(High Resolution Melting)HRM assays

HRM assays was carried out in a 384-well plate in the LightCycler® 480 Real-Time PCR System (Roche) under the same reaction system and conditions. The PCR reaction mixture was prepared in 6 μl total volume by adding 40 ng of genomic DNA, 3μl 2×GoldStar Taq Master Mix(CWBIO)and 0.3 μl 20×EvaGreen TMDye(Biotium), 5μM of forward and reverse primers. Thermocycling conditions for PCR included one cycle denaturation at 95°C for 10 min and 50 cycles consisted of denaturation at 95°C for 15 s, annealing at 59°C for 10 s and extension at 72°C for 10 s, and cooling at 40°C for 30 s.

Determination of genotypes

After the end of the reaction, the mutation was analyzed by Tm software calling and Gene Scaning in LightCycler480SW1.5.0.Melting of the PCR products was monitored by plotting the changes in fluorescence that occurred by gradual temperature-dependent releasing of a saturating double-strand DNA binding dye. Heterozygous DNA samples formed heteroduplexes, resulting in a different shape of the melting curve compared with a homozygous sample. Different genotypes of homozygous DNA samples, in contrast, were detected by a melting temperature (Tm) shift rather than an altered curve shape.