Characterization of Inherited Differences in Transcription of the Human Integrin α2 Gene*

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Inherited, single-base substitutions are found at only two positions, C-109T and C-99G, within the proximal 5'-regulatory region (within −1096 to +48) of the human integrin α2 gene. We recently reported that the T-52 substitution results in decreased binding of transcription factor Sp1 to adjacent binding sites, decreased transcription of the α2 gene, and reduced densities of platelet αβ1. In this study, we identify an additional Sp1-binding site at position −107 to −99 and show that the adjacent dimorphic sequence C-99G also influences the rate of gene transcription. In the erythropoiesis cell line Dami, transfected promoter-luciferase constructs bearing the G-92 sequence exhibit roughly a 3-fold decrease in activity relative to the C-92 constructs. In transfected CHRF-288-11 megakaryocytic cells, the corresponding activity decreases by 5-fold. DNase I footprinting of the promoter region with Dami nuclear extracts showed a protected segment at −107 to −99 that can be deprotected by coinubcation with molar excess of a consensus Sp1 oligonucleotide. Gel mobility shift assays and supershift assays with specific antibodies indicate that Sp1 binds to this region of the α2 gene promoter. Mutation of the Sp1 binding element within −107 to −99 in constructs containing either C-92 or G-92 abolishes basal promoter activity and eliminates the binding of Sp1. The G-92 sequence has a gene frequency of 0.15 in a typical Caucasian population, and the presence of this allele correlates with reduced densities of platelet αβ1. The combined substitution G-92/T-52 has an additive influence on gene transcription, resulting in an 8-fold decrease in transfected Dami cells or a 3-fold decrease in transfected CHRF-288-11 cells. In summary, the natural dimorphism C-92/G within the proximal 5'-regulatory region of the human integrin α2 gene contributes to the regulation of integrin αβ1 expression on megakaryocytes and blood platelets and must thereby modulate collagen-related platelet function in vivo.

The integrin αβ1 is a receptor for both laminin and collagen on most cell types but binds exclusively to collagen when expressed on megakaryocytes and blood platelets (1). A single copy of the α2 gene is present in the haploid genome, located on chromosome 5 (5q23–31) (2).

There is, on average, a 5-fold range in platelet αβ1 density among normal individuals that correlates with the inheritance of at least three α2 gene alleles and directly influences the rate of platelet adhesion to collagens ex vivo (3–7). The α2 gene allele 1 (A1 or 807T) is associated with a high density of platelet αβ1, whereas alleles 2 and 3 (A2 and A3) confer lower densities.

The expression of α2 is known to be regulated at the transcriptional level in megakaryocytes (8–10), epithelial cells (11, 12), and fibroblasts (13, 14). Fig. 1 summarizes the major features of the proximal 5'-regulatory region of the human α2 gene. Zutter et al. (11) have defined a "core" promoter region within positions −92 to −30 relative to the transcription start site (+1), and subsequent studies have determined that the proximal 961 bp of the 5'-flanking region of α2 isolated from the K562 cell line direct cell type-specific suppressor and enhancer activity in cells of epithelial origin (8, 11). Within the core promoter, two consensus Sp1/Sp3 binding elements are located on either side of the C-52T dimorphism previously described by us (15). A second dimorphism, C-92G, lies immediately downstream from the nonanucleotide sequence -GGGGCGGGG- with the general properties of another Sp1 and/or Sp3 binding element. Aside from this proximal 5'-regulatory region, additional distal sequences are thought to contain enhancer elements necessary for maximal transcription of the α2 gene in cells of megakaryocytic lineage (8, 9). Partial sequences of the human α2 gene have been reported (3), and a complete reference gene sequence can be assembled from overlapping genomic clones deposited at GenBankTM (accession numbers AC008773, AC008966, AC016619, AF035968, AF062039, AF113511, NM002203, L24121, and U31518).

We recently provided evidence that allelic differences in receptor density, initially observed on blood platelets, correlate with the C-52T dimorphism within the 5'-regulatory region of the α2 gene (15). This dimorphism influences transcription by altering the affinity of the flanking sequences for the transcription factors Sp1 and Sp3. Furthermore, the sequence T-52, which attenuates Sp1/Sp3 binding and transcriptional activity, is in linkage disequilibrium with one of the alleles (A3) known to be associated with diminished expression of the integrin on platelets.

