uation of 35 patients.[11] Results obtained from extra-medullary sites and HMCL, of which more than 85% of cases express CD221, always at high levels, suggest that CD221 expression could be upregulated during disease progression and associated with a more aggressive disease, and facilitates cell growth *in vitro*, in agreement with the biology of IGF-1 in mouse models.[4-5]

We found that CD221 expression was not random but correlated with t(4,14) and t(14,16) translocations, translocations generally associated with poorer prognosis in patients.[10] It also seemed that CD221 expression was related to disease severity, although given the small number of patients and their non-uniform treatment management, survival data should be interpreted cautiously. To conclude, the CD221 phenotype should be systematically evaluated in myeloma patients, and this receptor could be an ideal therapeutic target in patients, as recently shown.[11]

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Disorders of Hemostasis

The spectrum of mutations in Southern Spanish patients with hemophilia A and identification of 28 novel mutations

The aim of this study was to analyze the mutation pattern causing hemophilia A in a population from Southern Spain. Mutation analysis identified the mutation in 99 of the 109 unrelated patients enrolled in the Hemophilia Registry from Andalusia. About 54% of non-inversion mutations identified were previously unreported.

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Hemophilia A (HA) is an X-linked bleeding disorder caused by a wide spectrum of mutations in the coagulation factor VIII (*F8*) gene (MIM # 306700). In the severe phenotype, the most prevalent mutations are the intron 22 (IVS22) and intron 1 (IVS1) inversion accounting for 40-50% and 5% of the mutations, respectively.[1,2] Apart from these inversions, no mutation hotspots have been identified. Approximately 30% of all distinct point mutations in HA occur at CpG sites.[3]

The aim of this study was to analyze the mutation pattern causing HA in a population from Southern Spain. The study included a consecutive series of 109 unrelated males with HA (55 severe, 8 moderate and 46 mild phenotypes) enrolled in the Hemophilia Registry from Andalusia (Southern Spain). Forty were sporadic cases with no previous family history and 69 had a positive family history. Genealogical investigations conducted for each patient's family did not disclose any common ancestor in three generations.

Among the 55 patients with severe HA, of which 27 were sporadic cases (49%) and 28 had a positive family history (51%), the prevalence of IVS22[4] and IVS1[2]) inversion was 36% (20 patients) and 5% (3 patients), respectively. The IVS22 frequency was relatively low in comparison with the prevalence reported in other studies in the Spanish population.[1] Nevertheless, the number of patients in the group is too small to determine whether the frequency is statistically lower. When the correlation between familial or sporadic inheritance of the disease was analyzed, no significant differences were observed (11 and 9, respectively).

In patients with inversion-negative, severe or moderate-mild HA, we sequenced the *F8* gene (exons and intron/exon splice junctions) following standard protocols using previously described primers.[5] Among 32 patients with severe HA, the mutation was identified in 26 and 23 different mutations were found, 15 (65%) of which had not been previously reported;[6,7] none of these mutations affected the CpG sites. The novel mutations comprised 5 frameshift mutations, 4 nonsense mutations, 5 missense mutations and 1 mutation affecting the splicing sites (Table 1). Among 54 patients with moderate-mild HA, 12 sporadic cases (22%) and 42 with positive family history (78%), the mutation was identified in 50 patients and 29 different mutations were found (Table 2), all of them missense mutations. Thirteen (45%) of the 29 mutations identified were novel[6,7] and only one affected the CpG site. All the detected mutations were confirmed through two independent polymerase chain reaction

Table 1. Summary of *F8* mutations in severe hemophilic males. Novel mutations in boldface type. Transient: antibody that disappears over a period of 6 months.

