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FIBRINOGEN MILANO III, A NEW ABNORMAL HEREDITARY FIBRINOGEN VARIANT WITH A DEFECT IN THE Aα-CHAIN. C. Bögli (1), A. Hofer (1), M. Furlan (1), E.A. Beck (1), F. Baudo (2) and R. Redaelli (2). Central Hematology Laboratory, University of Bern, Inselspital, Bern, Switzerland (1) and Hematology Department, Niguarda Hospital, Milano, Italy (2).

A new congential dysfibrinogenemia, denoted as Milano III, was found in a 13-year-old girl with recurrent thrombophlebitis. Plasma of the patient exhibited prolonged thrombin and reptilase times. Plasma fibrinogen concentration, determined by a functional assay, was less than 0.2 g/l while the immunological method revealed normal fibrinogen levels. Fibrinopeptide release, induced by thrombin, was normal whereas polymerization of fibrin monomers was delayed. Under conventional conditions for normal fibrin aggregation, with and without added calcium, the final turbidity of abnormal fibrin was less than 10 % of normal fibrin turbidity. The abnormal fibrinogen strongly inhibited clotting of normal fibrinogen. Isoelectric focusing showed an abnormal Aα-chain with greatly increased anodic mobility suggesting that the net electric charge of the Aα-chain is similar to that of the γ-chain. SDS-PAGE as well as reversed phase HPLC of mercaptolyzed fibrinogen showed normal Bβ- and γ-chains whereas the Aα-chain was degraded. The susceptibility towards degradation appears to be related to the molecular defect in the variant Aα-chain, since normal Aα-chain was not affected under the same conditions. Intact size of Aα-chain was preserved during preparative chromatofocusing of reduced fibrinogen chains. Fibrinogen preparations from the proposita's mother and father contained approximately equal amounts of both normal and abnormal Aα-chain. We conclude that fibrinogen Milano III in the proposita is a new homozygous functionally abnormal variant with a structural defect in the Aα-chain which is not located in the amino terminus.

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STRUCTURE-FUNCTION RELATIONSHIPS IN ABNORMAL FIBRINOGEN WITH Bβ14 ARG→CYS SUBSTITUTION: FIBRINOGENS SEATTLE I AND CHRISTCHURCH II. H. Kaudewitz (1),A. Henschen (1), H. Pirkle (2), D. Heaton (3), J. Soria (4) and C. Soria (4). Max-Planck-Institute for Biochemistry, Martinsried/Munich, FRG (1), University of California, Irvine, USA (2), Christchurch Hospital, New Zealand (3), Hôtel Dieu and Hôpital Lariboisière, Paris, France (4).

Genetically abnormal, dysfunctional fibrinogen variants may be used as unique models for studies of structure-function relationships both in vitro and in vivo. Out of the over 40 so far structurally elucidated abnormal fibrinogens only 4 have an amino acid substitution in the Bβ-chain. These variants are named Fibrinogen Pontoise, New York I, Christchurch II and Seattle I.

Fibrinogens Seattle I and Christchurch II are slow-clotting fibrinogens which on thrombin-treatment release only half the normal amount of fibrinopeptide B and therefore were expected to contain an amino acid substitution close to the thrombin cleavage site in the Bβ-chain. In order to sequence the abnormal Bβ-chains the fibrinogens were cleaved with thrombin and cyanogen bromide. The abnormal Bβ-chain components were isolated from the mercaptolysed-pyridylethylated N-terminal disulfide knots. After pyroglutamyl-peptidase digestion the Bβ 14 Arg→Cys substitutions could be demonstrated for both variants by direct N-terminal sequence analysis. The form of the cyst(e)ine residue was determined by amino acid analysis of the alkylated native fibrinogen. As no alkylated cysteine was detected it was concluded that Bβ 14 Cys participates in a disulfide bridge.

Fibrinogens Seattle I and Christchurch II are the first two elucidated fibrinogens with substitutions at the Bβ-chain thrombin cleavage site. Surprisingly, both the thrombin and Reptilase times are prolonged. It may be assumed that the half-cystine residues in position 14 of the two Bβ-chains within one fibrinogen molecule are disulfide-linked to each other, in an analogous way to that already established for the half-cystine residues in position 16 of the Aα-chains of several abnormal fibrinogens.This additional disulfide bridge might change the conformation and charge in the N-terminal region sufficiently to explain the prolonged clotting times. Furthermore, this bridge would provide the evidence for the parallel arrangement of the Bβ-chains at the fibrinogen N-terminus in a similar way as previously shown for the Aα-chains.

