

Mannose-Binding Lectin and Mannose-Binding Lectin-Associated Serine Protease-2 Genotypes and Serum Levels in Patients with Sporotrichosis

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Abstract. Mannose-binding lectin (MBL) and MBL-associated serine protease-2 (MASP-2) are important proteins in the lectin pathway of the immune system. Mannose-binding lectin and MASP-2 deficiencies have been reported to be responsible for various fungal infections. We investigated the association of *MBL* and *MASP-2* variants with sporotrichosis in a Chinese population and revealed one rare heterozygous mutation in a disseminated cutaneous patient without immunosuppressive conditions (*MASP2*, p.156_159dupCHNH). We also found that sporotrichosis patients had decreased levels of MBL and MASP-2 in their serum samples compared with controls. Our findings linked, for the first time, *MASP-2* deficiencies with susceptibility to *Sporothrix* sp.

Sporotrichosis is a chronic granulomatous mycotic infection caused by species of the *Sporothrix schenckii* complex.¹ Such infections may progress into chronic cutaneous, subcutaneous, and/or even deeper infections involving the lymphatics, fascia, muscles, cartilage, and bones. The prognosis of the disease is related to the immune status of the host, and it is believed that genetic variation plays an important role in host susceptibility.²

The complement system is one of the major effectors of the innate immune system and an important bridge between innate and adaptive immunity. The MBL-associated serine protease-2 (MASP-2) is a key enzyme involved in the initiation of innate immune responses by binding to mannose-binding lectin (MBL) and activating the complement lectin pathway. The complex formed by MBL and MASP-2 cleaves C4 and C2 to form the C3 convertase (C4b2a). Subsequent complement activation leads to opsonization and phagocytosis of the target microbes, as well as formation of membrane attack complexes.^{3,4} Previous studies suggest that variants of *MBL* and *MASP-2* are associated with fungal infectious diseases such as invasive aspergillosis and recurrent vulvovaginal candidiasis.^{5–7} It remains unknown whether MBL and MASP-2 deficiencies are associated with sporotrichosis. This study explores the association between *MBL* and *MASP-2* variants and their corresponding serum levels in sporotrichosis patients.

The study was approved by the human medical and ethics committee of Shandong Provincial Institute of Dermatology and Venereology. We performed the mutation analysis of *MBL* and *MASP-2* gene in 61 cases with cutaneous sporotrichosis and 300 controls by Sanger sequencing (Table 1). After informed consent, genomic DNA was extracted from the peripheral blood of all participants. All the exons of *MBL* and *MASP-2* gene were amplified by polymerase chain reaction. After amplification, the products were purified and directly sequenced on ABI 3130xl Genetic Analyser (Applied Biosystems ABI, Carlsbad, CA). Mannose-binding lectin and MASP-2 concentrations were measured in the sera of 35 patients and 47 controls using commercial ELISA kits (Hycult Biotech, Uden, The Netherlands/HK326; Hycult Biotechnology). Minimum and

maximum concentration which can be measured is 0.16 ng/mL and 3.13 ng/mL, respectively. Color intensity was evaluated at 450 nm in an ELISA reader.

We identified one variant in exon 1 of the *MBL2* gene in patient group: p.G54D (c.161G>A). The control data are derived from Han Chinese (Beijing, China) in 1000 Genomes Project Phase 3 allele frequencies. No statistical significance of the allele frequencies and genotype frequencies was observed between the patient and control groups. The mean serum level of MBL in patients and controls were 304 ng/mL and 365 ng/mL, respectively ($P < 0.05$ *t*-test) (Figure 1A). There were no significant differences between the patients with and without p.G54D variant (Figure 1B).

Mannose-binding lectin-associated serine protease-2 sequencing revealed one rare heterozygous mutation in a disseminated cutaneous patient without immunosuppressive conditions: p.156_159dupCHNH (c.477_478insTGCCA-CAACCAC). This mutation was not detected in the other matched normal controls (0/300, $P = 0.169$, Fisher's exact test) and in the East Asian population (0/4290, Exome Aggregation Consortium database, $P = 0.014$, Fisher's exact test). The duplication of four amino acids within the epidermal growth factor domain (p.156_159dupCHNH) was predicted to cause damage to the MASP-2 protein and probably results in MASP-2 misfolding.^{8,9} In addition, four variants of *MASP-2* (p.H155R [c.464 A>G], p.D371Y [c.1111 G>T], p.V377A [c.1130 T>C], and p.S493 [c.1479 C>T]) were identified in patient group, and there were no significant differences in the allele frequencies and genotype frequencies between the patients and controls ($P > 0.05$ Fisher's Test) (Table 2). We found that sporotrichosis patients had decreased levels of MASP-2 in their serum samples compared with controls (median 244 versus 396 ng/mL, $P < 0.05$ *t*-test) (Figure 1C).