ID number	*Exon*	*Mutation*	*Amino acid substitution*	*Affected domain*	*CpG*	*Inhibitor*	*Family history*
A-304	**4**	**515G→T**	**C153F (TGC→TTC)**	**A1**	**No**	**No**	**Positive**
A-021	**4**	**515G→T**	**C153F (TGC→TTC)**	**A1**	**No**	**No**	**Positive**
A-195	**4**	**557_559delACT**	**D167fs**	**All**	**No**	**No**	**Positive**
A-113	**Intron 4**	**IVS4-1G→A**	**Splicing**	**All**	**No**	**No**	**Sporadic**
A-121	6	741G→A	W228X (TGG→TGA)	All	No	No	Positive
A-045	10	1487delC	P477fs	A2, B, A3, C1, C2	No	Transient	Sporadic
A50	14	2440C→T	R795X (CGA>TGA)	B, A3, C1, C2	Yes	No	Sporadic
A-232	**14**	**2526_2527delAG**	**G823fs**	**B, A3, C1, C2**	**No**	**No**	**Positive**
A-017	**14**	**3305_3306insAAAGAGGG**	**G1083fs**	**B, A3, C1, C2**	**No**	**No**	**Sporadic**
A-060	14	3637delA	I1194fs	B, A3, C1, C2	No	No	Sporadic
A-041	14	3637delA	I1194fs	B, A3, C1, C2	No	No	Positive
A-43	**14**	**4491_4492delTG**	**T1478fs**	**B, A3, C1, C2**	**No**	**No**	**Sporadic**
A-247	**15**	**5260T→C**	**F1735L (TTC→CTC)**	**A3**	**No**	**No**	**Sporadic**
A-019	**15**	**5291A→G**	**Q1745R (CAG→CGG)**	**A3**	**No**	**No**	**Positive**
A-011	**15**	**5301C→G**	**Y1748X (TAC→TAG)**	**A3, C1, C2**	**No**	**Transient**	**Positive**
A-359	**16**	**5508G→A**	**W1817X (TGG→TGA)**	**A3, C1, C2**	**No**	**No**	**Sporadic**
A-125	**17**	**5592delA**	**K1845fs**	**A3, C1, C2**	**No**	**No**	**Sporadic**
A-149	18	5878C→T	R1941X (CGA→TGA)	A3, C1, C2	No	No	Sporadic
A-111	**18**	**5881T→C**	**W1942R (TGG→CGG)**	**A3**	**No**	**No**	**Sporadic**
A-059	**18**	**5924T→A**	**I1956N (ATT→AAT)**	**A3**	**No**	**No**	**Positive**
A-005	**21**	**6250A→T**	**K2065X (AAG→TAG)**	**C1, C2**	**No**	**No**	**Sporadic**
A-293	21	6266G→A	W2070X (TGG→TAG)	C1, C2	No	No	Sporadic
A-028	23	6496C→T	R2147X (CGA→TGA)	C1, C2	Yes	Transient	Sporadic
A-288	23	6496C→T	R2147X (CGA→TGA)	C1, C2	Yes	Transient	Sporadic
A-065	**25**	**6748C→T**	**Q2231X (CAA→TAA)**	**C2**	**No**	**No**	**Positive**
A-042	26	6976C→T	R2307X (CGA→TGA)	C2	Yes	No	Positive

assays from different blood samples. R593C was the mutation most frequently found. The prevalence of this mutation in our population is unusually high and, although this may well be in part due a to founder effect, this hypothesis has not been studied.

About 30% of hemophiliac patients develop polyclonal IgG inhibitory antibodies directed against the exogenic factor VIII. In our patients, 5 of 55 patients with severe HA (9%) and 3 of 54 (5.5%) with moderate-mild disease developed inhibitors. Only one patient with IVS22 developed factor VIII inhibitors; therefore, the presence of IVS22 is not a major predisposing factor to inhibitor development in our population. Three patients with the R2150H mutation developed inhibitor antibodies. R2150H mutation may affect the tertiary structure of the molecule and alter the immunogenicity of the FVIII protein. R593C has also been reported in association with inhibitor; however, neither of our patients developed inhibitors.

We described 19 novel missense mutations and only one affecting the CpG sites. These mutations were identified in both the heavy and light chains, and in all but the B domain.[8] This fact reinforces the idea that single nucleotide substitutions within this domain are largely unimportant. In the present study the relationship between the novel missense mutations and the disease was indicated by several investigations: (i) mutations were not detected in 50 female controls; (ii) in hemophiliacs with a family history, we performed a segregation analysis of the mutation; (iii) in hemophiliacs with no previous family history, all missense mutations were at positions preserved in murine, pig, canine and human genomes.[6] In conclusion, we report here the results of an analysis of the *F8* gene mutations in Southern Spanish patients with HA. Mutation analysis identified the mutation in 99 of the 109 hemophilic males (91%). This frequency is similar to those previously described by other authors.[9,10] About 54% of non-inversion mutations identified were previously unreported. We failed to identify the mutation in ten patients. Rearrangements in introns other than 22 or 1, or mutations affecting the promoter or intronic regions could be responsible for the disease in these cases. Such mutations are not currently part of routine screening of *F8* gene.

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Key words: hemophilia A, factor VIII mutation, F8C.

Table 2. Summary of *F8* mutations in moderate-mild hemophilic males. Novel mutations in boldface type.

