Jpn. J. Infect. Dis., 57, 2004

Genetic Studies on Myeloperoxidase Deficiency in Italy

Caterina Marchetti[1,2], Pierluigi Patriarca[1], G. Pietro Solero[1], Francisco E. Baralle[2] and Maurizio Romano[1,2]*

[1]*Department of Physiology and Pathology, University of Trieste and*
[2]*International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*

SUMMARY: Hereditary myeloperoxidase (MPO) deficiency is the most common neutrophil biochemical defect characterized by the lack of peroxidase activity. In order to extend the epidemiological studies on hereditary MPO deficiency in Italy, approximately 40,000 individuals were analyzed and 7 partial and 8 total MPO deficient subjects were identified. The genetic characterization of the subjects showed the presence of 3 already-known mutations (c.752T>C, c.1705C>T and c.1566_1579del14) and 6 novel mutations: four missense mutations (c.995C>T, c.1112A>G, c.1715T>G and c.1927T>C), then a deletion of an adenine within exon 3 (c.325delA) and a mutation within the 3' splice site of intron 11 (c.2031−2A>C). The novel missense mutations cause the substitution of residues the p.A332V, p.D371G, p.L572W and p.W643R, respectively, and can cause potential structural changes. The c.325delA deletion causes a shift of the reading frame with the occurrence of a premature stop codon within the pro-peptide. An eukaryotic expression system was set up to investigate how the c.2031−2A>C mutation alters the MPO pre-mRNA splicing. The activation of a cryptic 3' splice site located 109nt upstream of the authentic 3' splice site was observed. The 109nt-insertion might cause the rapid degradation of the MPO mRNA or, alternatively, might lead to the generation of an abnormal MPO precursor lacking the enzymatic activity.

Hereditary myeloperoxidase (MPO) deficiency is the most common neutrophil biochemical defect characterized by the lack of peroxidase activity. MPO deficiency is not geographically restricted and an estimated prevalence of 1 in 2,000 - 4,000 individuals has been reported in the United States and Italy. This defect has been associated with higher susceptibility to infection and higher incidence of neoplasm. Previous studies have found that multiple genotypes likely underline human MPO deficiency. Four missense mutations within the MPO coding sequence (1: c.1705C>T; g.7906C>T; p.R569W. 2: c.518A>G; g.1215A>G; p.Y173C. 3: c.752T>C; g.1626T>C; p.M251T. 4: c.1501G>A; g.7208G>A; G501S) and one 14 base pairs deletion (Δ14; c.1566_1579del14;

*Corresponding author: E-mail: mromano@icgeb.org

Table 1. Genetic characterization of the group of 15 MPO deficient subjects

Patient Number	Sex	MPO Status	Genotype	Effects of Mutations
1	F	T	[c.2031−2A>C] + [c.2031−2A>C]	[r.2031-190_2031-2ins; r.2031−2A>C] + [r.2031-109_2031-2ins; r.2031−2A>C]
2	F	T	[c.1566_1579del14] + [c.1705C>T]	[r.1545_1622del] +[p.R569W]
3	F	P	[c.752T>C] + [WT]	[p.M251T] + [WT]
4	F	T	[c.1566_1579del14] + [c.1566_1579del14]	[r.1545_1622del] + [r.1545_1622del]
5	M	P	[c.995C>T] + [WT]	[p.A332V] + [WT]
6	F	P	[c.352delA] + [WT]	[p.A108fs5] + [WT]
7	F	T	[c2031−2A>C] + [[c.2031−2A>C]	[r.2031-109_2031-2ins; r.2031−2A>C] + [r.2031-109_2031-2ins; r.2031−2A>C]
8	M	T	[c.1705C>T] + [c.752T>C]	[p.R569W] + [p.M251T]
9	M	P	[c.752T>C] + [WT]	[p.M251T] + [WT]
10	M	T	[c.995C>T] + c.1715T>G	[p.a332V] + [p.L572W]
11	M	P	[c.1566_1579del14] + [WT]	[r.1545_1622del] + [WT[
12	F	P	[c.752T>C] + [WT]	[p.M51T] + [WT]
13	F	T	[c.1566_1579del14] + [c.2031−2A>C]	[r.1545_1622del] + [r.2031-109_2031-2ins; r.2031−2A>C]
14	F	P	[c.1112A>G] + [WT]	[p.D371G] + [WT]
15	F	T	[c.1566_1579del14[+ [c.1927T>C]	[r.1545_1622del] + [p.W643A]

