

Supplementary figure and tables

MME reference sequence and primers.

Reference sequence and numbering is according to *MME* ENSG00000196549. Exon numbering and variant position is according to *MME* NM_007289.3.

Exons are indicated in light blue, AT-repeat, c.439+33_439+48AT[8-15], in orange and the two *MME* mutations c.440-2A>C and c.467del in red. Primers used are indicated with black arrows and primer numbering.



Enzymes and conditions**Standard Sanger sequencing conditions at Telemark Hospital Trust (top) and the Medical University of Vienna (bottom)**

PCR enzyme	PCR primers	PCR reagents	PCR conditions	Comment	Result
Accuprime (ThermoFischer Scientific)	F1+R1	10x AccuPrime buffer II 1.25µl Primer F 2µM 1,13µl Primer R 2µM 1,13µl Accuprime 2U/ul 0,25µl dH ₂ O 7.75µl DNA ≈50ng/ul 1.00µl	95°C 2min + 95°C 30s, 61°C 30s , 72°C 1min x 35 cycles + 72°C 10 min + 4°C →	Standard conditions and Telemark Hospital Trust. PCR clean-up was performed with ExoSAP IT Express, sequencing with BigDye v.3.1 Terminator Cycle Sequencing Kit and sequencing clean-up with BigDye XTerminator Purification Kit (all ThermoFischer Scientific). The products were sequenced on the ABI3130XL (Life Technologies Ltd., Paisley, UK and analysed with CLC Main Workbench (CLC bio, Aarhus, Denmark).	Allele drop-out of 13AT allele
Phusion Green Hot Start II High-Fidelity DNA Polymerase (Thermo Scientific)	E5F+E7R	2x Phusion Green MM 10.00µl Primer F 1µM 0.50µl Primer R 1µM 0.50µl Nuclease free dH ₂ O 8.00µl DNA ≈50ng/ul 1.00µl	98°C 30s + 98°C 5s, 63°C 15s, 72°C 15s x 30 cycles + 72°C 5min + 4°C →	Standard conditions at the Medical University of Vienna PCR clean-up was performed with ExoSAP IT Express, sequencing with BigDye v.3.1 Terminator Cycle Sequencing Kit and sequencing clean-up with BigDye XTerminator Purification Kit (all Thermo Scientific). The products were sequenced on the ABI3130XL and analysed with Seqscape (Life Technologies Ltd., Paisley, UK)	Allele drop-out of 13AT allele

Ability of different PCR enzymes and conditions to amplify both the short and the long AT-repeat during Sanger sequencing.

The testing was performed on one individual homozygous for an 8AT repeat, one homozygous for a 13AT repeat and one heterozygous for an 8AT/13AT repeat. The test was repeated once for all individuals and additionally for four individuals heterozygous for an 8AT/13AT repeat.

Sanger sequencing conditions were similar for all enzyme and conditions tested. PCR clean-up was performed with ExoSAP IT Express, sequencing with BigDye v.3.1 Terminator Cycle Sequencing Kit and sequencing clean-up with BigDye XTerminator Purification Kit (all ThermoFischer Scientific). The products were sequenced on the ABI3130XL (Life Technologies Ltd., Paisley, UK and analysed with CLC Main Workbench (CLC bio, Aarhus, Denmark).

PCR enzyme	PCR primers	PCR reagents	PCR conditions	Comment	Result
Accuprime (ThermoFischer Scientific)	F1+R1	10x AccuPrime buffer II 1.25µl Primer F 2µM 1.13µl Primer R 2µM 1.13µl Accuprime 2U/ul 0.25µl dH ₂ O 7.75µl DNA ≈50ng/ul 1.00µl	95°C 2min + 95°C 30s, 61°C 30s , 69°C 1min x 35 cycles + 72°C 10 min + 4°C →	PCR program with 69 °C elongation temperature. Recommended from manufacturer for AT rich templates	Allele drop-out of 13AT allele
Accuprime (ThermoFischer Scientific)	F1+R1	10x AccuPrime buffer II 1.25µl Primer F 2µM 1.13µl Primer R 2µM 1.13µl 3 % DMSO 0.38µl Accuprime 2U/ul 0,25µl dH ₂ O 7.38µl DNA ≈50ng/ul 1.00µl	95°C 2min + 95°C 30s, 61°C 30s , 72°C 1min x 35 cycles + 72°C 10 min + 4°C →	PCR reaction with 3% DMSO Recommended by the manufacturer to loosen up secondary structures	Allele drop-out of 13AT allele
Platinum SuperFi II PCR Master MIX (ThermoFischer Scientific)	F1+R1	2x Platinum SuperFi II PCR mastermix 6.25µl Primer F 2µM 2.50µl Primer R 2µM 2.50µl dH ₂ O 0.25µl DNA ≈50ng/ul 1.00µl	98°C 30s + 98°C 10s, 61°C 10s , 72°C 30s x 35 cycles + 72°C 5 min + 4°C →	PCR enzyme recommended by ThermoFischer Scientific. PCR set-up and program based on manufacture recommendations.	Allele drop-out of 13AT allele
Herculase II Fusion DNA pol (Agilent Technologies)	F1+R1	5x herculase II buffer 2.50µl Primer F 2µM 1.13µl Primer R 2µM 1.13µl dNTP mix, 10mM each 0.31µl dH ₂ O 3.31µl Herculase II fusion DNA pol 0.13µl DNA ≈50ng/ul 1.00µl	95°C 2min + 95°C 30s, 61°C 30s , 72°C 1min x 35 cycles + 72°C 10 min + 4°C →	PCR set-up and program based on manufacture recommendations.	Allele drop-out of 13AT allele