In the current study, we provide additional characterization of the 5'-regulatory region of this gene. First, we provide direct evidence that the nonanucleotide -GGGGCGGGG- within positions −107 and −99 is indeed a Sp1-binding site. In addition, we show that the second dimorphism, C-92G, immediately

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∥ The abbreviations used are: bp, base pair; LUC, luciferase.
downstream from this site also modulates gene transcription and acts synergistically with C-^52T to vary transcription rates by more than an order of magnitude in megakaryocytic cell lines, such as Dami or CHRF-288-11. These two sequence dimorphisms represent the only naturally occurring differences within the proximal 5'-regulatory region of the gene (position 21-1096 to +48). These inherited sequence differences have a profound influence on expression levels of this gene in megakaryocytic cells.

MATERIALS AND METHODS

Cell and Tissue Samples—Platelets and mononuclear cells were isolated from citrated whole blood, as described previously (3). Personnel of the National Institutes of Health and processed in the GCRC. Six volumes of whole blood were mixed in informed consent, blood samples were collected from normal volunteers for determination of variance model were calculated.

Exon sequence numbering is based on the incomplete human sequence reported by Takada and Hemler (17).

The forward primer A2ProF2 (5'-CACACAGCCTCTTGAGGAGCC3') spans nucleotide 2160 through nucleotide 2191, was generated using forward primer A2ProF (5'-CACACAGCCTCTTGAGGAGCC3') and reverse primer A2ProR2 (5'-AC TAGCCTTACCCACAGTG3'). The second segment (800 bp), which extends nucleotide 2146 to 2244 and ends at nucleotide +191, was generated using forward primer A2ProF2 (5'-CACACAGCCTCTTGAGGAGCC3') and reverse primer A2ProR. From the third polymerase chain reaction product incorporating the sequence segment from nucleotide 191 to nucleotide +131.

The proximal 5'-promoter sequences—DNA binding activity of nuclear proteins in the supernatants was determined as described previously (22, 23).

The Dami and CHRF-288-11 cells were transfected by electroporation using a Cell-Porator (Life Technologies, Inc.). Approximately 1 x 10^6 cells were transfected in 500 μl of Iscove's modified medium containing 20 μg of plasmid DNA and 20 μg of pSV-β-galactosidase DNA by electroporation at 300 V and 1180 microfarads.

Plasmid Constructions—The proximal 5'-regulatory region of the α2 gene corresponding to 244 to +40 was amplified by polymerase chain reaction from a human megakaryocyte DNA by electroporation at 300 V and 1180 microfarads.

Interaction between genotype and phenotype were assessed from the data for the single-base substitutions T to G at positions 92 or T92G, and C to A at position 85 of OEB-1 (13). The OEB-1 DNA fragment was excised using the restriction enzymes BglII and HincII.

The desired fragment was cloned in pGL2-enhancer to form p244-LUC. The insert sequence was confirmed in both directions using primers supplied by the manufacturer (Promega; GLprimer1 and GLprimer2). The plasmids p244D32T-LUC, p244D92G-LUC, and p244A92G32T-LUC were generated from p244-LUC as follows: the p244 insert was excised using SacI and HindIII restriction sites and cloned into the pALTER-1 vector (Promega, Madison, WI). The primer sequences were as follows (restriction sites are underlined): 5'-A2PROFORP1, 5'-GAGGGTACCCAGAAGAAGCTTGCCAGAGGGC-3' and 5'-A2PROREV2, 5'-GGACATCTGGAACTGCTCCAGAGGGC-3'.

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then extracted with phenol:chloroform, and DNA was precipitated with ethanol. Sequencing reactions of the DNA segment corresponding to the DNA probe used for footprinting were carried out by the dideoxynucleotide chain termination method of Sanger et al. (24) using the fmol sequencing kit (Promega) according to the manufacturer’s instructions. The sequencing primer was 5′-GTTTCTGGCCAGCTCTGGCACG-3′. Reaction products were analyzed using 6% denaturing (urea) polyacrylamide gel electrophoresis (QuickPoint; Invitrogen, Carlsbad CA) followed by autoradiography.

RESULTS

The 5′-regulatory Region Sequence—The naturally occurring dimorphism C → T at position 92 is located precisely in the middle of tandem Sp1/Sp3 binding elements of the core promoter (15), whereas the C → G dimorphism lies closest downstream from the non-analogue -GGGGCGGGG- at 107/99 (Fig. 1). Our findings, summarized below, indicate that the 107/99 sequence represents an additional Sp1-binding site.

