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Report

The Gene for May-Hegglin Anomaly Localizes to a <1-Mb Region on Chromosome 22q12.3-13.1

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The May-Hegglin anomaly (MHA) is an autosomal dominant platelet disorder of unknown etiology. It is characterized by thrombocytopenia, giant platelets, and leukocyte inclusion bodies, and affected heterozygotes are predisposed to bleeding episodes. The MHA gene has recently been localized, by means of linkage analysis, to a 13.6-cM region on chromosome 22, and the complete chromosome 22 sequence has been reported. We recently performed a genome scan for the MHA gene in 29 members of a large, multigenerational Italian family, and we now confirm that the MHA locus is on chromosome 22q12.3-13.1. The maximal two-point LOD score of 4.50 was achieved with the use of marker D22S283, at a recombination fraction of .05. Haplotype analysis narrowed the MHA critical region to 6.6 cM between markers D22S683 and D22S1177. It is of note that the chromosome 22 sequence allowed all markers to be ordered correctly, identified all the candidate genes and predicted genes, and specifically determined the physical size of the MHA region to be 0.7 Mb. These results significantly narrow the region in which the MHA gene is located, and they represent the first use of chromosome 22 data to positionally clone a disease gene.

The May-Hegglin anomaly (MHA [MIM 155100]) is a rare autosomal dominant disorder characterized by thrombocytopenia, giant platelets, and leukocyte inclusions. The syndrome was first described by May (1909) in an asymptomatic woman with giant platelets and pale-blue cytoplasmic inclusion bodies in the granulocytes. Subsequently, in 1945, Hegglin reported a father and two sons with hematologic findings similar to those previously described by May. Since MHA was last reviewed (Bizarro 1997), an additional 19 patients from 10 families have been reported in the world's literature (Noris et al. 1998; Urato and Repke 1998; Kinsella et al. 1999; Pajor et al. 1999), resulting in a total number of 263 patients from 66 families. The clinical manifestations of MHA are highly variable, with the majority of affected heterozygotes being either asymptomatic or having mild bleeding tendencies, including epistaxis, easy bruisability, and prolonged menstrual periods. Some patients, however, have significant bleeding that can require platelet and blood transfusions. It is of note that patients are at risk for iatrogenic harm secondary to misdiagnosis and inappropriate treatment with corticosteroids, immunoglobulins, and/or splenectomy. In the absence of a family history, MHA is most often diagnosed in the evaluation of thrombocytopenia following minor surgical procedures, childbirth, or routine hematologic evaluation. However, the pathophysiology and underlying etiology of MHA remain unknown.

In addition to the obvious quantitative and qualitative platelet abnormalities, MHA is notable for the characteristic sky-blue inclusion bodies found in the cytoplasmic periphery of neutrophils, eosinophils, basophils, and monocytes. Named after the neutrophil-specific transiently appearing Döhle bodies seen with acute infections (Döhle 1911), the nature of these MHA inclusions is not known. On the basis of results of electron microscopy, Jenis et al. (1971) suggested that the highly parallel paracrystalline arrays, which are first evident developmen-

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tally at the stage of myelocyte formation, were composed of depolymerized ribosomes. Given the definitive but subtle ultrastructural differences between Döhle bodies and the MHA inclusions, Cawley and Hayhoe (1972) suggested that the MHA inclusions were not Döhle bodies but, rather, were "Döhle-like." Regardless of their nature, evidence for the functional impairment of polymorphonuclear leukocytes is limited to an in vitro report (Cabrera et al. 1981), and there have been no reports on altered immune function in MHA.

The autosomal dominant transmission of MHA, although suggested by Hegglin (1945), was first clearly established by Wassmuth et al. (1963), in their analysis of a five-generation Danish family with 13 affected members. The results of karyotypic analyses have not revealed any translocations or other abnormalities that indicate an MHA locus (Buchanan et al. 1964; Lusher et al. 1968; Gausis et al. 1969). Recently, Kunishima et al. (1999) mapped the disease locus to a 13.6-cM region on chromosome 22q12.3-q13.2, and they excluded the platelet GPIb gene as a possible disease candidate.

