Hereditary Myeloperoxidase Deficiency Due to a Missense Mutation of Arginine 569 to Tryptophan*

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William M. Nauseef†, Susan Brigham, and Melissa Cogley

From the Department of Medicine, Veterans Administration Medical Center and the University of Iowa, College of Medicine, Iowa City, Iowa 52242

Hereditary deficiency of myeloperoxidase (MPO) is a common disorder but its genetic basis is unknown. We have reported that neutrophils from individuals with MPO deficiency lack enzymatic and immunochemical evidence for mature MPO but have a 90-kDa precursor protein. We have thus hypothesized that hereditary MPO deficiency reflects a defect in processing of a mutated primary translation product.

Genomic DNAs from normal subjects digested with BgIII and probed with radiolabeled cDNA for MPO have a 2.6-kilobase (kb) band. Previously we described the presence of an aberrant 2.1-kb fragment in BgIII digests from most individuals with either partial or complete MPO deficiency. We describe here the responsible mutation. The substitution of thymidine for cytosine in exon 10 at nucleotide 8,089 of the genomic sequence results in generation of a recognition site for BgIII not present normally and converts the normal 2.6-kb BgIII fragment to the 2.1-kb fragment associated with MPO deficiency. At the amino acid level this mutation would replace arginine at codon 569 with tryptophan. Six of seven patients with complete MPO deficiency had this mutation. One subject was homozygous for this mutation whereas five others were heterozygous at this locus. The seventh patient was the only completely deficient subject without this mutation. Thus, at least two mutations and three genotypes can produce the phenotype of MPO deficiency.

Myeloperoxidase (MPO; donor H2O2 oxidoreductase, E.C. 1.11.1.7)† is a lysosomal hemoprotein located in the azurophilic granules of polymorphonuclear leukocytes (PMNs) and monocytes (1). The MPO-H2O2-halide system constitutes an extremely efficient and potent component of the host defense system of human PMNs, expressing microbicidal activity against a wide range of organisms (1).

MPO is a dimeric protein and each dimer contains a 59-kDa heavy subunit and a 13.5-kDa light subunit (2). The primary translation product is a single 80-kDa protein (3–6) which undergoes co-translational glycosylation, proteolytic processing (6–12), and lysosomal targeting (13, 14) during the promyelocytic stage of myeloid development (2, 15–19). Both cDNA and genomic clones for MPO have been isolated and sequenced (20–24) and the gene for MPO has been localized to the long arm of chromosome 17, at q22–23 (25).

Hereditary deficiency of MPO is relatively common, occurring at a frequency of 1 in 2,000–4,000 of the population (26). Nonetheless, a genetic mutation for this disorder has not been identified. We have shown previously (27) that PMNs from subjects with inherited MPO deficiency lack enzymatic, immunochemical, and spectroscopic evidence for mature MPO but do contain a protein that is immunochemically related to MPO and is 90-kDa, the size of the biosynthetic precursor for MPO (6). Based on these studies, we hypothesized that a defect in post-translational processing is the basis for inherited MPO deficiency (27). In contrast, Tobler et al. (28) described a completely MPO-deficient subject whose PMNs lacked evidence of the precursor protein, suggesting that a pre-translational defect caused MPO deficiency in that patient.

Subsequently we found that BgIII digests of genomic DNA from subjects with MPO deficiency, probed with cDNA for MPO, possess a 2.1-kb fragment, in contrast to the 2.6-kb fragment present in BgIII digests of genomic DNA from individuals with normal MPO activity (29). Based on these results, we hypothesized that the mutation underlying hereditary MPO deficiency creates a BgIII site not normally present in the genomic sequence for MPO and that the presence of this new BgIII site provides a marker for one of the genotypes that produces MPO deficiency.

We present data indicating that the substitution of thymidine for cytosine at nucleotide 8,089 in the genomic sequence of MPO is present in most patients with MPO deficiency. This

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† Clinical Investigator in the Department of Veterans Affairs. To whom correspondence should be addressed: Dept. of Medicine, University of Iowa, 200 Hawkins Dr., Iowa City, IA 52242. Tel.: 319-356-1759; Fax: 319-356-4600.

