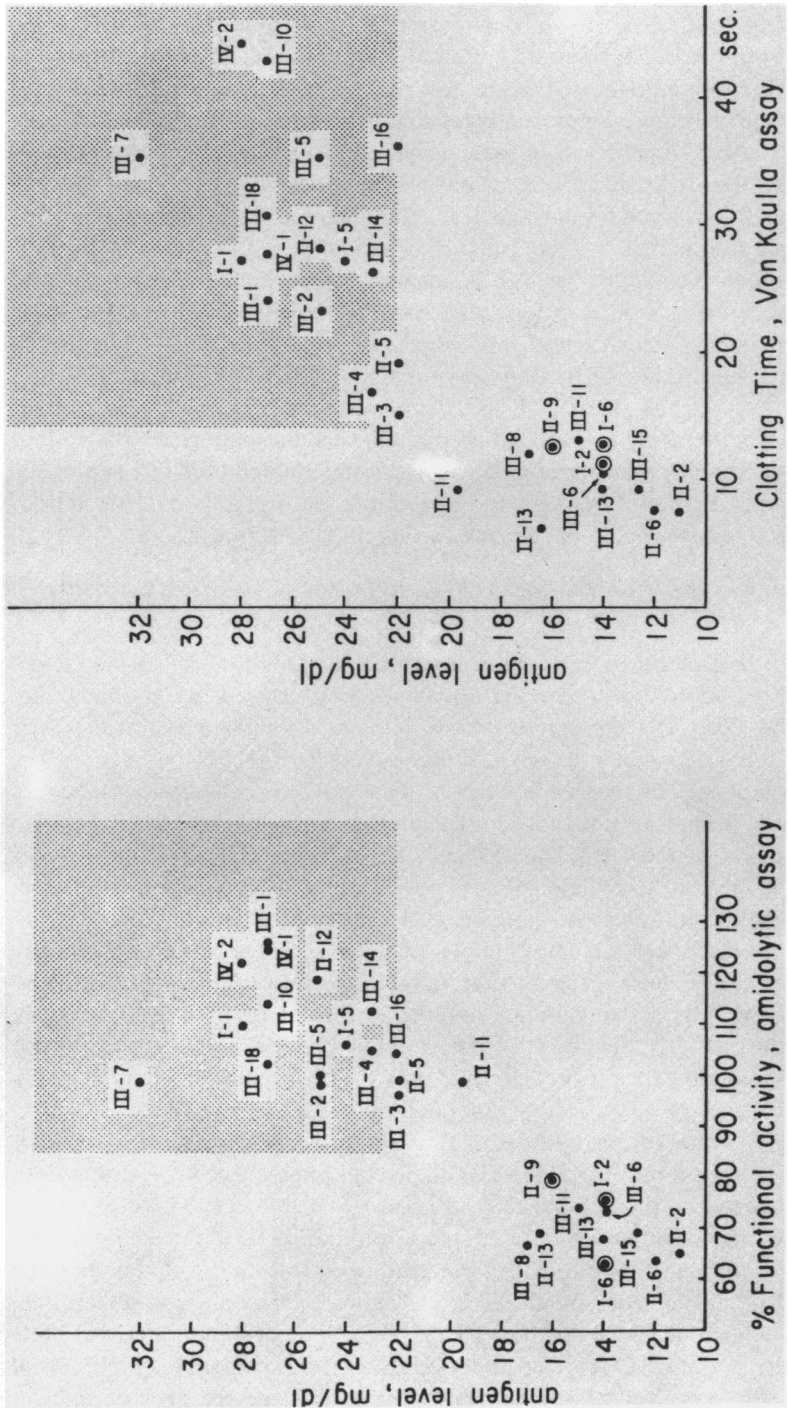

FIG. 2.—ATIII functional activity and antigen levels in members of the Utah pedigree. Antigen levels measured by radial immunodiffusion of plasma are plotted against functional activity levels in plasma (*A, left figure*) and serum (*B, right figure*). Functional activity was measured in plasma samples using an amidolytic assay and in serum by clot inhibition. *Data points that are circled* were obtained from affected individuals who were receiving sodium warfarin. *Shaded area in each plot* indicates the range of assay values observed for normal controls. Mean values for normals were 28 mg/dl in the RID assay, 100% in the amidolytic assay, and 30 sec. in the von Kaulla assay.