We have also used a positional-cloning strategy to identify the MHA gene. The availability of a large and clinically well-characterized family from Veneto, Italy —the largest reported family with MHA, with more than 112 family members and 31 affected members (Bizzaro 1997, 1999)—faciliated these studies. After informed consent was given, blood samples were obtained from a total of 29 family members and their relevant spouses, for use in a genomic scan. The clinical status of each patient had previously been determined (Bizzaro 1997). Figure 1 shows the entire pedigree, the subjects in the present report, and their clinical status. Immortalized lymphoblastoid cells lines were established with the use of standard techniques (Anderson and Gusella 1984). Genomic DNA was isolated with the use of a PureGene DNA isolation kit, according to the manufacturer's recommendations. Concentrations were determined spectrophotometrically, and working concentrations of each specimen, 10 ng/μl, were prepared.

Table 1

Two-Point Linkage Data between the MHA Locus and Chromosome 22 Polymorphic Markers

	LOD Score at θ =				
Locus	.00	.05	.10	.20	.30
D22S345	−∞	−3.86	−2.17	−1.28	−.39
D22S277	−∞	3.00	2.90	2.31	1.51
D22S1142	−∞	2.67	2.52	1.89	1.13
D22S683	−∞	−3.80	−2.06	−.65	−.12
D22S283	−∞	4.50	4.23	3.30	2.17
D22S1177	−∞	4.34	4.06	3.14	2.03
IL2RB	−∞	1.99	2.20	1.89	1.24
D22S445	−∞	2.85	2.66	2.05	1.31
D22S272	−∞	2.24	2.43	2.09	1.39
D22S284	−∞	−1.34	−.29	.31	.33
D22S423	−∞	−.96	−.19	.20	.17
D22S444	−∞	.41	.71	.73	.49

Microsatellite markers from the Human Screening Panel, version 9.0 (Research Genetics), were used for the genome scan. Additional markers (Research Genetics; Integrated DNA Technologies) were obtained to refine the critical region. PCR amplifications with the use of fluorescently end-labeled primers were performed with Ampli*Taq* Gold (PE Biosystems), according to the manufacturer's suggestions, and all reactions were performed on an ABI catalyst engine. Samples were multiplexed on the basis of expected sizes and fluorescent labels, and they were then electrophoretically separated on a 4.2% denaturing polyacrylamide gel, with use of an ABI 377 sequencer. The data were analyzed by the ABI GENESCAN and GENOTYPER software packages. Genotype data were entered into the Labman (Adams 1994) custom data-management system, which generated the input files for linkage analysis. Two-point linkage analysis

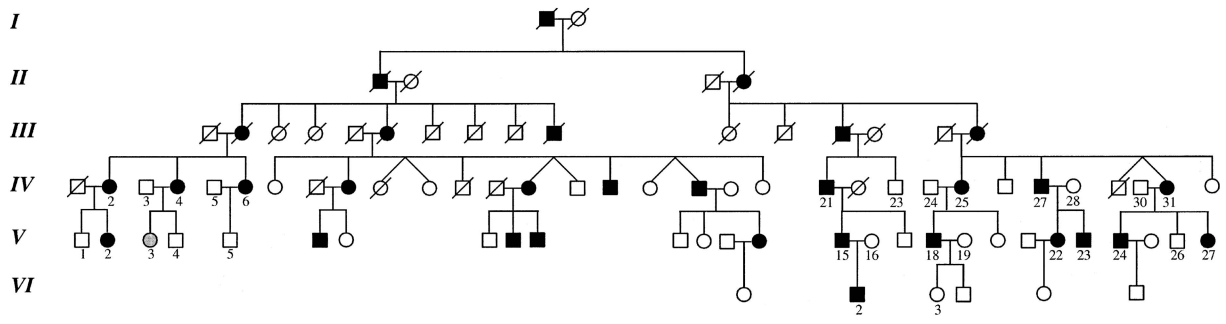

Figure 1 Pedigree of the Italian kindred with MHA. This family was previously described elsewhere (Bizzaro 1997), and we have herein updated its members' disease status. Family members in the present study are numbered. Blackened symbols denote affected individuals; unblackened symbols denote unaffected individuals. Affected individual V.3 (*gray-shaded circle*) also has von Willebrand disease.

