CONCISE REPORT

Structural Integrity of the Glycoprotein IIb and IIa Genes in Glanzmann Thrombasthenia Patients From Israel

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Glanzmann thrombasthenia is an autosomal recessive disorder of the platelet glycoproteins (GP) IIb and IIa. These glycoproteins normally serve as receptors for other adhesive glycoproteins, including fibrinogen, von Willebrand factor, and fibronectin. Most patients affected by Glanzmann thrombasthenia have low levels of GPIIb and GPIIIa; however, the separate mechanisms responsible for the deficiency in each remain to be determined. cDNA clones coding for the GPIIb and GPIIIa have been recently isolated, and their corresponding genomic sequences have been colocalized to the long arm of chromosome 17. Since a deletional event involving one or both of these structural genes could explain the disease phenotype, we have studied the DNA of two previously well-characterized cohorts of Glanzmann thrombasthenia patients from Israel. We performed Southern analysis with near full-length cDNA probes on genomic DNA obtained from 20 individuals. Four restriction enzyme digests were completed on each DNA sample. The similarity of banding patterns among probes, family members, and controls indicated that there were no major insertions or deletions in either the GPIIb or GPIIIa genes. Thus, the genetic defect in these patients with Glanzmann thrombasthenia is most likely due to either a small change in the nucleotide sequence of the coding region or a defect in the regulatory region of one or both genes.

The platelet receptor for adhesive glycoproteins, a calcium-dependent heterodimer composed of glycoproteins (GP) IIb and IIa, is a member of the integrin cyto-adhesion receptor superfamily. Patients with Glanzmann thrombasthenia have a spectrum of quantitative or qualitative defects in GPIIb and GPIIIa that lead to a clinically significant bleeding disorder. Two groups in Israel with a high incidence of the disease, one Iraqi-Jewish and the other Arab, have been well characterized. Although platelets from the two groups showed marked deficiencies in both proteins, trace amounts of GPIIb could be detected by immuno blotting in platelets from the Arab population but not in those from the Iraqi-Jewish population. The genetic basis for the disease phenotype remains obscure. Given the unusual colocalization of the genes for these two proteins at the terminus of the chromosome, it is possible that loss of all or part of the loci coding for these receptor proteins could account for the disease. An unbalanced translocation resulting in loss of these loci might be well tolerated, depending on the genetic information distal to these genes. The recent cloning of cDNAs for GPIIb and GPIIIa has made it possible to analyze the genomic loci of the two genes in patients with Glanzmann thrombasthenia. In this report, we show by genomic Southern blotting that the structural genes coding for GPIIb and GPIIIa in these Glanzmann thrombasthenia patients do not contain major deletions or insertions.

MATERIALS AND METHODS

Sample population. The 12 Arab subjects included five affected individuals, five relatives, and two geographic controls. Among these subjects were two subgroups: family A included two patients, two unaffected siblings and the mother, and family B included an affected child and both unaffected parents. The six Iraqi-Jewish subjects included four unrelated patients and two ethnic controls. Clinical and laboratory characterization of these 18 subjects has been previously reported. As a control, Southern analysis was performed on genomic DNA from two American subjects.

Genomic DNA preparation. Peripheral blood was collected from Glanzmann thrombasthenia patients in accordance with accepted guidelines regarding patients’ rights and approval; normal peripheral blood was obtained from volunteers. The mononuclear leukocyte fraction was collected and genomic DNA was prepared as previously described.

Southern blotting. Genomic DNA was digested with restriction enzymes HindIII, PstI, MspI, EcoRI, BamHI, XbaI, or BglII, electrophoresed on a 1% agarose gel, and transferred to nitrocellulose. Hybridization was performed with random-primed or nick-translated probes as previously described. The GPIIIa cDNA was isolated from a human endothelial cell library by L.A. Fitzgerald, who kindly provided it for our use. A 2.3-kb EcoRI fragment containing all of the GPIIIa coding sequence and part of the 3' untranslated sequence was used in these studies. The GPIIb cDNA was cloned from a library that had been constructed from mRNA of the human erythroblast cell line (HEL) cell line. The cDNA contained all of the coding block except 340 base pairs (bps) at the 5' end. Both the GPIIb and GPIIIa probes are thought to contain sequence identical to the platelet receptor mRNAs. The deduced endothelial cell GPIIIa sequence is identical to the known amino acid sequence for much of the platelet receptor, and in situ hybridization for chromosomal localization is consistent with GPIIIa being a single copy gene. The GPIIb protein found in HEL cells has been shown to be identical to that found in platelets.