TABLE 1

ATIII DNA POLYMORPHISM ALLELE FREQUENCIES IN A POPULATION OF NORTHERN EUROPEAN ANCESTRY

Polymorphism	Allele	Frequency	n =
Sequence (*PstI*)	−	.66	80
	+	.33	
Length	*F*	.75	38
	S	.25	

polymorphisms is indicated in the pedigree drawing of figure 1*A*. Parts *B*, *C*, and *D* of figure 1 show actual immunoblot and Southern blot data for one nuclear family. Maximum likelihood analysis of linkage between the DNA polymorphisms at the structural locus and ATIII deficiency trait was performed. The criteria used to assign ATIII deficiency trait to a given individual were: (1) occurrence of the electrophoretically abnormal ATIII species in plasma of living family members and (2) medical history for deceased persons. Application of maximum likelihood analysis established that ATIII deficiency is tightly linked to the antithrombin structural locus in the Utah kindred and that the abnormal gene is associated with a chromosome of the +,*S* DNA polymorphism haplotype. Table 2 shows that the lod score for linkage is 3.35 at $\theta = 0$. When the logarithm base 10 of the odds ratio for the hypothesis of linkage vs. nonlinkage, or lod score, is greater than 3, the significance level of the linkage test is $< .001$ and the linkage result is accepted [17]. Therefore, these data indicate that the mutation causing ATIII deficiency in the Utah kindred is very tightly linked to the structural gene encoding the protein moiety of antithrombin III.

DISCUSSION

Descriptions of more than 50 different kindreds with hereditary antithrombin III deficiency have appeared in the medical literature during the past 20 years (see Thaler and Lechner [18] for review). Familial ATIII deficiencies may be divided into two broad categories. The majority of cases are ones in which ATIII antigen levels and anticoagulant activity levels are both reduced to approximately 50% of normal values. However, several families with reduced (~50%) activity levels but normal (~100%) antigen levels have also been described, and it has even been possible to identify and characterize an abnormal ATIII species in some of these cases. A priori consideration of molecular genetic mechanisms that could lead to the two ATIII deficiency phenotypes described above would include complete or partial gene deletions and mutations affecting anticoagulant

TABLE 2

LOD SCORES FOR LINKAGE OF ATIII DEFICIENCY AND ATIII DNA POLYMORPHISMS

θ = ...	0.0	.1	.2	.3	.4
Lod	3.35	2.78	2.09	1.35	0.6

biosynthesis or degradation in the former case and structural mutations in the latter case. Lesions in unlinked genes encoding molecules involved in the processing, modification, or expression of ATIII could also lead to both of the observed phenotypes.

Complete deletion of one ATIII allele has been established as the molecular basis for antithrombin deficiency in three kindreds where antigen and activity levels are both reduced to ~50% [13, 19]. The Utah kindred described here belongs to this same phenotypic category (reduced antigen *and* activity); however, in contrast to the previously cited cases, heterozygosity of ATIII DNA polymorphisms in a number of affected individuals (I-2, -6; II-2, -6, -9, -13; and III-11, -13, -15; see fig. 1) indicates that hemizygosity of the structural gene is not responsible for anticoagulant deficiency in this family. Since both ATIII genes in affecteds appear normal at the resolution of whole genome Southern blotting, anticoagulant deficiency must result from (1) a subtle mutation in the antithrombin locus itself or (2) a mutation in an unlinked gene that influences ATIII production. The presence of the $+,S$ DNA polymorphism haplotype in all affected members of the family suggested a linkage relationship between the deficiency trait and a specific chromosome bearing an abnormal ATIII allele. This hypothesis was confirmed by a maximum likelihood linkage study that established that the deficiency trait and the $+,S$ haplotype chromosome are tightly linked (lod = 3.35 at $\theta = 0$). This result, combined with evidence for an electrophoretically abnormal ATIII in affected plasmas, strongly suggests that antithrombin deficiency in the Utah kindred is caused by a dysfunctional ATIII gene.

The protein product of the dysfunctional ATIII gene was identified by electrophoresis in a polyacrylamide gradient gel system and immunoblotting. It is qualitatively different from wild-type antithrombin III and appears to be present in reduced amounts. The existence of a qualitatively and quantitatively abnormal ATIII may be explained by the presence of a single structural mutation that also reduces the rate of anticoagulant biosynthesis or increases its rate of degradation. The leu-125 to pro substitution postulated to destabilize the alpha-globin chain is an example of this kind of mutation [20]. Alternatively, the qualitative and quantitative differences in immunoreactive ATIII species from affected Utah plasmas may be due to the presence of two tightly linked mutations on the abnormal chromosome, one of which is in the structural portion of the gene and leads to aberrant electrophoretic behavior, and the other of which is in a regulatory region and leads to reduced synthesis.