Q5 Hi Fidelity DNA pol (New England BioLabs)	F1+R1	5x Q5 Reaction buffer Primer F 2µM Primer R 2µM dNTP, 10mM dH ₂ O Q5 HiFid DNA pol DNA ≈50ng/ul	2.50µl 3.13µl 3.13µl 0.25µl 2.38µl 0.13µl 1.00µl	98°C 30s + 98°C 10s, 61°C 30s , 72°C 30s x 35 cycles + 72°C 2 min + 4°C →	PCR set-up and program based on manufacture recommendations.	Allele drop-out of 13AT allele
OneTaq Hot Start DNA polymerase (New England BioLabs)	F1+R1	5x OneTaq Std Rxn buffer Primer F 2µM Primer R 2µM dNTP, 10mM dH ₂ O OneTaq Hotstart DNA pol DNA ≈50ng/ul	2.50µl 1.13µl 1.13µl 0.25µl 6.44µl 0.13µl 1.00µl	94°C 30s + 94°C 30s, 61°C 30s , 68°C 1min x 35 cycles + 68°C 5 min + 4°C →	PCR set-up and program based on manufacture recommendations.	Both 8AT and 13AT alleles detected
Type iT Microsatellite PCR kit (QIAGEN)	F1+R1	2x Type-it PCR mix Primer F 2µM Primer R 2µM dH ₂ O DNA ≈50ng/ul	6.25µl 0.63µl 0.63µl 3.00µl 1.00µl	95°C 5min + 95°C 30s, 60°C 90s, 72°C 30sec x 28 cycles + 60°C 30min + 4°C →	Enzyme used at Telemark Hospital for expansion analysis. PCR set-up and program based on manufacture recommendations.	Both 8AT and 13AT alleles detected
Advantage GC LA Polymerase (Takara)	E5F+E7R	2x GC Melt Buffer dNTP-Mix (20mM each) Primer F 1µM Primer R 1µM Nuclease free dH ₂ O LA Polymerase Mix (5U/µl) DNA ≈50ng/ul	12.50µl 0.50µl 1.00µl 1.00µl 8.75µl 0.25µl 1.00µl	94°C 1min + 94°C 30s, 63°C 30s, 72°C 1min x 30 cycles + 72°C 5min + 4°C →	PCR set-up and program based on manufacturer's recommendations	Both 8AT and 13AT alleles detected

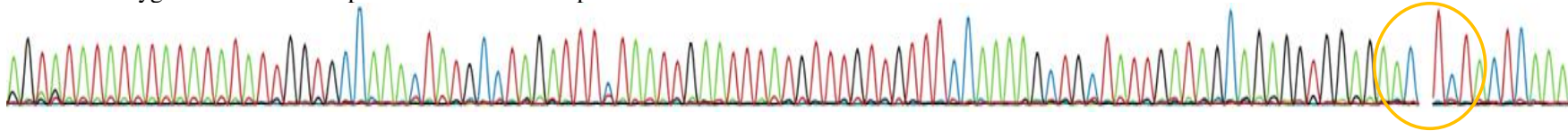
Fragment Analysis (FLA)

Electrophoretic length separation of the PCR products was performed on 3130XL Genetic Analyzer (Life Technologies). Data were analyzed by GeneMarker v. 1.85 (SoftGenetics LLC).

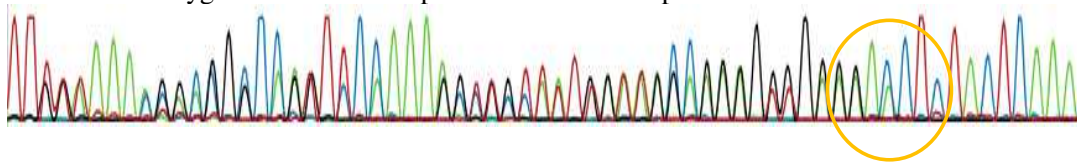
PCR enzyme	PCR primers	PCR reagents	PCR conditions
Accuprime (ThermoFischer Scientific)	F1-FAM tagget+R1	10x AccuPrime buffer II 1.25µl Primer F 2µM 1.13µl Primer R 2µM 1.13µl Accuprime 2U/ul 0.25µl dH ₂ O 7.75µl DNA ≈50ng/ul 1.00µl	95°C 2min + 95°C 30s, 61°C 30s , 72°C 1min x 35 cycles + 72°C 10 min + 4°C →
Type iT Microsatellite PCR kit (QIAGEN)	F1-FAM tagget+R1	2x Type-it PCR mix 6.25µl Primer F 2µM 0.63µl Primer R 2µM 0.63µl dH ₂ O 3.00µl DNA ≈50ng/ul 1.00µl	95°C 5min + 95°C 30s, 60°C 90s, 72°C 30sec x 28 cycles + 60°C 30min + 4°C →