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RESULTS

Identical restriction fragment patterns were obtained with the DNA from all 20 individuals, which was tested with four restriction enzymes and both the GPIIb and GPIIIa probes. Figure 1 shows blots of representative DNA samples from the Arab and Iraqi-Jewish patients and the control subjects obtained with the *HindIII* and *PstI* digests. The identical restriction patterns indicate that there has been no significant loss of DNA in the GPIIb and GPIIIa loci. From this data it is also unlikely that an insertional mutational event could have disrupted the loci. Because the inclusion of more enzymes would increase the sensitivity of the analysis, we studied five more digests in one of the Arab families. Figure 2 shows blots obtained with three of these enzymes. The additional digests revealed no differences in restriction pattern, providing further support for the conclusion that the genomic loci in these patients are conserved.

It is worth noting that we observed no polymorphisms in these genomic blotting studies. This absence of polymorphism indicates a high degree of conservation of sequence, despite the apparently large genomic sequence covered by each of the structural genes.

DISCUSSION

Southern blot analysis of genomic DNA from control subjects and subjects with Glanzmann thrombasthenia failed to reveal abnormalities indicative of gross structural alterations at the GPIIb and GPIIIa loci. Such data also exclude the possibility that significant amounts of genetic material could have been lost by unbalanced translocation or some other deleterious event. Therefore, the genetic defect in these patients must be due to small mutations in the coding region or a defect in regulatory regions not studied with the GPIIb and GPIIIa probes. An example of the former would be a nonsense mutation resulting in a premature termination codon. This could produce a truncated GPIIb or GPIIIa chain that would fail to be processed correctly. In this situation, the normal partner glycoprotein could also fail to be processed correctly and could thus undergo degradation without having been transported to the cell surface. The immunoglobulin molecule provides one example of a situation in which the normal member of a heterodimer fails to be processed correctly because its partner is defective. A mutation in a cis-acting sequence important to the transcriptional regulation of one or both of the GPIIb and GPIIIa genes could also affect expression of the normal partner.
Further study of Glanzmann thrombasthenia is greatly hindered by the inaccessibility of the mRNA for the GPIIb and GPIIIa glycoproteins. The availability of mRNA from tissue expressing these genes would allow cDNA cloning and would markedly simplify nucleotide sequence analysis of the coding region. Also, with GPIIb and GPIIIa mRNA, abnormalities in transcriptional regulation or message size could be identified by simple blotting procedures. The GPIIIa gene is expressed in endothelial cells and constitutes the β-chain of the vitronectin receptor. We have also observed that GPIIIa is expressed in fibroblasts in conjunction with the vitronectin α-chain (M.E.R. and T.Q., unpublished data). Thus, skin biopsy could provide a means with which to obtain mRNA for the GPIIIa chain. Access to GPIIb mRNA might be possible through the use of placental tissue. A complete understanding of the genetic defect in these patients with Glanzmann thrombasthenia probably awaits completion of the laborious task of cloning and sequencing both normal and diseased GPIIb and GPIIIa alleles.

genes could also account for the phenotype observed in these patients with Glanzmann thrombasthenia. Such a regulatory region mutation would not be detected with these cDNA probes.

A single defective allele is most likely responsible for the disease in these high-prevalence groups in which consanguinity is common. Individuals affected with Glanzmann thrombasthenia would thus be homozygous at the defective locus. However, we did consider the possibility of more than one diseased allele being present in these patient groups. The pattern of bands on a genomic Southern blot might not reveal a significant loss of genetic material on a single chromosome. This would be the case if one of the diseased alleles carried a point mutation. To rule out this possibility, we performed densitometric analysis on a number of blots probed with GPIIb, GPIIIa, and a third control probe. The results were consistent with the presence of a constant allelic dose in all persons studied (data not shown).

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