Identification of Transcription Factor-binding Sites Surrounding the 92 Dimorphism—To determine the identity of the transcription factors that might bind to the promoter sequence surrounding the dimorphic position at −92, DNase I footprinting analysis was performed with nuclear extracts from Dami, K562, or CHRF-288-11 cells and a promoter probe representing the region −246 to −14 on the plus strand. Comparable results were obtained with nuclear extracts from each of the three cell lines, and an example of the results obtained with Dami cell extracts is depicted in Fig. 2. The footprint spanned the promoter region from about −200 to roughly −30 on the plus strand. Incubation with Dami cell extracts showed protected segments at −107 to −99 and −61 to −43 (Fig. 2).

The Influence of the C → G Substitution at Position −92 on the Binding of Nuclear Proteins—Gel mobility shift analyses were conducted with nuclear proteins from either Dami or CHRF288-11 cells using a 29-bp core promoter fragment (bp −111 to −82) that contains either 92C or 92G as probe (Fig. 3). In addition, the same segments synthesized with mutations in the upstream Sp1-like binding element to create Cm or Gm. In a representative gel mobility shift assay using a nuclear extract from Dami cells (Fig. 4), a major complex is formed in the presence of either 92C (lane 1) or 92G (lane 2). Three nonspecific minor complexes appear with faster relative mobilities. Mutation of the Sp1-like binding element in either Cm (lane 3) or Gm (lane 4) eliminated the formation of the major complex. Based on supershift analyses with polyclonal antibodies, including anti-nuclear factor-κB (lanes 5 and 6), anti-AP2 (lanes 7 and 8), anti-Sp1 (lanes 9 and 10), anti-Sp3 (lanes 11 and 12), or anti-Egr-1 (lanes 13 and 14), this complex is formed by the binding of Sp1 only. The C → G base substitution at −92 has only a small effect, if any, on the binding of Sp1 to the flanking segment, such that the intensity of the complex formed with −92G is usually less intense than that formed with −92C (for example, compare lane 2 versus lane 1). Lastly, complex formation with either 92C (lane 15) or 92G (lane 16) was essentially abolished by preincubation with a 200-fold molar excess of the unlabeled Sp1 consensus oligonucleotide (Sp1c; Promega).

Additional gel mobility shift assays were conducted to examine whether the effect of the −92C or −92G substitution has an effect on the binding of Sp1 and Sp3 to the downstream elements at position −52. This was accomplished using the long probes depicted in Fig. 5. Note that these probes contain the −92C or −92G dimorphism and the two tandem Sp1/Sp3 binding elements that flank −52C, but not the Sp1 binding element located upstream at −107/99. As shown in Fig. 6, the same four complexes are formed between Dami cell nuclear proteins and the −92C long probe (lane 2) or −92G long probe (lane 7): the slowest mobility complex (arrowhead) is given by binding of Sp1 and/or Sp3; the remaining three complexes are nonspecific (see below). In the absence of Dami cell nuclear proteins, no complexes are formed (lanes 1 and 6). Addition of either anti-Sp1 (lanes 3 and 8) or anti-Sp3 (lanes 4 and 9) results in a supershifted complex (asterisk) in the case of either probe. Coincubation with a 200-fold molar excess of the consensus Sp1-binding oligonucleotide (Sp1c) inhibits formation of the specific complex (lanes 5 and 10), whereas the nonspecific complexes remain. Repeated attempts to generate specific complexes in gel mobility shift assays using even longer probes containing both the −52 Sp1-binding elements and the Sp1-binding element upstream at −107/99 consistently failed, most likely because of the sheer length of these longer oligonucleotide probe sequences.

Thus, the nuclear protein from Dami cells that forms a specific complex with either the −92C or the −92G probe is the Sp1 transcription factor, and the C/G substitution at −92 does not affect the binding of Sp1 to the downstream elements surrounding the −52 C/T dimorphism. Essentially identical results are obtained with nuclear proteins from the CHRF288-11 cell line (data not shown).

