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ABSTRACT N. CO35

MALATTIE EMORRAGICHE CONGENITE E ACQUISITE

BLEEDING PHENOTYPE CHARACTERIZATION IN PATIENTS WITH CONGENITAL BLEEDING DISORDERS: A MULTIGENIC APPROACH USING NEXT GENERATION SEQUENCING TO IDENTIFY COAGULATION-MODULATING VARIANTS.

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Background and aims. Congenital bleeding disorders, including hemophilia A (HA), hemophilia B (HB), and rare bleeding disorders (RBDs), often display variable clinical phenotypes not fully explained by a single pathogenic variant. Additional genetic variants modulating the coagulation cascade may influence bleeding severity. This study aimed to investigate such variants using a multigenic sequencing approach.

Methods. Eighteen patients with a previously confirmed diagnosis of congenital bleeding disorder were selected for analysis. The cohort included individuals with HA, HB, FV deficiency, FXI deficiency, and plasminogen deficiency, all followed at the Hemophilia Center of Padua University Hospital. Genetic analysis was performed using a custom Next-Generation Sequencing (NGS) panel (xGen™ Hyb Panel, Integrated DNA Technologies) targeting 33 coagulation-related genes: *A2M, CPB2, F2, F3, F5, F7, F8, F9, F10, F11, F12, F13A1, F13B, FGA, FGB, FGG, KLKB1, KNG1, PLAT, PLG, PROC, PROCR, PROS1, PROZ, SERPINA5, SERPINA10, SERPINC1, SERPIND1, SERPINE1, SERPINF2, TFPI, THBD, VWF*. Sequencing was performed on the Illumina NextSeq550 platform. Variants with a minor allele frequency <0.1% were classified according to ACMG criteria.

Results. The study population consisted of 10 patients with HA (1 mild, 1 moderate, 8 severe), 2 with HB (1 mild, 1 severe), 1 with FV deficiency, 1 with FXI deficiency, and 4 with plasminogen deficiency. On average, 150 variants per patient were observed. NGS identified class 5 pathogenic variants in 8 patients with HA (*F8*, including intron 22 inversion) and in 2 with HB (*F9*), and class 4 likely pathogenic variants in 3 patients (*F8* [n=2] and, *F11* [n=1]). Although only class 3 variants were identified in patients with plasminogen deficiency (n=4) and FV deficiency (n=1), these were located in the gene associated with the clinical phenotype (*PLG* and *F5*, respectively). Moreover, two plasminogen-deficient patients harboured two distinct variants in *PLG*. Beyond the causative mutations, 11 out of 18 patients (61%) harboured at least one variant of class 3 or 4 with potential modulatory effect. These were found in *F2, F5, F10, F13A1, KNG1, VWF, SERPINA5, SERPINC1, PROS1*, and *PLG* and included well-characterized variants such as FV Leiden, the prothrombin G20210A mutation (prothrombotic), and the Friuli FX variant (bleeding phenotype). Moreover, two predisposing variants were identified in *F13A1* (n=2, pro-hemorrhagic) and *F2* (n=1, prothrombotic). The distribution of class 3, 4, and 5 variants and predisposing variants is illustrated in Figure 1.

Conclusions. NGS identified the putative causative mutation in all patients and revealed additional coagulation-related variants in the majority of the cohort. These results support a multigenic model influencing the bleeding phenotype in congenital bleeding disorders. Functional and segregation studies are warranted to clarify the role and clinical impact of the predisposing variants identified.

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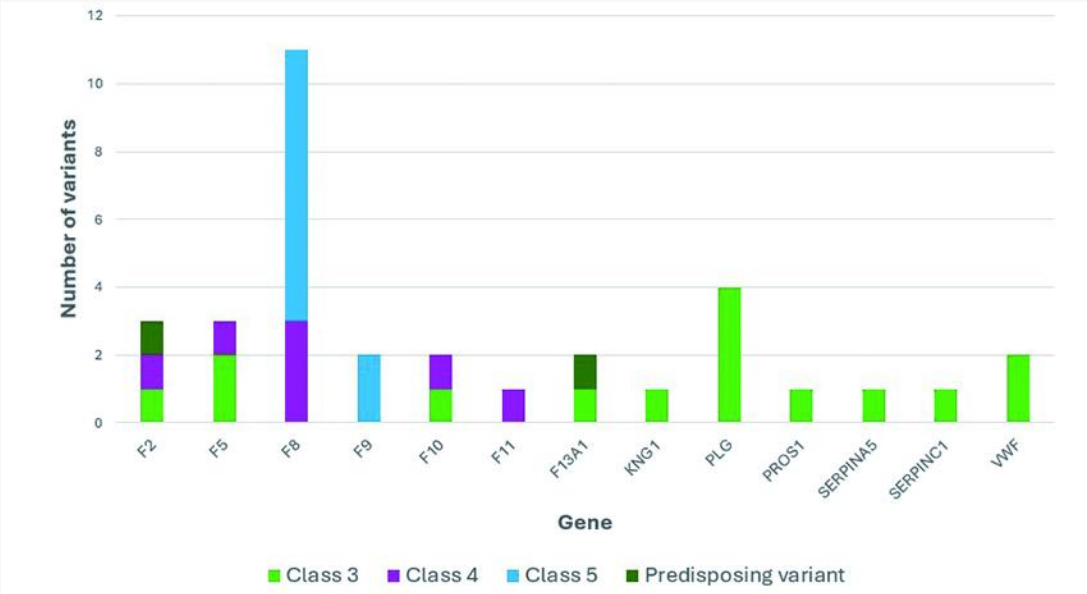

Number of variants identified per gene, categorized by ACMG classification. Pathogenic (class 5), likely pathogenic (class 4), variants of uncertain significance (class 3) and predisposing variants are shown in separate colors.