1 The abbreviations used are: MPO, myeloperoxidase; PCR, polymerase chain reaction; PMNs, polymorphonuclear leukocytes; kb, kilobase pair(s).

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<th>Patient</th>
<th>Deficiency</th>
<th>BgIII digest of genomic DNA</th>
<th>BgIII susceptibility of Exon 10</th>
<th>C→T mutation at nucleotide 8,089</th>
<th>WT* Mutation</th>
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(n = 21)

* WT, wild type.
mutation creates the BglII site associated with this inherited defect and is predicted to replace the arginine at codon 569 in exon 10 with tryptophan. Our data also indicate that other mutations exist as well, supporting the previous speculation that multiple genotypes result in the phenotype of MPO deficiency.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and reaction buffers, proteinase K, HindIII λ phage, Escherichia coli DNA polymerase I, specific primers for polymerase chain reactions, and TaqI polymerase were obtained from the DNA Core Facility at the University of Iowa; dextran and Hypaque-Ficoll were obtained from Pharmacia Fine Chemicals (Piscataway, NJ); [α-32P]dCTP was obtained from Amersham Life Sciences Products (Arlington Heights, IL).

Cells—PMNs were isolated from heparinized venous blood by dextran sedimentation and Hypaque-Ficoll density centrifugation followed by hypotonic lysis of erythrocytes as previously described (29). All studies were conducted in accordance with a protocol approved by the Human Subjects Committee of the Iowa City Department of Veterans Affairs Medical Center and the University of Iowa. Genomic DNA was isolated from PMNs using sodium dodecyl sulfate-proteinase K digestion, as previously described (29). Genomic DNA was thoroughly digested with BglII, separated electrophoretically in 0.8% agarose gels, and blotted to nylon filters as previously described.

![Image](https://example.com/image.png)

**Fig. 1.** Immunochemical and genetic characterization of subjects studied. Immunoblots (panel A) of PMNs isolated from individuals were probed with monospecific anti-MPO antiserum followed by 125I-protein A and Southern blots (panel B) of BglII digests of genomic DNA isolated from individuals were probed with a 32P-labeled full-length clone for MPO (pMP062-2). In immunoblots (panel A), both control and MPO-deficient subjects had immunoreactivity of a 90-kDa protein. Completely deficient subjects (RT, KB, DC, and FF) lacked any immunoreactivity for mature MPO subunits (59- and 13.5-kDa), whereas the partially deficient subject (TMa) had some immunoreactivity with mature MPO. Restriction digests (panel B) with BglII demonstrate that DNA from MPO-replete subjects had a 2.6-kb band but lacked the 2.1-kb band. Both bands were present in digests from completely deficient subjects RT, FF, and DC, and the partially deficient subject TMa. LH is the only completely deficient subject whose DNA possessed exclusively the 2.1-kb fragment and KB is the only completely deficient subject whose digest lacked the 2.1-kb fragment.
Subjects—The MPO status of individuals was assessed enzymatically and immunologically. The peroxidase activity of solubilized PMNs was quantitated spectrophotometrically using the oxidation of ortho-di-anisidine (30). Immunochemical evaluation was performed on solubilized PMNs separated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) and blotted to nitrocellulose filters. As done previously, filters were probed with a monospecific polyclonal antibody to MPO protein in immunoblots. Only PMNs from partially deficient subjects, as judged enzymatically, had immunological evidence of mature MPO subunits.

Polymerase Chain Reaction (PCR)—The assignment of numbers for various nucleotides is based on previously published data (31). Primers for amplification of exon 10 (nucleotides 8,006–8,176 in genomic sequence) by PCR were 5'-AGGTGGCA'M'GACCCCATCCT-3' (forward primer) and 5'-TCCCTGCTGCTGCGTACGTCA-3' (reverse primer). Amplification of products of the desired size was optimized using “touchdown” PCR, as previously described (32). The annealing temperature was decreased 1° every second cycle from 65 °C to 55 °C, at which level 25 cycles were carried out. Amplicons were directly cloned into pCRll, using the TA-cloning system (Invitrogen, San Diego, CA), following procedures recommended by the manufacturer. For each subject separate PCR amplifications of exon 10 were performed (n ≥ 3). Multiple clones from each amplification were sequenced on both strands.