Reporter Assays—To investigate how decreased affinity of the G → T and T → C sequences might influence in situ transcription rates, we compared the relative activities of promoter-LUC.
The 29-bp core promoter fragment (bp 111–141) of the α2 gene was labeled and incubated (left panel) alone (lane 1); in the presence of 20 (lane 2), 40 (lane 3), or 80 μg (lane 4) of Dami cell nuclear protein; or in the presence of a 50-fold molar excess of unlabeled consensus Sp1-binding oligonucleotide (Sp1c) plus 20 (lane 5), 40 (lane 6), or 80 μg (lane 7) of Dami cell nuclear protein. The two vertical bars to the left of the gel represent two major protected areas, the first at −107 to −99 (top bar) and the second within the region from −61 to −43 (bottom bar). The protected nucleotides were mapped by comparison with a sequence generated using a primer originating at nucleotide −14 (right panel), and selected nucleotide positions are indicated to the left of the figure.

**Fig. 2.** DNase I footprinting analysis of the proximal promoter region. A 233-nucleotide DNA fragment from the 5′-regulatory region (−246 to −14) of the α2 gene was labeled and incubated (left panel) alone (lane 1); in the presence of 20 (lane 2), 40 (lane 3), or 80 μg (lane 4) of Dami cell nuclear protein; or in the presence of a 50-fold molar excess of unlabeled consensus Sp1-binding oligonucleotide (Sp1c) plus 20 (lane 5), 40 (lane 6), or 80 μg (lane 7) of Dami cell nuclear protein. The two vertical bars to the left of the gel represent two major protected areas, the first at −107 to −99 (top bar) and the second within the region from −61 to −43 (bottom bar). The protected nucleotides were mapped by comparison with a sequence generated using a primer originating at nucleotide −14 (right panel), and selected nucleotide positions are indicated to the left of the figure.

**Fig. 3.** Oligonucleotide probes at position −92 for gel mobility shift assays. The 29-bp core promoter fragment (bp −111 to −83) that contains either −92C (C) or −92G (G) was used. The upstream Sp1-binding element at −105 to −99 is underlined. Oligonucleotides mutated in this Sp1-binding element represented the probes Cm and Gm. The Sp1 consensus oligonucleotide (Promega) is designated Sp1c.

Derived from a comparison of 62 haplotypes, is not identical to the published α2 promoter sequence (11) but can be considered the allele sequence most equivalent to that published sequence. The remaining plasmid constructs are as follows: pα244A92G-LUC, which contains the replacement G at −92; pα244A52T-LUC, which contains the replacement T at −52; and pα244A92G52T-LUC, which contains both replacements. Each construct was ligated into the vector pGL2-basic or pGL2-enhancer (Promega). Identical results were obtained with either vector, so the cumulative results were averaged. Promoter activities were compared after transient transfection of Dami cells or CHRF-288-11 cells. Cotransfection with the vector pSV-β-galactosidase (Promega) was used to normalize for transfection efficiency. The results of these assays are summarized in **Fig. 7**.

Dramatic differences were observed in both Dami cells (Fig. 7A) and CHRF-288-11 cells (Fig. 7B). In Dami cells (five separate experiments), the mutation A92G results in a 3-fold decrease in promoter activity, 52T also results in a 3-fold decrease in activity, and the combination A92G52T decreases activity by 5-fold. This finding is consistent with an additive effect of each substitution on gene transcription. In Dami cell transfectants, this translates into a decrease in reporter gene activity. Even more dramatic differences were obtained for CHRF-288-11. In three experiments, A92G caused roughly a 5-fold decrease in promoter activity, 52T resulted in about a 4-fold decrease in activity, and the combination A92G52T decreased activity by 20-fold (Fig. 7). Again, the combined effect of the two substitutions was additive. In the case of either cell line, individual nonparametric tests of C versus T were statistically significant at the 0.05 level, using the Wilcoxon test.