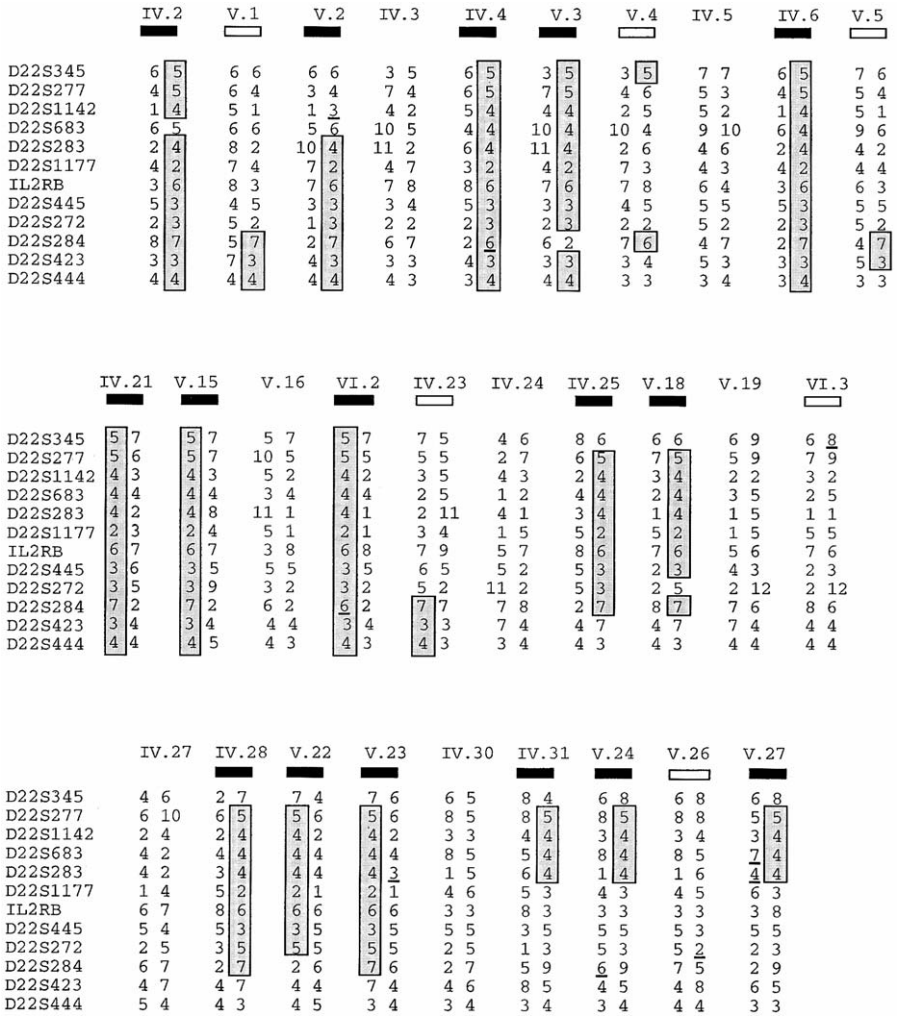

Figure 2 Haplotypes of the kindred with MHA. Twelve markers (D22S345, D22S277, D22S1142, D22S683, D22S283, D22S1177, IL2RB, D22S445, D22S272, D22S284, D22S423, and D22S444) from within and flanking the linkage region are shown. Family numbers and individual numbers are the same as those in figure 1. Affected individuals are underlined with a blackened bar; unaffected individuals are underlined with an unblackened bar. Spouses are shown to determine phase.

was performed by use of the computer program LINKAGE, version 5.1, and its associated subprograms (Ott and Terwilliger 1994). MHA was assumed to be a completely penetrant, genetically homogeneous, autosomal dominant disease, with an allele prevalence of .00001. Sex-averaged genetic-recombination maps were used to derive the intermarker distances.

A genomewide search was performed with the use of PCR-based microsatellite screening markers at a density of 10 cM. Initial suggestion of linkage to chromosome region 22q was obtained with the use of marker D22S445, which gave a LOD score of 2.85, at recombination fraction (θ) .05. As shown in table 1, additional markers were analyzed, resulting in a maximal LOD score of 4.50, at θ = .05, for marker D22S283. Marker D22S1177 had a maximal positive LOD score of 4.34, at θ = .05. Thus, each of the two markers had a LOD score >3.3, which is the suggested minimal threshold for linkage (Lander and Kruglyak 1995).