Sequencing—The DNA sequence of cloned amplicons was determined directly for each strand, using 32P-dATP and the dideoxynucleotide-chain termination method (Sequenase, version 2.0, U. S. Biochemical Corp.). Primers included both M13 reverse primer and T7 DNA polymerase.

Probes—Blots of digested genomic DNA were probed for MPO-related sequences using full-length cDNA for MPO (pMP062-2) or partial length clones. Partial clones were generated by digesting pMP062-2 with XbaI or SphI, separating the fragments by electrophoresis in low melting point agarose, and excising the separated fragments. In the cDNA for MPO, there is one XbaI site (nucleotide 625) and one SphI site (nucleotide 2,319). Probes were labeled by random priming (Boehringer Mannheim), using [32P]dCTP, as previously described.

RESULTS AND DISCUSSION

With the recruitment of additional subjects with either complete or partial MPO deficiency, we have confirmed and extended our previous observations (Table 1). PMNs from all patients studied had a 90-kDa protein immunologically related to MPO (Fig. 1, panel A). PMNs from patients with partial MPO deficiency contained 30–60% of the normal amount of mature MPO subunits, as judged enzymatically and immunologically, whereas those with complete deficiency lacked any evidence of mature MPO. Most patients with complete MPO deficiency (6 of 7) had a 2.1-kb band in BglII digests of genomic MPO which was absent from normal subjects (Fig. 2).

1, panel B; Table I). In addition, one of the partially deficient subjects (CN) possessed this 2.1-kb fragment (29). No normal subject studied to date had the 2.1-kb BglII fragment (n = 21). Most of the completely deficient (6 of 7) and all of the partially deficient subjects also had a 2.6-kb BglII fragment similar to that seen in BglII digests of genomic DNA isolated from individuals with normal amounts of MPO activity.

Because all affected subjects had immunological reactivity consistent with the presence of a normal size precursor protein (27), we hypothesized that MPO deficiency was due to a post-translational defect in processing the precursor to normal, mature MPO. Furthermore, we reasoned that the underlying mutation was likely a single base mutation which altered the sequence but not the size of the MPO precursor. Assuming that the mutation involved a single nucleotide change and that this change created a new BglII site, we searched the genomic sequence for sites where a single mutation would create a new recognition site not present in the normal sequence. Sites where a single base mutation would create a BglII recognition site not present in the normal sequence are indicated and numbered (1 to 24). The region of genomic DNA identified by hybridization with partial clones 5' SphI and 3' XbaI extends from nucleotide 1,407 to 10,096 of the sequence and is indicated by the horizontal bar below the schematic of the MPO gene.

![Diagram showing potential locations for new BglII sites after single base mutations in the genomic sequence for MPO.](image)

**PROBE:** pMP062-2  5' SphI  3' SphI  5' XbaI  3' XbaI

![Diagram showing probes and indicated sites.](image)
ment arising between nucleotides 1,407 and 10,096 in the genomic DNA.

Based on this analysis, we eliminated mutations 5, 7, 8, 9, 11, 15, and 16. Eliminating those mutations which would give the incorrect size of BglII fragment, we reduced the number of feasible sites to mutations 6, 18, and 24. Mutations 6 and 18 would occur in introns, whereas mutation 24 would occur in exon 10 at nucleotide 8,089 in the genomic DNA (nucleotide 1,868 in the cDNA). This mutation in the genomic DNA would convert the normal 2.6-kb BglII fragment to fragments of 458 and 2,030 nucleotides not present in BglII digests of normal genomic DNA.