**Donor Differences in Platelet α2β1 Expression**—As summarized under “Introduction,” there is a 5-fold range in platelet α2β1 density among normal subjects. In this study, the surface
content of platelet α2β1 in whole blood was measured by flow cytometry, using one of two murine monoclonal anti-α2β1 antibodies, SC12 or 12F1. Identical findings were made with each antibody, and the results are depicted in Fig. 8. A total of 71 donors who expressed either allele A1 or allele A2 were subdivided by genotype into three categories (Fig. 8A), denoted 1/1 (homozygous for A1), 1/2 (heterozygous for A1 and A2), and 2/2 (homozygous for A2). Donors were also classified (Fig. 8B) as expressing none (0), one (1), or two or more (≥2) of the G−92 or T−52 substitutions. For each donor, platelet α2β1 density was determined, as reflected by relative binding of both the antibody 8C12 (□) and the antibody 12F1 (●). Two-way analyses of variance were performed with these relative binding data from each antibody to simultaneously evaluate the effects of α2 genotype and number of G−92 or T−52 substitutions on platelet α2β1 density. Virtually identical findings were made with each antibody; in both cases, differences in densities between α2 genotypes (Fig. 8A) were highly statistically significant (with the 12F1 antibody, F2,62 = 24.90, p < 0.001; with the 8C12 antibody, F2,62 = 45.01, p < 0.001), as were differences in densities between the numbers of promoter substitutions (Fig. 8B) (with the 12F1 antibody, F2,62 = 7.42, p = 0.001; with the 8C12 antibody, F2,62 = 4.87, p = 0.011). Group means and standard errors of the mean for the relative binding data are depicted in Fig. 8. In the Fig. 8A, we note that the 1/1 genotypes have the highest α2β1 densities, the 1/2 heterozygotes have intermediate α2β1 densities, and the 2/2 homozygotes have the lowest α2β1 densities, with little quantitative difference between the 12F1 and the 8C12 data. In Fig. 8B, we find an inverse relationship between the number of inherited substitutions within the promoter regions and the platelet density of α2β1, with the platelet integrin level of donors who express 2 or more of G−92 or T−52 consistently lower than that of donors who express a single or no substitutions at these positions.

**DISCUSSION**

Previous studies of Sp1 binding elements within the proximal promoter region of the human α2 gene have focused on two tandem elements at positions −61 to −53 and −51 to −43. These sites have been found to be essential for the transcriptional activity of this gene (9, 12). In a recent report (15), we showed that the inherited dimorphism C−92T significantly influences the binding of Sp1 to these flanking elements, such that the substitution T−52 results in decreased binding of Sp1 and decreased Sp1-dependent transcription in megakaryocytic cell lines. In this study, we identify an additional Sp1-binding site at positions −107 to −99 and show that the nearby naturally occurring base substitution C−92→G markedly impairs Sp1-dependent transcription of this gene. The C−92G dimorphism cooperates with the previously defined C−52T dimorphism to regulate the level of transcription of human α2 genes through mechanisms that undoubtedly involve the activity of both Sp1 and Sp3.

Direct participation of Sp1 and Sp3 at the −52 binding site was apparent in gel mobility shift assays because the substitution C−T resulted in an obvious decrease in the binding of

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**Fig. 5.** Long oligonucleotide probes encompassing −92 and −52 for gel mobility shift assays. The 61-bp core promoter fragment (bp −98 to −38) that contains either −92C (C) or −92G (G) was used. The downstream Sp1-binding elements at −61 to −53 and −51 to −43 are underlined.

**Fig. 6.** Binding of nuclear proteins from Dami cells to long oligonucleotide probes. Radiolabeled long oligonucleotide probes containing either −92C (lanes 1–5) or −92G (lanes 6–10) were incubated alone (lanes 1 and 6) or with a nuclear protein extract from Dami cells, followed by no additions (lanes 2 and 7), addition of polyclonal anti-Sp1 antibody (lanes 3 and 8), addition of polyclonal anti-Sp3 antibody (lanes 4 and 9), or addition of a 200-fold molar excess of unlabelled consensus Sp1-binding oligonucleotide (Sp1c). An arrowhead to the left of the gel indicates the major Sp1-containing complex. Supershifted complexes are designated by an asterisk.