Sequence traces for MME: c.467del**Individual with one short and one long AT-repeat**

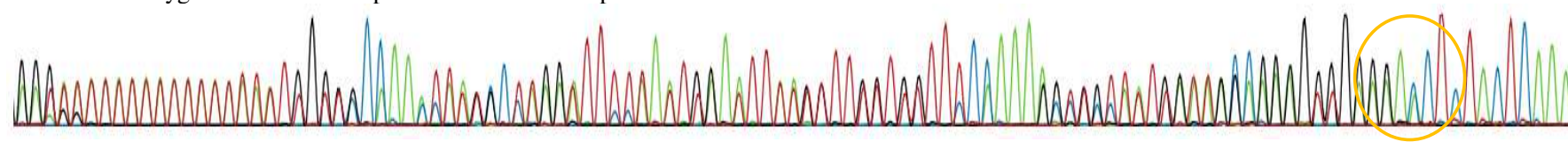
False homozygous deletion with primers outside AT-repeat



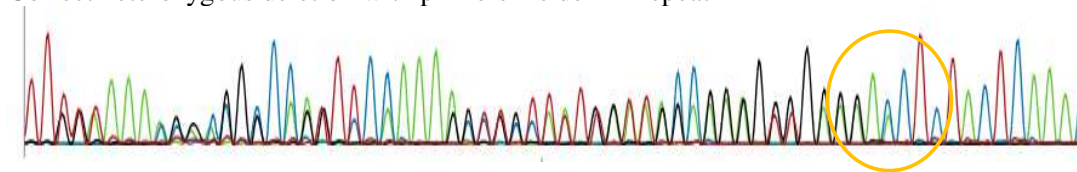
Correct heterozygous deletion with primers inside AT-repeat

**Individual with two short AT-repeats**

Correct heterozygous deletion with primers outside AT-repeat

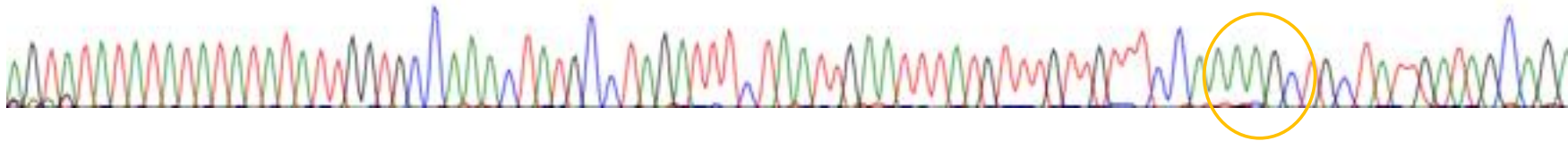


Correct heterozygous deletion with primers inside AT-repeat

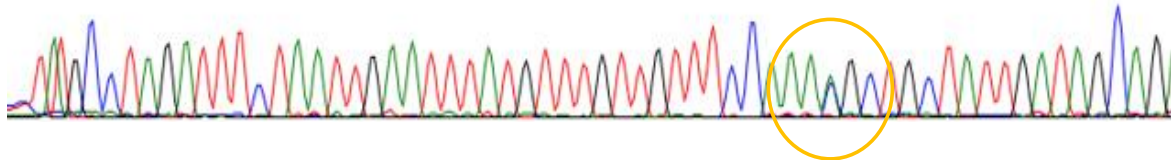


Sequence traces for MME: c.440-2A>C**Individual with one short and one long AT-repeat**

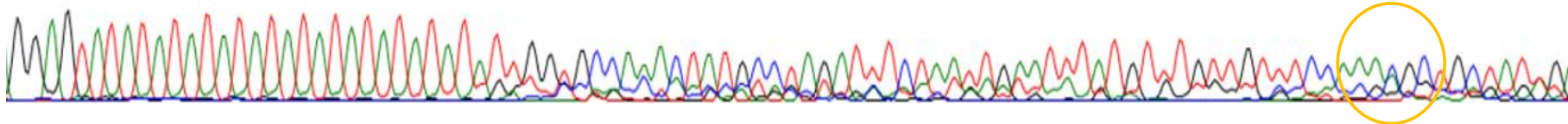
False wildtype with primers outside AT-repeat



Correct heterozygous missense variant with primers inside AT-repeat

**Individual with two long AT-repeats**

Correct heterozygous with primers outside AT-repeat



Correct heterozygous with primers inside AT-repeat

