Myeloperoxidase (MPO) Gene Mutation in Hereditary MPO Deficiency

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Myeloperoxidase (MPO), present in the azurophilic granules of polymorphonuclear leukocytes, is a myeloid enzyme whose synthesis is restricted to promyelocytes. Complete hereditary MPO deficiency affects 1 in 2,000 to 4,000 individuals; however, the genetic cause of this defect is unclear. We have determined the molecular basis of MPO deficiency in one individual (SQ). Granulocytes of SQ had no MPO activity, and had complete absence of mature and precursor MPO protein by Western blotting. Scanning MPO gene structure by Southern blotting detected a novel Bgl II fragment in SQ; no other alteration in gross gene structure was detected. We hypothesized that a single base pair mutation formed a new Bgl II restriction site, and that this occurred in exon 10 of MPO gene. As predicted, exon 10 from SQ was cleaved by Bgl II, but DNA from the normal patients and five other MPO-deficient patients was not cleaved by this enzyme. Direct sequencing of the polymerase chain reaction (PCR) product of exon 10 showed a C to T substitution at codon 569 in exon 10, resulting in arginine (CGG) to tryptophan (TGG) substitution and creating a novel Bgl II site. The mutation was homozygous, as shown by both sequencing and Southern blotting, and no other alterations in base sequence were detected. To determine the frequency of this mutation, DNA was collected from 400 normal individuals, and the presence of the mutation was examined by digesting with Bgl II after amplifying exon 10 by PCR. No other case with the novel Bgl II site was detected, suggesting that this is not a restriction fragment length polymorphism. The rest of the coding region of the MPO gene was sequenced in DNA from SQ, as well as from the five other MPO-deficient individuals and one normal person; no other mutations were found. Our results suggest that a point mutation at codon 569 of MPO gene represents one molecular form of MPO deficiency.

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Materials and Methods

Patients. A 65-year-old Hispanic woman was found to have MPO deficiency during routine blood examination by automated flow cytometry. Complete MPO deficiency was confirmed by histochemical staining procedures and Western blot analysis of the neutrophils using heterologous rabbit antiserum raised against purified MPO. The patient had no history of recurrent infections and was not charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

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isopropanol. DNA blotting was performed as described and probed with $^{32}$P-oligolabeled (random primed) restriction fragments. After hybridization, the filters were washed to a final stringency of 0.25× standard saline citrate at 65°C and exposed to Kodak XAR film (Eastman Kodak). The MPO probe was a 1-kb Smal I fragment excised from cDNA clone pMPO2 (Fig 1).16

Polymerase chain reaction (PCR) and direct sequencing. PCR were performed using 1 μg of genomic DNA. The strategy for PCR amplification of the 12 exons of MPO and the sense and antisense primers of exon 10 of MPO gene are shown in Fig 1. The entire coding region of MPO gene was amplified by PCR using primers complementary to the intron sequences flanking each exon. Template DNA was amplified using 1 μmol/L primers, 200 μmol/L deoxynucleotide triphosphates (Pharmacia, Piscataway, NJ) in 10 mmol/L Tris HCl (pH 8.3), 50 mmol/L KCl, and 2.5 mmol/L MgCl₂; total reaction volume was 100 μL with 0.5 U of Taq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT) and 30 cycles of amplification for 1 minute at 94°C, 1 minute at 55°C, and 2 minutes at 72°C using a thermal cycler (Eppendorf Inc, Fremont, CA). Amplified products were separated on 3% NuSieve (FMC, Rockland, ME) gels and purified using glass beads (GeneClean; Bio 101 Inc, LaJolla, CA). Puriﬁed, ampliﬁed DNA was sequenced by dideoxynucleotide termination (US Biochemicals, Cleveland, OH).

RESULTS

Patient SQ was detected during routine automated blood analysis as having PMNs with no histochemical staining for MPO activity. Western blot analysis of PMNs from SQ using rabbit anti-MPO serum found an absence of precursor (89 kD), heavy (55 kD), and light (19 kD) subunits of MPO; and similar ﬁndings were found in the ﬁve other MPO-deﬁcient patients, as previously reported.20

MPO gene integrity in MPO deﬁciency. To determine whether MPO deﬁciency resulted from gross alteration of the MPO gene, restriction analysis was performed by Southern blotting. Genomic DNA from normal and MPO-deﬁcient patients was digested with various restriction enzymes (Bgl II, Pvu II, EcoRI, BamHI) and probed with the 1.2-kb Smal I insert from pMPO2 cDNA (Figs 1 and 2). Normal genomic DNA digested with Bgl II results in two fragments of 10 and 2.5 kb (lane 1). Two MPO-deﬁcient patients had a pattern identical to normal DNA (lanes 2 and 4), suggesting that no major alteration of the MPO gene was present in these MPO-deﬁcient patients. However, SQ (lane 3) showed loss of the 2.5-kb fragment and presence of a new 2-kb fragment after digestion with Bgl II. On longer exposure, a faint 0.5-kb band was detectable in this lane. Complete loss of the 2.5-kb Bgl II fragment shows a homozygous alteration of the MPO gene. Digests of DNA with other restriction enzymes showed the same restriction fragment length pattern among the samples.