**Fig. 7.** Functional analysis of the Sp1-binding sites adjacent to positions −92 and −52. Either Dami cells (A) or CHRF-288-11 cells (B) were transfected with each of the promoter-LUC reporter gene constructs, and the relative luciferase activity (ordinate) was determined. The constructs examined contained the α2 5′-regulatory sequence extending from −244 through +48 cloned into the minimal promoter construct, pGL2-basic. The construct Δ92GΔ52T is indicated by G T; construct Δ52 is indicated by G C, construct Δ52T is represented as C T, and C C represents the reference sequence. The mean (solid bars) and one standard deviation are shown for each data group; in the case of Dami cells, n = 5; and for CHRF-288-11 cells, n = 3
both Sp1 and Sp3 (15). In the case of the −92 site, the substitution C→G only partially impairs the ability of Sp1 to bind to the adjacent upstream binding site at −107/−99. At the same time, supershift assays failed to demonstrate any involvement of Sp3 at this site. Nonetheless, the same substitution markedly decreases transcriptional activity of promoter-LUC constructs in transfection assays. Our evidence for this conclusion is substantial. In transient transfection assays, promoter-LUC constructs that contain the C→T substitution at nucleotide −52 exhibit 3-fold and 4-fold decreases in activity in Dami and CHRF-288-11 cells, respectively. In similar assays, the C→G substitution at nucleotide −92 causes a 3-fold and a 5-fold decrease in activity in Dami and CHRF-288-11 cells, respectively. The combination of these substitutions has an additive effect, resulting in 8-fold and 20-fold decreases in transcription in either cell line. In a population of 71 normal donors, the expression of both T−92 and G−92 correlates with decreased activity in Dami and CHRF-288-11 cells, respectively. In similar assays, the C→G position has only a weak direct effect. Because both Sp1 and Sp3 are present in Dami cell nuclear extracts, this finding implies that there is a preferential binding of Sp1 to the upstream site at −107/−99. Whereas this result would not be a typical finding with the majority of Sp1/Sp3-binding sites, there is precedent for differential binding of Sp1 and Sp3. Two examples involve the human major intrinsic protein gene (25) and the cyclin D gene (32). A CT box located at position −147/−152 within the major intrinsic protein gene interacts with purified Sp1 or Sp3 present in mouse lens nuclear extracts (25). A proximal CT box located at position −49/−56 in the human major intrinsic protein gene and conserved in the mouse gene can react with purified Sp1 but cannot interact with Sp3 in lens nuclear extracts, reacting instead with Sp3 (25). This is a case in which auxiliary factors within the nuclear extract are likely modulating the binding of Sp1 without an effect on Sp3. On the other hand, Wang et al. (32) have observed an opposite effect, in which preferential binding of Sp1 to a segment of the cyclin D3 promoter was observed in the presence of ample levels of Sp3 by gel mobility shift assay. Such binding differences are always influenced by the ratio of Sp1 to Sp3 in the nucleus at a particular time, and additional studies to determine the effect of the relative levels of Sp1 and Sp3 on control of α2 gene transcription are warranted.

Expression differences in platelet integrin α2β1 result from the additive effects of multiple regulatory elements within and
outside of the ITGA2 5'-regulatory region. The molecular basis for the expression differences associated with ITGA2 alleles (A1, A2, and A3) remains to be determined, but our results certainly indicate that it does not involve the proximal 5'-regulatory region. The molecular differences responsible for the distinction of alleles A1, A2, and A3 are not likely to be located within the 5'-regulatory region of the gene because the allele frequencies of the −52 and −92 dimorphisms are radically different from those of the intragenic dimorphisms that distinguish alleles A1, A2, and A3. In a “typical” Caucasian population, they are as follows: 0.85 for −92C, 0.15 for −92G, 0.65 for −52C, and 0.35 for −52T. On the other hand, the allele frequencies of A1, A2, and A3 are 0.39, 0.53, and 0.08, respectively. Because we have found no further dimorphisms between position −52 and the beginning of intron 1, the molecular basis for expression difference between alleles A1, A2, and A3 must be found somewhere 3' to the beginning of intron 1. Our subsequent efforts will be directed toward the characterization of intragenic regulatory elements in the proximal portion of the gene, particularly within the unusually large 37-kilobase intron 1.

In the case of the human α2 gene, allelic differences have a profound effect on expression of the platelet integrin α2β1, and these expression differences can influence morbidity and risk for fatality in certain disease states, where the adhesion function of platelets is critical. Those alleles that are associated with low receptor density, alleles A2 and A3, are overrepresented in symptomatic patients with type 1 von Willebrand disease and may thus predispose these individuals to increased risk for bleeding (33). On the other hand, the inheritance of allele A1, associated with high receptor density, represents a risk factor for diabetic retinopathy (34) and for acute coronary disease (e.g., myocardial infarction) or stroke in younger patients (35, 36). In this report, we show that additional molecular differences within the 5'-regulatory and promoter region of the α2 gene also contribute to transcriptional regulation of this gene. Combined with the underlying genetic basis for the existence of multiple alleles, the molecular differences described in this report help to explain the diversity of receptor densities among normal subjects and the absence of an apparent gene dosage effect. These findings will pave the way for a more thorough characterization of the mechanisms that control expression of this important receptor.

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