The immunoblotting procedure developed during the course of this work proved very useful for determining the deficiency status of an individual (II-11) whose plasma yielded ambiguous values in three standard biological assays for ATIII. It may be useful to ascertain whether this methodology can be used to identify electrophoretically abnormal inhibitor molecules in other kindreds where determination of ATIII deficiency status for selected individuals has been problematic using conventional analytic methods. In addition, application of this procedure has revealed the presence of a minor ATIII immunoreactive component of increased electrophoretic mobility in normal plasma (see fig. 1*B*). Concomitant reduction in the intensity of both the major and minor normal bands observed in plasma

from affected heterozygotes of the Utah family suggests that the minor band is probably not the product of artifactual immunoprecipitation with an antibody to a contaminant in the ATIII preparation used to raise the antiserum.

The combined results of this study (which establishes linkage of the ATIII structural gene and ATIII deficiency) and previous studies of the Utah family [21] and other kindreds [19, 22, 23] (which showed genetic linkage of ATIII-deficiency trait and the 1q markers *Fy* [19, 21, 22] and *1qh* [23]) place the ATIII structural gene on the long arm of chromosome 1. This assignment has been confirmed cytogenetically. Using in situ hybridization and ATIII gene-dosage studies on DNA isolated from fibroblasts with characterized 1q deletions, the ATIII structural gene locus has been mapped to subregion 1q23-1q25 [24].

Our present study represents a solid foundation from which to begin molecular cloning of the dysfunctional ATIII Utah gene. We have established that ATIII deficiency in the Utah kindred is the result of a dysfunctional antithrombin III gene rather than an unlinked mutation. It was important to eliminate the possibility of an unlinked mutation, since this type of lesion would not be amenable to investigation with the ATIII cDNA probe. The existence of unlinked mutations has not yet been demonstrated for ATIII deficiency, but seemed reasonable since antithrombin III is a glycoprotein whose encoded polypeptide undergoes several maturation steps prior to its appearance in plasma as an active anticoagulant. Furthermore, precedents for unlinked mutations do exist in other systems: for instance, several cases of familial isolated growth-hormone deficiency type 1 that are *not* associated with growth-hormone gene mutations have been described [25]. Thus, the demonstration of tight linkage between the ATIII structural locus and ATIII deficiency in the Utah kindred established the presence of a dysfunctional ATIII gene and showed that an ATIII cDNA probe can be productively used to study hereditary thrombosis in the Utah family. The linkage study was also useful in that it identified individuals who are triply heterozygous for the deficiency trait and the two ATIII DNA polymorphisms (e.g., II-9, III-13, and III-15). Genomic DNA will be cloned from one of these individuals, and ATIII gene-containing recombinants isolated. DNA polymorphism analysis will be used to distinguish between clones originating from the dysfunctional ATIII gene and those originating from the wild-type allele. Finally, the identification of an electrophoretically abnormal antithrombin species in plasma of affected individuals of the Utah kindred will expedite molecular genetic work. Although the polypeptide-encoding portion of the ATIII gene is only 1,395 nucleotides, the entire structural locus spans some 14,000 base pairs (bp) of DNA. Isolation of the dysfunctional ATIII Utah molecule and identification of an abnormal peptide will allow us to target sequencing efforts and to more rapidly and efficiently determine the molecular basis of hereditary thrombosis in this family.

ACKNOWLEDGMENTS

We thank members of the Utah family for their cooperation, Dr. L. Rubin for helpful advice, and Drs. D. Lawn and B. Knowles for critical reading of the manuscript. The encouragement and support of Prof. E. T. Kaiser is gratefully acknowledged.