MPO status: T: complete deficiency. P: partial deficiency. WT: wild-type allele. The genotype of each patient (fifth colum) and the effects of mutations (sixth column) at RNA (plain text) or at protein level (bold text) are shown.

g.6801_7272del14) within MPO exon 9 were identified as responsible for the defect (1-5). These studies indicated that MPO deficiency is an autosomal recessive disease and that most patients are compound heterozygotes, which means that they have a different mutation on each allele of the MPO gene.

The epidemiological studies on hereditary MPO deficiency were extended in the Italian Veneto region by performing a population screening in collaboration with the Clinical Chemistry laboratory of Borgo Roma Hospital in Verona. This was the first systematic genetic screening carried out in Italy in order to find mutations in the MPO gene responsible for MPO deficiency. We identified 7 partial and 8 total MPO deficient subjects out of approximately 40,000 individuals analysed. The genetic characterization of the subjects showed the presence of 3 known mutations (c.1705C>T, c.752T>C and c.1566_1579del14) and 6 novel mutations associated with MPO deficiency: four missense mutations in exons 7, 10 and 11 (1:c.995C>T; p.A332V. 2: c.1112A>G; p.D371G. 3: c.1715T>G; p.L572W. 4: c.1927T>C; p.W643A), then the deletion of an adenine within exon 3 (c.325delA; p.A108fs5) and a mutation within the 3' splice site of intron 11 (c.2031 −2A>C;IVS11 −2A->C) (5). The study revealed the presence of 7 heterozygous, 3 homozygous and 5 compound heterozygous subjects for the mutations within the MPO gene. We observed that most of the amino acids involved in the novel missense mutations were localized within the heme-binding pocket of the MPO molecule. The novel mutations might cause a local change in the MPO secondary structure thus altering the MPO enzymatic activity. The genotypes of the MPO deficient subjects are summarized in Table 1.

Two mutations, IVS11 −2A->C and Δ14 deletion, were present in high frequency among the analysed MPO deficient subjects. Then, we have characterized both in vivo and in vitro, how these mutations cause the disruption of MPO pre-mRNA splicing.

Using a minigene expression system, we showed that the result of the IVS11 −2A->C mutation is the activation of a cryptic 3' splice site located 109 nucleotides upstream of the authentic 3' splice site of intron 11 (Fig. 1). The 109nt insertion might cause the rapid degradation of the MPO mRNA or, alternatively, might causes a shift in the reading frame with the generation of a stop codon after codon 749 that should be translated into a MPO precursor with a completely subverted sequence of the 72 amino acids carboxy-terminal tail downstream of the activated cryptic splice site.

Previous studies on neutrophil precursors showed that the Δ14 deletion can be considered an exonic regulatory element of pre-mRNA processing since it affects exon 9 splicing causing the selection of a cryptic 5' splice site (GGT 9935) located 77 nucleotides upstream of the authentic 5' splice site of MPO exon 9. In order to evidence the possible trans-acting factors that interact specifically with MPO exon 9 across the Δ14 region, we carried out both electrophoretic mobility shift (EMSA) and UV cross-linking assays. EMSA experiment indicated that an RNA-protein(s) complex was formed in presence of the wild type exon 9 sequence and was not formed in presence of the Δ14 exon 9. In UV cross-linking assay, a 40 kDa protein was then shown to specifically bind to the wild type exon 9 and not to the Δ14 one (Fig. 2).