ID number	*Exon*	*Mutation*	*Amino acid Substitution*	*Affected Domain*	*CpG*	*Inhibitor*	*Family history*
A-025	4	396A→C	E113D (GAA→GAC)	A1	No	Yes	Sporadic
A-151	4	538C→T	H161Y (CAT→TAT)	A1	No	No	Positive
A-084	6	755C→T	T233I (ACA→ATA)	A1	No	No	Positive
A-128	**7**	**854T→C**	**V266A (GTG→GCG)**	**A1**	**No**	**No**	**Positive**
A-089	**7**	**878A→T**	**H274L (CAC→CTC)**	**A1**	**No**	**No**	**Positive**
A-266	7	923C→T	S289L (TCG→TTG)	A1	Yes	No	Positive
A-342	**8**	**1195A→G**	**F380V (AAA→GAA)**	**A2**	**No**	**No**	**Positive**
A-221	**10**	**1505T→A**	**V483D (GTC→GAC)**	**A2**	**No**	**No**	**Positive**
A-009	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-039	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-040	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Sporadic
A-061	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-260	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-298	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-365	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-189	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Positive
A-173	12	1834C→T	R593C (CGC→TGC)	A2	Yes	No	Sporadic
A-035	**13**	**2043G→A**	**M662I (ATG→ATA)**	**A2**	**No**	**No**	**Sporadic**
A-392	14	2167G→A	A704T (GCC→ACC)	A2	Yes	No	Positive
A-183	**14**	**2213A→G**	**Y719C (TAC→TGC)**	**A2**	**No**	**No**	**Sporadic**
A-358	14	3780C→G	D1241E (GAC→GAG)	B	No	No	Positive
A-368	14	3780C→G	D1241E (GAC→GAG)	B	No	No	Positive
A-136	**14**	**5144G→A**	**R1696Q (CGA→CAA)**	**A3**	**Yes**	**No**	**Positive**
A-387	15	5305G→A	G1750R (GGA→AGA)	A3	No	No	Positive
A-336	16	5399G→A	R1781H (CGT→CAT)	A3	Yes	No	Positive
A-281	**16**	**5420G→C**	**S1788T (AGC→ACC)**	**A3**	**No**	**No**	**Sporadic**
A-096	**16**	**5428T→C**	**S1791P (TCT→CCT)**	**A3**	**No**	**No**	**Positive**
A-215	**16**	**5428T→C**	**S1791P (TCT→CCT)**	**A3**	**No**	**No**	**Positive**
A-390	16	5527G→A	A1824T (GCA→ACA)	A3	No	No	Positive
A-037	**16**	**5531C→T**	**P1825L (CCC→CTC)**	**A3**	**No**	**No**	**Positive**
A-309	**16**	**5531C→T**	**P1825L (CCC→CTC)**	**A3**	**No**	**No**	**Positive**
A-027	18	5954G→A	R1966Q (CGA→CAA)	A3	Yes	No	Positive
A-030	18	5954G→A	R1966Q (CGA→CAA)	A3	Yes	No	Positive
A-417	18	5954G→A	R1966Q (CGA→CAA)	A3	Yes	No	Positive
A-155	18	5954G→A	R1966Q (CGA→CAA)	A3	Yes	No	Positive
A-323	19	6046C→T	R1997H (CGG→TGG)	A3	Yes	No	Positive
A-064	19	6046C→T	R1997H (CGG→TGG)	A3	Yes	No	Positive
A-134	19	6046C→T	R1997H (CGG→TGG)	A3	Yes	No	Positive
A-046	23	6506G→A	R2150H (CGT→CAT)	C1	Yes	No	Positive
A-194	23	6506G→A	R2150H (CGT→CAT)	C1	Yes	Yes	Positive
A-209	23	6506G→A	R2150H (CGT→CAT)	C1	Yes	No	Positive
A-410	23	6506G→A	R2150H (CGT→CAT)	C1	Yes	Yes	Positive
A-245	23	6506G→A	R2150H (CGT→CAT)	C1	Yes	No	Positive
A-014	23	6506G→A	R2150H (CGT→CAT)	C1	Yes	Yes	Positive
A-078	**23**	**6551A→T**	**E2165V (GAG→GTG)**	**C1**	**No**	**No**	**Sporadic**
A-129	24	6623A→G	Q2189R (CAG→CGG)	C2	No	No	Sporadic
A-068	24	6622C→G	Q2189E (CAG→GAG)	C2	No	No	Positive
A-024	25	6744G→T	W2229C (TGG→TGT)	C2	No	No	Sporadic
A-120	**25**	**6821T→A**	**M2255K (ATG→AAG)**	**C2**	**No**	**No**	**Positive**
A-081	**26**	**7028T→C**	**L2324P (CTG→CCG)**	**C2**	**No**	**No**	**Positive**

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Platelets

Increased glycocalicin index and normal thrombopoietin levels in patients with idiopathic thrombocytopenic purpura with a decreased rate of platelet production

Platelet kinetic studies in idiopathic thrombocytopenic purpura (ITP) have shown that in a subgroup of patients a shortened mean platelet life (MPL) is associated with a decreased platelet production rate (PPR).[1] Other methods of studying certain aspects of thrombocytopoiesis are the plasma concentrations of thrombopoietin and glycocalicin.