TABLE 1
Clinical information

| Characteristics | Patients (n = 61) | Controls (n = 300) |
|------------------------|-------------------|--------------------|
| Female | 22 (36%) | 133 (44%) |
| Male | 39 (64%) | 167 (56%) |
| Mean age at diagnosis | 62.81 | 54.56 |
| Clinical presentations | | |
| Fixed cutaneous | 35 (57%) | – |
| Lymphocutaneous | 21 (35%) | – |
| Disseminated cutaneous | 5 (8%) | – |

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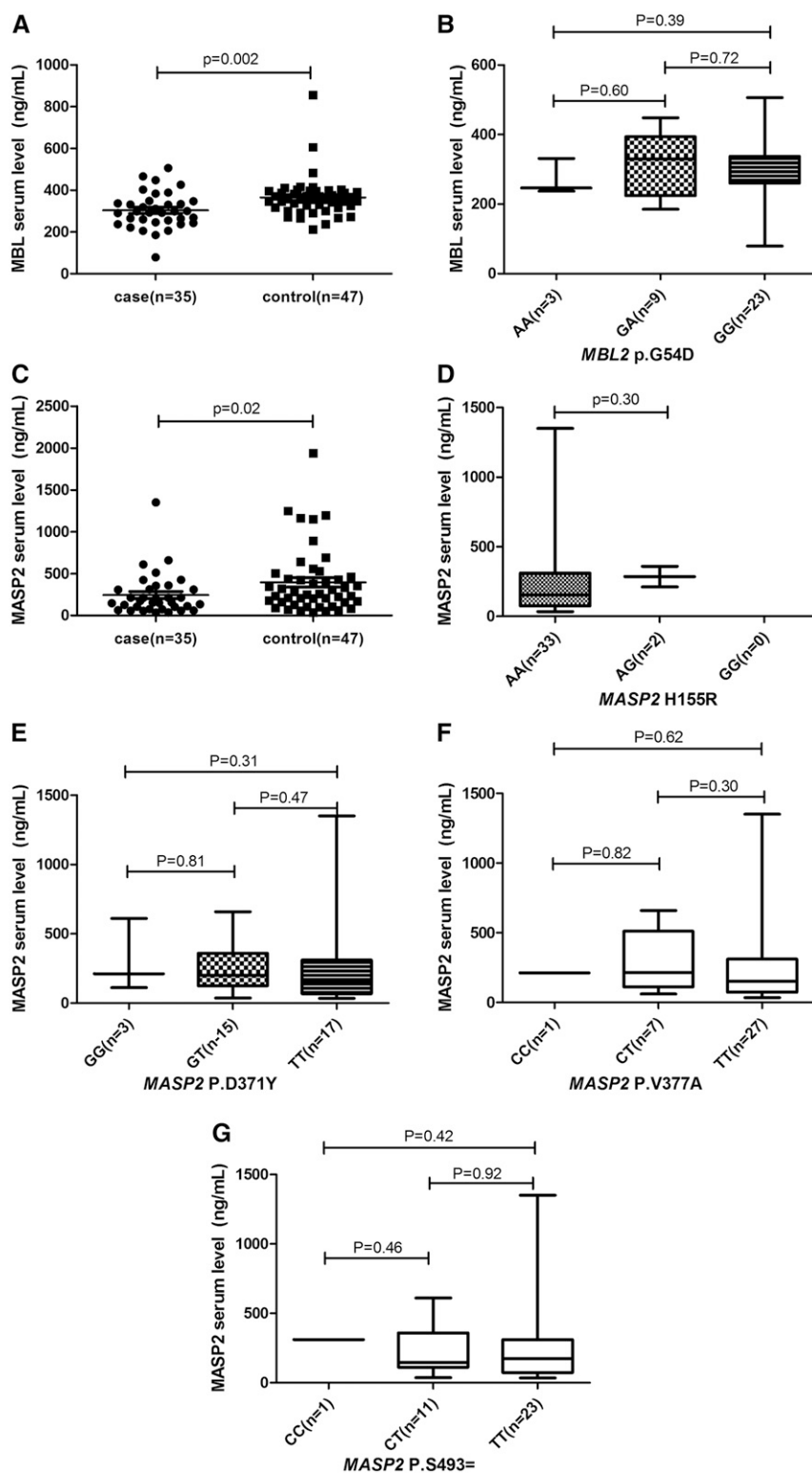