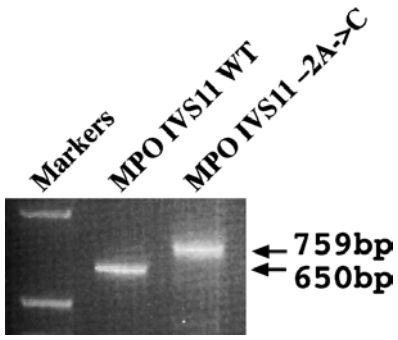

Fig. 1. Effects of the mutation IVS11 −2A->C on MPO intron 11 splicing. RT-PCR assay performed on transfection experiments of Hep3B cell line using the WT MPO construct or the IVS11 −2A->C construct. The RT-PCR products are resolved on 2% agarose gel electrophoresis. Sample 1 (MPO IVS11 WT): 650bp RT-PCR fragment corresponding to exon 10 +11+12 mRNA. Sample 2 (MPO IVS11 −2A-> C): 759bp RT-PCR fragment corresponding to exon 10 +11+12 mRNA plus the inclusion of 109nt of intron 11. Sample 3: RT-PCR negative control. M: molecular weight marker.

The protein was purified by setting up an RNA-affinity purification protocol. Microsequencing by mass spectrometry identified the isolated protein as the heterogeneous ribonuclear protein E2 (hnRNP E2). Recombinant hnRNP E2 was expressed in *E. coli* and was used to confirm the specificity of interaction with the MPO exon 9 as well as to explore its sequence binding requirements. Then, functional experiments showed that the cryptic GGT 9935 5' splice site of MPO exon 9 can be effectively selected by the splicing machinery in particular conditions. All together these results would suggest that hnRNP E2 might regulate the selection of the authentic MPO exon 9 5' splice site blocking sterically the access to the GGT 9935

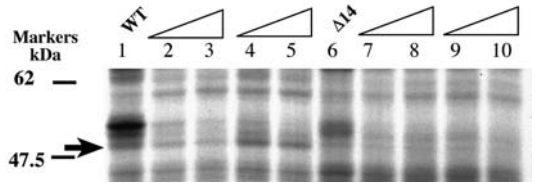

Fig. 2. UV cross-linking to WT and Δ14 RNA. UV cross-linking assay were carried out using HeLa nuclear extract with uniformly labeled WT (lane 1) and Δ14 RNA (lane 6). The arrow shows the position of the 48 kDa complex that is detected only in the presence of WT-RNA and not in the presence of Δ14-RNA. Competition analysis confirmed the binding specificity of the 48 KDa complex following addition of increasing amounts of cold WT and Δ14 RNAs to labeled WT (lane 2-3, 4-5) and to labeled Δ14 (lane 7-8, 9-10) in the presence of HeLa nuclear extract. The molar ratios of cold/labelled RNA were 3 and 6. The competition analysis shows that the 48 kDa band is competed away from the labeled WT-RNA only when cold WT-RNA (and not Δ14) is used.

splice site by its interaction with the wild type MPO exon 9. In the presence of the Δ14 deletion hnRNP E2 cannot bind to MPO exon 9 and the cryptic 5' splice site might be selected. Moreover, other trans-acting factors specific of some stages of neutrophil maturation could be recruited, even through hnRNP E2, to regulate the selection of the MPO exon 9 5' splice site. Our study represents the first demonstration that hnRNP E2 is able to bind to an intraexonic sequence and supports the hypothesis that such a protein might be relevant for the pre-mRNA processing in the leukocite precursors.

REFERENCES

1. Nauseef, W. M., Brigham, S. and Cogley, M. (1994): Hereditary myeloperoxidase deficiency due to a missense mutation of arginine 569 to tryptophan. J. Biol. Chem., 269, 1212-1216.
2. Romano, M., Dri, P., Dadalt, L., Patriarca, P. and Baralle, F. E. (1997): Biochemical and molecular characterization of hereditary myeloperoxidase deficiency. Blood, 90, 4126-4134.
3. DeLeo, F., Goedken, M., McCormick, S. and Nauseef, W. (1998): A novel form of hereditary myeloperoxidase deficiency linked to endoplasmic reticulum/proteasome degradation. J. Clin. Invest., 101, 2900-2909.
4. Ohashi, Y. Y., Kameoka, Y., Persad, A. S., Koi, F., Yamagoe, S., Hashimoto, K. and Suzuki, K. (2004): Novel missense mutation found in a Japanese patient with myeloperoxidase deficiency. Gene, 327, 195-200.
5. Marchetti, C., Patriarca, P., Solero, G. P., Baralle, F. E. and Romano, M. (2004): Genetic characterization of myeloperoxidase deficiency in Italy. Hum. Mutat., 23, 496-505.