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Clinical studies have shown elevated plasma levels of thrombopoietin in conditions with diminished megakaryocyte production.[2] Glycocalicin is the soluble, external part of membrane glycoprotein Ibα (GPIbα). The glycocalicin-index, normalized for individual platelet count, has been introduced as a parameter of platelet turnover.[3] We investigated thrombopoietin and glycocalicin levels in ITP patients and correlated them to the platelet kinetic parameters MPL and PPR. *Platelet kinetic studies*. In order to study platelet kinetics, autologous platelets were labeled with Indium-111 tropolonate according to the recommendations of the International Committee for Standardization in Hematology.[4] The platelet production rate (PPR) is defined as the number of platelets entering the circulation to maintain the platelet count. The normal values of PPR is 223×10⁹/day (158–268) and the normal mean platelet life (MPL) is 9.2±1.4 days (8.9–9.4).[5]

Plasma thrombopoietin concentrations were determined with an enzyme-linked immunosorbent assay (Quantikine, R&D systems, Minneapolis, USA). The normal value in this assay is 114 pg/mL (93-146). Plasma glycocalicin concentrations were measured by enzymatic immunoassay (Takara Shuzo Co, Ltd). The glycocalicin index is derived from the glycocalicin value (mg³/mL) ×250×10⁹/L divided by the individual platelet count. The normal value is 0.7 (0.6-0.9).

Data are presented as the median with 25th and 75th percentiles. Statistical analysis was performed using Kruskal-Wallis non-parametric analysis of variances and the Wilcoxon two-sample test. Correlations were assessed with Spearman's rank correlation procedure. A *p*-value of <0.05 was considered statistically significant,

Table 1. Characteristics of patients.

	Production decreased	*Production normal or increased*	*p*
Patients, n	9	26	
Female	4	16	
Age, years	62 (30-68)	44 (32-67)	0.9
Platelet count at diagnosis,×10⁹/L	22 (13-46)	63 (43-89)	0.02
Mean platelet life, days	2.6 (1.4-3.7)	1.9 (1.1-3)	0.5
Platelet production rate, 10⁹/d	100 (88-145)	255 (188-325)	0.004
Thrombopoietin, pg/mL	109 (71-172)	111 (64-171)	0.8
Glycocalicin-index	12 (7-25)	5 (3-10)	0.03

Results are expressed as median (25th/75th percentile).

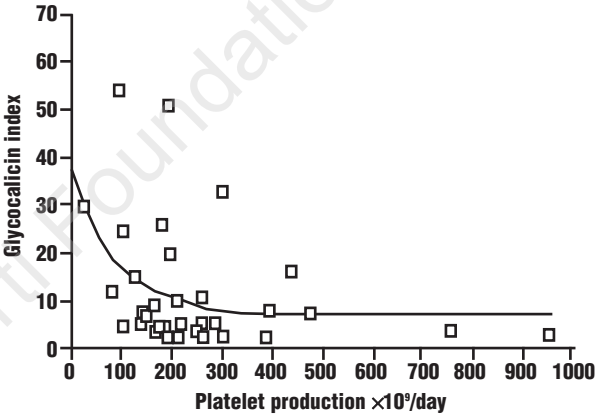

Figure 1.

and all tests were two-sided. After informed consent, 35 patients (20 women) with ITP were studied. Their mean age was 45 years (32-66) and platelet count at diagnosis was 58×10⁹/L (22-85). MPL was 2 days (1.1-3) and PPR was 195×10⁹/day (150-300). PPR was reduced in 9 patients whereas in 26 patients it was normal (n=17) or increased (n=9; median 395, min: 300, max: 950×10⁹/day). The PPR were not correlated to changes in MPL. Thrombopoietin plasma levels (110 pg/mL, 68-171) were measured in all the studied patients and compared to those in controls (114 pg/mL, 93-146). No statistical difference was observed (p=0.7). In addition, there was no significant difference in thrombopoietin plasma levels in patients with a normal or increased PPR, (111 [64-171]) *vs* a reduced PPR (median 109 [71-172], p=0.8).

The glycocalicin index was 5 (4-13). A significant correlation was observed between this index and PPR (Figure 1; p=0.03). In patients with a normal or increased PPR, the glycocalicin index was 5 (3 -10), whereas it was 12 (7-25) in patients with a decreased PPR (p=0.03). No significant correlation was observed between the glycocalicin index and MPL (p=0.08). Patients with a MPL ≤2 days demonstrated a glycocalicin index of 7 (3-26) compared to 5 (4-