To define the boundaries of the MHA locus, haplotype analysis was performed. A common disease haplotype was defined, as is shown by the highlighted boxes in figure 2. In affected individual IV.31 and her two affected descendants, individuals V.24 and V.27, the telomeric boundary was defined by recombinant events between markers D22S283 and D22S1177. In both affected individual IV.2 and her affected daughter, individual V.2, the centromeric boundary was defined by the informative recombinant event between markers D22S683 and D22S283. Affected individuals IV.4, V.3, IV.6, IV.21,

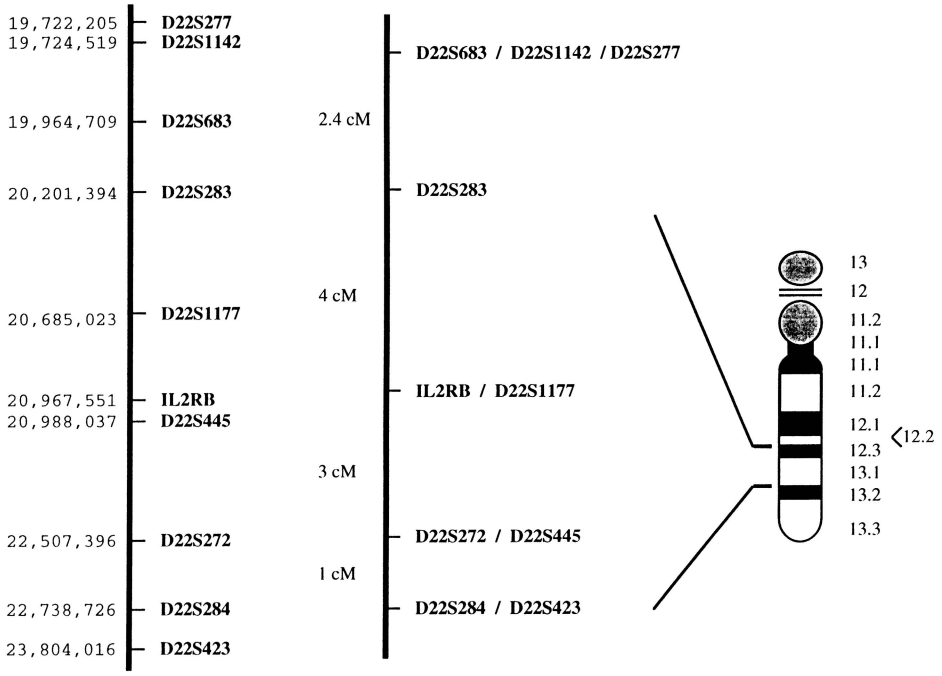

Figure 3 Chromosome 22 ideogram. The markers used in this study are shown with their relative locations and genetic distances, as previously defined (Broman et al. 1998), and with the actual physical distances based on chromosome 22 sequence data (Dunham et al. 1999).

V.15, and VI.2, who shared a large number of identical haplotypes throughout the region, provided no additional information. Therefore, on the basis of data from affected heterozygotes, the MHA locus was limited to the region bounded by markers D22S683 and D22S1177.

On the basis of the 1998 human genetic map from the Center for Medical Genetics, Marshfield Medical Research Foundation (Broman et al. 1998), the genetic distance between these markers is 6.6 cM. Interestingly, the actual physical distance between these markers, on the basis of the chromosome 22 sequence, is ~700 kb (fig. 3). The high degree of genetic recombination in this region is further suggested by the large number of single and multiple crossover events—both informative and noninformative—in each subfamily. These findings are in accord with the results of Dunham et al. (1999), who found, in their sequence analysis of chromosome 22, that the region extending from marker D22S424 (4 cM centromeric to marker D22S683) to marker D22S1177 had a relatively increased recombination rate per unit of physical distance. However, no specific sequence characteristics were found to explain these results (Dunham et al. 1999). Additionally, a number of markers were found to undergo mutation, most likely secondary to strand slippage, giving rise to new alleles. All of these "evolving" alleles, which were confirmed at least twice, are underlined in figure 2. Characterization of the mutation rates of these microsatellite markers may have importance for biostatistical purposes—for example, for paternity testing and forensic identification.