FIGURE 1. Mannose-binding lectin (MBL) and MBL-associated serine protease-2 (MASP-2) levels. (A) MBL levels in patients and controls. (B) Mannose-binding lectin levels according to the genotypes of *MBL-2* (p.G54D) in patients. (C) MBL-associated serine protease-2 levels in patients and controls. (D–G) MBL-associated serine protease-2 levels according to the genotypes of *MASP-2* (p.H155R, p.D371Y, p.V377A, and p.S493) in patients.

There were no significant differences between patients with and without the aforementioned variant (Figure 1D–G). Our studies showed that the heterozygous mutation p.156_159dupCHNH did not affect the protein expression (212

ng/mL), but whether the mutation affected the protein function needs further experimental exploration.

To summarize, *MASP-2* sequencing in sporotrichosis patients revealed one rare mutation. Various inborn errors of

TABLE 2
MBL and MASP-2 gene polymorphism

| Gene region | SNP ID | Allele | mRNA | Genotype | Case | Control | Allele frequency (in case) | Allele frequency (in control) | P-value (Fisher's test) |
|----------------|------------|---------|--------------|----------|------------|-------------|----------------------------|-------------------------------|-------------------------|
| MASP-2 exon 4 | rs2273343 | p.H155R | c.464 A > G | AA | 59 (96.7%) | 282 (94.0%) | A: 98.4% | A: 97.0% | 0.216 |
| | | | | AG | 2 (3.3%) | 18 (6.0%) | G: 1.6% | G: 3.0% | |
| | | | | GG | 0 | 0 | – | – | |
| | | | | GG+AG | 2 (3.3%) | 18 (6.0%) | – | – | |
| MASP-2 exon 10 | rs12711521 | p.D371Y | c.1111 G > T | GG | 5 (8.0%) | 49 (16.3%) | G: 31.1% | G: 36.8% | 0.311 |
| | | | | GT | 28 (46.0%) | 123 (41.0%) | T: 68.9% | T: 63.2% | |
| | | | | TT | 28 (46.0%) | 128 (42.7%) | – | – | |
| | | | | TT+GT | 56 (92%) | 251 (83.7%) | – | – | |
| MASP-2 exon 10 | rs2273346 | p.V377A | c.1130 T > C | TT | 46 (75.4%) | 204 (68.0%) | T: 86.1% | T: 80.8% | 0.398 |
| | | | | CT | 13 (21.3%) | 77 (25.7%) | C: 13.9% | C: 19.2% | |
| | | | | CC | 2 (3.3%) | 19 (6.3%) | – | – | |
| | | | | CC+CT | 15 (24.6%) | 96 (32.0%) | – | – | |
| MASP-2 exon 12 | rs1782455 | p.S493= | c.1479 C > T | CC | 2 (3.3%) | 3 (1.0%) | C: 18.0% | C: 14.3% | 0.701 |
| | | | | CT | 18 (29.5%) | 80 (26.7%) | T: 82.0% | T: 85.7% | |
| | | | | TT | 41 (67.2%) | 217 (72.3%) | – | – | |
| | | | | TT+CT | 59 (96.7%) | 297 (99.0%) | – | – | |
| MBL-2 exon 1 | rs1800450 | p.G54D | c.161G > A | GG | 42 (68.9%) | 217 (72.3%) | G: 81.1% | G: 84.8% | 0.312 |
| | | | | AG | 15 (24.6%) | 75 (25.0%) | A: 18.9% | A: 15.2% | |
| | | | | AA | 4 (6.5%) | 8 (2.7%) | – | – | |
| | | | | AA+AG | 19 (31.1%) | 83 (27.7%) | – | – | |

MASP-2 = MBL-associated serine protease-2; MBL-2 = mannose-binding lectin-2; SNP = single nucleotide polymorphism.

MASP-2 gene have been reported to be responsible for various infections. Our findings linked, for the first time, MASP-2 deficiencies with susceptibility to *Sporothrix* sp. However, more ethnic lines and many studies are needed to affirm the consideration in future.

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