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PCR-Restriction Fragment Length Polymorphism Method to Detect the X/Y Polymorphism in the Promoter Site of the Mannose-binding Lectin Gene

To the Editor:
Mannose-binding lectin (MBL), a pattern-recognition molecule produced by the liver and present in serum, is an important player in the innate immune system. MBL acts by binding various carbohydrate structures on microbial surfaces, after which it activates the complement system via the lectin pathway. In addition, MBL can promote direct opsonophagocytosis of microorganisms and modulate diverse inflammatory mediators (1).

Deficiency of MBL was first identified in association with a common defect of opsonization in children. Additional studies have identified MBL deficiency as a risk factor for diverse infectious diseases (1) and, recently, atherosclerosis (2). MBL deficiency is caused by mutations in the coding and promoter regions of the MBL gene, which have a profound effect on plasma concentrations of the MBL protein.

Three point mutations have been found in the structural region of the MBL gene: at codons 54, 52, and 57 in exon 1. These mutations cause allelic variants designated B, C, and D, respectively. In addition to these exon 1 mutations, there are three major polymorphisms in the promoter region of the MBL gene, which are expressed as four haplotypes called HYP, LYP, LXP, and LYQ. The X/Y polymorphism at position −221 is important because the X haplotype is highly prevalent (~24% in Caucasian populations (3)) and is associated with low circulating MBL concentrations, comparable to those of the structural variants B, C, and D (4). Therefore, determination of the exon 1 point mutations and the X/Y promoter polymorphism is adequate to assess MBL deficiency in a certain individual.

Several methods have been described for the detection of mutations in the structural region, such as PCR followed by restriction fragment length polymorphism (RFLP) analysis (4). This method is simple and gives unambiguous results, but such a method has not been published for the detection of the X/Y promoter polymorphism mentioned above. Here we describe a PCR-RFLP method for the X/Y polymorphism. The method fits well with the PCR-RFLP method we implemented for the detection of the three point mutations in exon 1.

PCR was carried out in a final volume of 50 μL containing 1 μL (~30–100 ng) of genomic DNA, 2.5 mM MgCl2, 50 μM deoxynucleotide triphosphates, 0.4 μM upstream primer (5’-GTITTCCACTCATCTTCATTTCCCTAAG-3’), 0.4 μM downstream primer (5’-GAAAATCAGGGAGTTAATCCTG-3’), and 1 U of AmpliTaq Gold (Applied Biosystems) in a buffer containing 100 mM Tris-HCl (pH 8.3) and 500 mM KCl.

The PCR was initiated by a 10-min denaturation and enzyme activation step at 95 °C and completed by a 10-min extension step at 72 °C. The temperature cycles were as follows: 35 cycles of 30 s at 95 °C, 30 s at 60 °C, and 45 s at 72 °C.

The amplification product, with a length of 350 bp, was digested by BsaJI according to the manufacturer’s instruction (New England Biolabs Inc.). The PCR product contained one nonpolymorphic BsaJI restriction site that served as an internal control for enzyme digestion, producing two fragments of 242 and 108 bp. In the Y variant, the 242-bp fragment was cleaved into two fragments of 166 and 76 bp. Because of the substitution of the base cytosine for guanine at position −221 of the MBL gene, this does not occur in the X variant. PCR restriction fragments were separated by electrophoresis in 2% Response Research (Biozym) agarose gels (Fig. 1).

To assess the validity of the method, we tested a series of 17 different DNA samples (partially provided by Prof. Dr. M. Turner, Institute of Child Health, London, UK) with our newly developed PCR-RFLP method. Haplotype frequencies were 1 XX, 6 XY, and 10 YY. The observed haplotypes were confirmed by a heteroduplex method using PCR-amplifiable synthetic DNA (5).

In conclusion, our newly developed PCR-RFLP method for the X/Y promoter polymorphism of the MBL gene works with the previously described PCR-RFLP method for the three exon 1 variants to detect the mutations in the MBL gene that cause MBL deficiency.

References
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Is Cystatin C a Marker of Glomerular Filtration Rate in Thyroid Dysfunction?

In the recent literature, cystatin C has been advocated as a new and more accurate estimate of glomerular filtration rate (GFR) (1). Cystatin C is a 13-kDa endogenous cysteine proteinase inhibitor produced by all nucleated cells at a constant rate and broken down completely in the renal tubuli (2). Cystatin C concentrations are independent of age and body weight, and there is no need for urine collection for clearance estimations. Furthermore, serum concentrations of cystatin C are not influenced by malignancy or inflammation. In contrast, the often-used serum creatinine concentration is supposedly influenced by dietary intake, renal tubular metabolism, age, and variations in muscle mass. There are also various analytical difficulties with the widely used Jaffe colorimetric assay for creatinine. A slight decrease in GFR has been found in patients with hypothyroidism, which improved significantly after treatment (3–5). We wondered whether cystatin C would also be a good marker of renal function in case of thyroid dysfunction. Because thyroid hormones have general metabolic effects, the thyroid state could influence plasma cystatin C concentrations.

We reanalyzed patient data from earlier trials. All patients gave written informed consent, and the earlier studies were approved by the local ethics committee. The study groups consisted of consecutive patients seen at our clinics for primary hypothyroidism based on autoimmune thyroiditis (n = 37; 10 males and 27 females; median age, 46 years; range, 22–72 years) and for hyperthyroidism caused by Graves disease (n = 14; 1 male and 13 females; median age, 41 years; range, 23–73 years). Blood samples were taken at diagnosis, before start of treatment, and after euthyroidism had been regained for at least 3 months. Samples were assayed for thyroid-stimulating hormone (range, 0.4–4.0 mIU/L), free thyroxine (10–24 pmol/L), and serum creatinine (ranges, 40–80 μmol/L for females and 45–90

![Fig. 1. Relationships between thyroidal state and estimated GFR (A) and cystatin C concentrations (B).](image-url)