Online-appendix only

DNA was extracted from whole blood using the Maxwell 16 System Blood DNA Purification Kit (Promega, Madison, WI, USA).
Six single nucleotide polymorphisms (SNPs) located within the promoter or coding regions of the MBL2 gene (MBL2 –D (codon 52, rs5030737), MBL2 –C (codon 57, rs1800451), MBL2 –B (codon 54, rs1800450), MBL2 –H/L(-550, rs11003125), MBL2 –X/Y(-221, rs7096206) and MBL2 –P/Q(+4, rs12780112) were analyzed using real-time polymerase chain reaction (rt-PCR) with TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) (13-15). For all TaqMan assays, DNA amplifications were carried out in 25µl polymerase chain reactions containing 20 ng DNA, 0.9µM primers and 0.2µM probes (final concentrations) amplified in 96-well plates. Reactions were performed with the following protocol on a GeneAmp PCR 9700 or a 7900 HT Sequence Detection System: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The genotyping was determined by measuring the end-point fluorescence on a 7900 HT Sequence Detection System using the SDS version 2.3 software. The presence of one of the three structural mutations within exon 1 at codons 52, 54 or 57 in the MBL2 gene, the D, B and C variants (designated as "O"), significantly reduces circulating MBL. Of the promoter polymorphisms, comprising two variants in the 5' regulatory region at positions -550 (H/L) and -221 (X/Y) and one in the 5' untranslated region at position -4 (P/Q), only the X/Y polymorphism influences S-MBL, causing reduced MBL levels (19).
In the present report, genotypes were categorized according to polymorphisms in the MBL2 gene as AA (wild type), AO (heterozygote) or OO (homozygote). After the addition of information on the promoter region, genotypes were classified as high (including all AA with the exclusion of homozygotes for the X promotor) or low, as performed previously by Hansen et al. (16).