We used PCR to amplify a 171-nucleotide fragment of genomic DNA encoding exon 10 from LH, the completely MPO-deficient subject who had the 2.1-kb BglII fragment and lacks the 2.6-kb band seen in normal subjects. The PCR product was digested with BglII and the size of the products assessed by electrophoresis in agarose (Fig. 4, left panel). The exon 10 amplicon from the normal subject was not digested with BglII, as predicted from the genomic DNA sequence. However, the exon 10 amplicon from LH was completely cut by BglII, indicating the presence of a BglII site in both alleles of her genomic DNA which is absent from that of normal subjects. Similar PCR amplification of genomic DNA from RT, a completely MPO-deficient subject whose BglII digested genomic DNA had both 2.6- and 2.1-kb fragments, revealed partial digestion of the exon 10 amplicon, consistent with the presence of both normal and mutated exon 10 alleles in this subject. As shown (Table I), the PCR amplicons of exon 10 from the genomic DNA of each of the subjects who had the 2.1-kb fragment in BglII digests of genomic DNA were susceptible to digestion with BglII. Only the amplicon from LH, whose BglII digest of genomic DNA possessed only the 2.1-kb fragment, was completely susceptible to BglII. In analysis of all other MPO-deficient subjects, both complete and partial, who had a 2.1-kb band, BglII digestion of the amplicon of exon 10 was incomplete, consistent with the presence of two populations of amplicons, those sensitive and those resistant to BglII digestion. The amplicon from control subjects and from MPO-deficient subjects whose genomic DNA lacked the 2.1-kb BglII fragment were resistant to digestion with BglII.

To locate the mutation responsible for BglII susceptibility of exon 10, the amplicons were cloned and sequenced. To minimize the chances of misinterpreting errors secondary to Taq polymerase as true mutations, amplifications of genomic DNA from each subject were replicated and multiple clones from each amplification sequenced. Because normal exon 10 lacks a BglII site and the vector pCRII contains a single BglII site the PCR-amplified exon 10 was 171 nucleotides and the normal amplicon was approximately half of the clones from each subject were linearized whereas those containing a mutated exon 10 were easily screened by digestion with BglII. After BglII digestion, clones containing a normal exon 10 were linearized whereas those containing a mutated exon 10 produced two bands, approximately 3,017 and 1,054 nucleotides, after BglII digestion. For all subjects heterozygous for the 2.1-kb band, approximately half of the clones from each subject were linearized and the remaining clones had two bands after digestion with BglII (Fig. 4, right panel).

Exon 10 cloned from LH (n = 4) possessed a mutation (C to T) at nucleotide 8,089 (Fig. 5, panel B), producing a cleavage site for BglII (AGATCT) not present in exon 10 from normal individuals (AGATCC, Fig. 5, panel A). The same mutation was found in exon 10 derived from every patient who possessed a 2.1-kb fragment in BglII digests of genomic DNA (Table I). For patients heterozygous for the 2.1-kb BglII fragment, approximately half of their clones had normal sequence in this region and the remainder had the C to T mutation. None of the clones derived from MPO-deficient subjects KB, CW, MM, and SS or from normal subjects possessed the mutation at nucleotide 8,089.

Previously it had been speculated that MPO deficiency is inherited as an autosomal recessive trait (26). Based on these studies, at least three distinct genotypes produce complete MPO deficiency. A mutation of cytosine to thymidine at nucleotide 8,089 in exon 10 creates a recognition site for BglII not
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It is noteworthy that all patients studied had immunochemical evidence of an MPO precursor, indicating that all of the genotypes studied (i.e. those with and without the mutation in exon 10) produced the same functional defect in some post-translational event in MPO biosynthesis. We previously suggested that heme insertion triggers a conformational change in apo-MPO that is critical for exit of pro-MPO from the endoplasmic reticulum and subsequent proteolytic processing to mature MPO and targeting to the lysosome (13). We speculate that the nature of the conformational requirement is such that there is little tolerance in the precursor sequence of MPO for mutations. Accordingly, changes at a number of different sites may violate these restrictions and result in abnormally folded apoprotein, unable to accept heme and proceed as pro-MPO down the processing pathway. Studies of the effects of this mutation on MPO gene expression will clarify important early events in the MPO biosynthetic pathway.

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