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TWO ABNORMAL FIBRINOGENS DESIGNATED AS OSAKA II AND MORIOKA WITH A HITHERTO UNIDENTIFIED AMINO ACID SUBSTITUTION; γ ARG-275 BY CYS. S. Terukina (1), M. Matsuda (1), N. Yoshida (1), K. Yamazumi (1), Y. Takeda (2) and T. Takano (3). Institute of Hematology, Jichi Medical School, Tochigi, Japan (1), Clinical Laboratory, Osaka Kosei-Nenkin Hospital, Osaka, Japan (2) and Department of Pediatrics, Iwate Medical School, Iwate, Japan (3).

A hitherto unidentified amino acid substitution of γ Arg-275 by Cys has been found in two abnormal fibrinogens, Osaka II and Morioka. The propositi are both asymptomatic heterozygotes for the abnormality characterized by altered polymerization of fibrin monomers. Reducing SDS-PAGE revealed that fibrinogens derived from the propositi both consist of two populations; one with a normal and the other with an abnormal longer γ-chain by 0.5 Kd. The γ-γ cross-linking took place nearly normally, however. Analyzing plasmic digests of fibrinogen by SDS-PAGE, we located the abnormality residing in the γ-chain remnant of fragment D. Chromatofocusing of D_1 obtained by plasmic digestion in 5 mM Ca^{++} of purified fibrinogen separated the variant D_1 (vD_1) from the normal one (nD_1) distinctly, as confirmed by SDS-PAGE and functional studies. As anticipated, vD_1 failed to interfere with normal fibrin polymerization and thrombin clotting of normal fibrinogen, whereas nD_1 inhibited these reactions significantly. After reduction and pyridylethylation, vD_1 and nD_1 were individually digested with lysylendopeptidase (lysEP). Analyzing the digests by reverse phase HPLC, we noted a single peak present in the digests of vD_1 but missing in those of nD_1, and vice versa. Analysis of N-terminal five cycles of these peptides suggested that both of them corresponded to the peptide with residues 274-302 based on the known sequence data. Primary sequence and total amino acid analyses revealed that γ Arg-275 has been substituted by Cys in both of these abnormal fibrinogens. Analysis of the lysEP-digests of the isolated γ-chain also gave the same result. Since no free SH has been identified at the γ Cys-275 substitute, the variant γ-chain may be endowed with some additive by an S-S linkage. Even if so, elucidation of an apparent elongation by SDS-PAGE of the γ-chain variant must await further investigation. In any case, however, the substitution of γ Arg-275 by Cys may have induced critical alterations in the γ-chain-dependent polymerization site in the D domain in these two abnormal fibrinogens.

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FIBRINOGENS KYOTO AND TOCHIGI, EACH WITH AN APPARENT ABNORMAL MOL. WT. γ CHAIN, ARE CHARACTERIZED BY REPLACEMENT OF γ ASN-308 BY LYS AND γ ARG-275 BY CYS, RESPECTIVELY. N. Yoshida (1), S. Terukina (1), M. Matsuda (1), M. Moroi (2), M. Okuma (3) and N. Aoki (4). The Institute of Hematology (1) and Department of Biochemistry II (2), Jichi Medical School, Tochigi, the First Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto (3) and the First Department of Internal Medicine, Tokyo Medical and Dental University, Tokyo (4), Japan.

Congenital inherited abnormal fibrinogens (Fbgs) Kyoto and Tochigi showed prolonged thrombin- and reptilase-time, normal release of fibrinopeptides A and B, normal crosslinking ability and impaired polymerization of the fibrin monomer.

Purified Fbg analyzed on SDS-PAGE under the reduced condition in the system of Laemmli contained 50 % of an apparent lower mol. wt. γ chain (γ Kyoto)(mol. wt.= 48,000 compared with 50,000 for the normal) in Fbg Kyoto and an apparent higher mol. wt. γ chain (γ Tochigi)(mol. wt.= 50,500) in Fbg Tochigi. Apparent mol. wt. differences were also detected in reduced and carboxymethylated Fbg, Fbg fragment D_1, and D_2, but not in D_3. This suggested that the abnormality of γ chains in both Fbgs is in γ 303-356.

Amino acid sequence analysis was performed for CNBr- or lysylendopeptidase-digested peptides of the γ chain or D_1 peptides after fractionation on HPLC. In Fbg Kyoto, γ Asn-308 was substituted by Lys, and a deletion of short peptides corresponding to the mol. wt. difference of 2,000 could not be detected. In Fbg Tochigi, γ Arg-275 was substituted by Cys, and no abnormality of amino acid sequence was found in γ 303-356.

These results suggest that some lesions or conformations containing γ 275 and γ 308 will directly or indirectly affect polymerization of fibrin monomers. Although the reason for apparent mol. wt. differences is not known yet, SDS-PAGE in the system of Laemmli will be useful for the analysis of abnormal Fbgs.

Fbg Kyoto could not be separated into two or three populations and may contain hetero-dimer molecules, but Fbg Tochigi had unclottable Fbg with predominant γ Tochigi and may contain abnormal homo-dimer molecules and normal molecules.