Amplification and sequencing of MPO gene. Human MPO gene has three Bgl II restriction sites (Fig 3). From the results of Southern blot analysis of SQ, we hypothesized that a single base pair mutation formed a new Bgl II restriction site resulting in a new 2.0-kb fragment. University of Wisconsin GCG software (Genetics Computer Group, Madison, WI) was used to design primers that would amplify the 2.5-kb fragment. Ampliﬁcation and sequencing of the 2.5-kb fragment was performed, and no alteration of the MPO gene was detected.
Fig 3. Potential Bgl II sites in human MPO gene. Three normal Bgl II digestion sites occur in MPO and are indicated by arrowheads; potential Bgl II sites that could be created by a single base change are indicated by arrows. The positions indicated by kilobase on the top line are based on GENBANK file HSMPOG (accession no. X15377; NCBI, Bethesda, MD). Exon positions are shown on the bottom line.

son, WI) was used to determine locations of potential Bgl II sites resulting from one nucleotide mismatch (Fig 3). From this analysis, we predicted that a new Bgl II site occurring in exon 10 of MPO gene would result in a 2-kb fragment (Fig 3).

To confirm the putative mutation in SQ, a 500-bp fragment including exon 10 was amplified by PCR using primers (shown on Fig 1) from genomic DNA from SQ, from additional MPO-deficient patients who had no additional Bgl II site, and from a normal individual. The resulting PCR products, both before (−) and after (+) digestion with Bgl II, were analyzed on a 3% NuSieve agarose gel stained with ethidium bromide. As predicted, DNA amplified from SQ was cleaved by Bgl II (Fig 4). No normal band persisted after Bgl II digestion, indicating a homozygous alteration of the MPO gene. DNA from the normal and other MPO-deficient patients were not cleaved by this enzyme.

Direct sequencing of the PCR product of exon 10 was performed by the dideoxy chain termination method for DNA from SQ, from the five other MPO-deficient patients, and from one normal individual (Fig 5). A homozygous C to T transition at base 569 in exon 10 was found in SQ DNA, replacing arginine (CGG) by tryptophan (TGG). Sequencing of both the coding (Fig 5A) and noncoding (data not shown) strands gave identical results. Repeat PCR and sequencing of the DNA gave identical results. We sequenced all of the remaining exons of the MPO gene from both the coding and noncoding strands of SQ, from the other five MPO-deficient samples, and from the normal individual using a strategy outlined in Fig 1. No alteration in base sequences was detected.

Frequency of point mutation of the MPO gene at base 569 in the general population. We exploited the presence of a novel Bgl II site in amplified exon 10 to determine the frequency of this point mutation in the general population. Genomic DNA was isolated from 400 unrelated individuals from diverse ethnic origins. Exon 10 of MPO gene was amplified by PCR, subjected to Bgl II digestion, and separated on 3% agarose gel (Fig 6). DNA from SQ was processed simultaneously as a positive control, and a blank
mutant changing a C to T at amino acid 569, resulting in tryptophan (TGG) replacing an arginine (CGG). Substitution of a hydrophilic, basic arginine with a tryptophan residue would likely change the charge of the large subunit of MPO. A possible explanation for the lack of MPO antigen in Western blots is that the arginine-to-tryptophan mutation may result in a precursor that does not fold properly and that may then be subject to degradation at this point in biosynthesis. Of note, the codon at the site of the mutation is very conserved and normally encodes an arginine in peroxidases from various tissues and species: human and mouse MPO and human, rat, and porcine thyroperoxidase. In human lactoperoxidase, the positive charge is conserved by a lysine at this position; therefore, it would not be surprising if the requirement for arginine (or lysine) at this position is linked to folding and stability of the precursor.

The DNA from 400 normal individuals was analyzed for a C-to-T alteration at codon 569 of MPO; this was accomplished by Bgl II digestion after PCR amplification of exon 10. None of the normal individuals had the novel Bgl II restriction site found in SQ, supporting our belief that this alteration results in MPO deficiency and that codon 569 does not represent a polymorphism in the general population. Of note, Nauseef has frequently observed the novel Bgl II restriction site (and presumably the mutation identified in this study); in contrast, five of six MPO-deficient individuals described here do not have this mutation. The explanation for this discrepancy is unclear and requires analysis of additional patients. Besides SQ, we sequenced the exons of the MPO gene from five additional MPO patients and found no abnormalities. Perhaps these individuals have mutations at their RNA splice sites or outside the coding sequences of the gene. Further studies of these samples will be required.

The incidence of homozygous MPO deficiency has been suggested to be on the order of 1 per 2,000 to 4,000 individuals; therefore, 1 in 50 individuals will be heterozygous for MPO deficiency. None of 400 individuals had the same alteration found in SQ. The missense mutation found in SQ probably represents only one of a variety of alterations leading to MPO deficiency. Of the seven individuals that we have studied in depth, none expressed MPO enzymatic activity; six of seven had no MPO protein by Western blot; one individual expressed the 55-kD heavy chain of MPO but not the light chain. Normal size RNA for MPO was identified in one patient, but no MPO protein precursors were found. An additional patient had heterogeneous nuclear RNA in promyelocytes that hybridized with MPO cDNA, but the cells contained no mature MPO RNA, suggesting a lesion affecting processing of MPO mRNA. Nauseef et al have found that some patients with MPO deficiency express the high molecular weight MPO precursor but not the mature products on Western blot. Therefore, MPO is similar to thalassemia and other monogenic diseases, in which the phenotypic defect may result from a variety of genotypic abnormalities. This report identifies, for the first time, the genotype of one individual with MPO deficiency.
Fig 6. Amplification of exon 10 by PCR and digestion with Bgl II from 400 individuals from the general population. The first two lanes in all cases show amplified products of an MPO-deficient patient SQ before (−) and after (+) Bgl II digestion (positive control). The other lanes show DNA amplified products of exon 10 digested with Bgl II from 216 individuals. DNA were electrophoresed and stained with ethidium bromide, and the gel was photographed. Data are not shown for an additional 184 samples.

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