Sequence analysis of chromosome 22, in addition to haplotype analysis of family members with MHA, had the additional benefit of ordering several polymorphic markers that had colocalized on the genetic map from the Center for Medical Genetics, Marshfield Medical Research Foundation (fig. 3). Thus, the order of these markers, from centromere to telomere, was established as D22S277-D22S1142-D22S683-D22S283-D22S1177-IL2RB-D22S445-D22S272-D22S284-D22S423.

In addition to MHA, two other hereditary forms of thrombocytopenia with giant platelets and leukocyte inclusion bodies have been identified. Fechtner syndrome (MIM 153640) was first described by Peterson et al. (1985) as an autosomal dominant disorder transmitted through four generations of a family in which nephritis, high-tone sensorineural deafness, congenital cataracts, macrothrombocytopenia, and leukocyte inclusions were variably present. Soon thereafter, similar findings were reported for an additional family with 16 affected members (Gershoni-Baruch et al. 1988). On the basis of their ultrastructural evaluation, Heynen et al. (1988) have suggested that the disorder may result from a cytoskeletal defect in megakaryocytes. The other autosomal dominant disorder, Sebastian syndrome, was reported as

a unique entity (Greinacher et al. 1990). Affected individuals had the same hematologic findings (macrothrombocytopenia and leukocyte inclusions) as did those with Fechtner syndrome, but the Alport-like symptoms (renal disease, deafness, and cataracts) were not present. Additional reports have documented Sebastian syndrome in a number of additional families and have revealed its panethnic nature; these additional families included Saudi Arabian (Khalil and Qari 1995), Japanese (Tsurusawa et al. 1999), and African American families (Young et al. 1999). Interestingly, Rocca et al. (1993) described a family in which 10/14 members had findings that were consistent with the diagnosis of Fechtner syndrome but in which younger members had reduced expression of Alport-like manifestations. Therefore, low expressivity may explain the clinical variability between Sebastian and Fechtner syndromes. Most relevant to this report is the fact that the gene for Fechtner syndrome has recently been mapped to chromosome 22q11-13 (Toren et al. 1999), suggesting that these three autosomal dominant macrothrombocytopenic disorders with leukocyte inclusions (MHA, Sebastian syndrome, and Fechtner syndrome) may indeed be allelic.

Since the underlying genetic defect of MHA is not known, the diagnosis remains one of exclusion, and MHA must be differentiated from a number of acquired forms. Secondary thrombocytopenias can be associated with abnormal platelet sequestration (hypersplenism), decreased platelet production, and increased destruction (immune and drug-induced disseminated intravascular coagulation). The presence of Döhle bodies in a peripheral blood smear necessarily prompts the differential diagnosis to include acute infections.

Among the inherited platelet disorders, one form of isolated thrombocytopenia has been identified and a second form has recently been mapped. Ho et al. (1996) localized the gene for the familial platelet disorder with associated myeloid malignancy (MIM 601399) to chromosome 21q22. On the basis of this localization, mutational analysis of candidate genes showed that haploinsufficiency of the hematopoietic transcription factor CBFA2 caused the disease (Song et al. 1999). Another autosomal dominant thrombocytopenia—characterized by moderate platelet counts, minimal symptoms, and normal platelet and megakaryocyte morphology—was recently mapped to chromosome 10p11.1-12 (Savoia et al. 1999). This gene, designated THC2, has not yet been identified.

Use of the chromosome 22 sequence (Dunham et al. 1999) has resulted in the identification of a number of candidate genes within the MHA region, including myosin heavy polypeptide 9, nonmuscle; tumor necrosis factor–inducible protein CG12-1; apolipoprotein L; calcium-channel voltage-dependent γ subunit 2; eukaryotic translation-initiation factor 3 subunit 7; thioredoxin 2; Hermansky-Pudlak syndrome–similar protein; parvalbumin; neutrophil cytosolic factor 4; and colony stimulating factor 2 receptor β low affinity. Direct sequence analysis of these candidates should identify the MHA gene, which in turn should yield insights into the fundamental molecular mechanisms underlying megakaryocyte and platelet formation or function.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/ (for the human genetic map)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MHA [MIM 155100], Fechtner syndrome [MIM 153640], and familial platelet disorder with associated myeloid malignancy [MIM 601399])

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