REFERENCES

1. Bock SC, Wion KL, Vehar G, Lawn RM: Cloning and expression of the endogenous anticoagulant protein, human antithrombin III. *Nucleic Acids Res* 10:8113–8125, 1982
2. Cosgriff TM, Bishop DT, Hershgold EJ, et al.: Familial antithrombin III deficiency: its natural history, genetics, diagnosis and treatment. *Medicine* 62:209–220, 1983
3. Bock SC, Harris JF, Schwartz C, Skolnick MH: DNA polymorphisms in the human antithrombin III gene. *Cytogenet Cell Genet* 37:422–423, 1984
4. von Kaulla E, von Kaulla, KN: Antithrombin III and diseases. *Am J Clin Pathol* 48:69, 1967
5. Mancini G, Carbonara AO, Heremans JF: Immunochemical quantitation of antigens by single immunodiffusion. *Immunochemistry* 2:235–254, 1965
6. Bell GI, Karam JH, Rutter WJ: Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. *Proc Natl Acad Sci USA* 78:5759–5763, 1981
7. Bock SC, Levitan DJ: Characterization of an unusual DNA length polymorphism 5′ to the human antithrombin III gene. *Nucleic Acids Res* 11:8569–8582, 1983
8. Southern EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517, 1975
9. Laemmli UK: Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 277:680–685, 1970
10. Burnette WN: "Western blotting." Electrophoretic transfer of proteins from SDS-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 112:195–203, 1981
11. Hawkes R, Niday E, Gordon G: A dot-immunobinding assay for monoclonal and other antibodies. *Anal Biochem* 119:142–147, 1982
12. Hasstedt SJ, Cartwright PE: PAP: *Pedigree Analysis Package*. Technical Report No. 13, Dept. of Medical Biophysics and Computing, Univ. of Utah, Salt Lake City, 1979
13. Prochownik EV, Antonarakis S, Bauer KA, Rosenberg RD, Fearon ER, Orkin SH: Molecular heterogeneity of inherited antithrombin III deficiency. *N Engl J Med* 308:1549–1552, 1983
14. Skolnick M: The Utah genealogical data base: a resource for genetic epidemiology, in *Banbury Report No. 4: Cancer Incidence in Defined Populations*, edited by Skolnick M, Lyon JL, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, 1980, pp 285–297
15. Botstein D, White RL, Skolnick M, Davis RW: Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 32:314–331, 1980
16. Skolnick MH, Bishop DT, Cannings C, Hasstedt SJ: The impact of RFLPs on human gene mapping, in *Genetic Epidemiology of Coronary Heart Disease*, edited by Rao DC, Elston RC, Kuller LH, Feinleib M, Carter C, Havlik R, New York, Alan R. Liss, 1984, pp 271–292
17. Morton NE: Sequential tests for the detection of linkage. *Am J Hum Genet* 7:277–318, 1955
18. Thaler E, Lechner K: Antithrombin III deficiency and thromboembolism. *Clin Haematol* 10:369–390, 1981
19. Winter JH, Bennett B, Watt JL, et al.: Confirmation of linkage between antithrombin III and Duffy blood group and assignment of ATIII to 1q22-q25. *Ann Hum Genet* 46:29–34, 1982
20. Goossens M, Lee KY, Liebhaber SA, Kan YW: Globin structural mutant alpha-125 leu to pro is a novel cause of alpha thalassemia. *Nature* 296:864–865, 1982
21. Bishop DT, Martin B, Baty B, Cosgriff T, Hershgold E, Skolnick M: Linkage of antithrombin III deficiency to Duffy blood group. *Am J Hum Genet* 30:48A, 1978

22. LOVRIEN EW, MAGENIS RE, RIVAS ML, GOODNIGHT S, MORELAND R, ROWE S: Linkage and study of antithrombin III. *Cytogenet Cell Genet* 22:319–323, 1978
23. MAGENIS RE, DONLON T, PARKS M, RIVAS ML, LOVRIEN EW: Linkage relationships of dominant antithrombin III deficiency and the heterochromatic region of chromosome 1. *Cytogenet Cell Genet* 22:327–329, 1978
24. BOCK SC, HARRIS JF, BALAZS I, TRENT JM: Assignment of the human antithrombin III structural gene to chromosome 1q23-q25. *Cytogenet Cell Genet.* In press, 1985
25. PHILLIPS JA III, PARKS JS, HJELLE BL, ET AL.: Genetic analysis of familial isolated growth hormone deficiency type 1. *J Clin Invest* 70:489–495, 1982

Errata

In the paper "Duplication of Chromosome 10p: Confirmation of Regional Assignments of Platelet-Type Phosphofructokinase and Hexokinase I by Gene-Dosage Studies," by S. Schwartz, M. M. Cohen, S. R. Panny, J. H. Beisel, and S. Vora (*Am J Hum Genet* 36:750–759, 1984), the title of this paper that appears on p. 750 should have read: Duplication of Chromosome 10p: Confirmation of Regional Assignments of Platelet-Type Phosphofructokinase and Hexokinase I by Gene-Dosage Studies.

In the paper "Human Minimal Androgen Insensitivity with Normal Dihydrotestosterone-Binding Capacity in Cultured Genital Skin Fibroblasts: Evidence for an Androgen-Selective Qualitative Abnormality of the Receptor," by L. Pinsky, M. Kaufman, D. W. Killinger, B. Burko, D. Schatz, and R. Volpé (*Am J Hum Genet* 36:965–978, 1984), the running heads appearing on the even-numbered pages should have read: Pinsky et al.

The editoral staff of the *American Journal of Human Genetics* apologizes to the respective authors for these errors.