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.

II OIGCK	arrows and pri	mer manneer	<u>s</u> .							
				<u>E5</u> F						
92661	$\tt CATCACTGTTCATAAAAAAAAAAAAAAAAAAAAAAAAAA$									
92761	AATCTGGTAAGGGGTTTGGTTTTTCCTATAATGTTTTGGAACTGGGGGGGAGAAATGAATG									
92861	CTTTGCAAATGTTAATTACTGCAAATGAGCAATTATGTTTGCATAGTGCAAATGAGCAATTATGTTGATGCATTTTATTAAATGTCCTATTTCAG ATGTC									
92961	Exon 5 CTTCAAGAACCCAAAACTGAAGATATAGTAGCAGTGCAGAAAGCAAAAGCATTGTACAGGTCTTGTATAAATGAATG									
		T-repeat		F2 MME: c.440-2A>C						
93061	TTAGGAGTATATATATATATATTGGTGCCAAACTATGCCTAGATTTCTAATTGAATTTATGTTTGTT									
	1 MME	c.467del Exo	n 6							
93161	GGAGAACČTCTA	CTCAAACTGTT	ACCAGACATATATGGGTGGCCAGT	AGCAACAGAAAACTGGGAGCAAAAATATGGTAAGGCAATTTTCCTACTAAAAA						
				R2₄R1						
93261	AGAAATTTCCAT	GTAAAATCTAT	GTTATAATATCATTAGTGAACTAAA	AGCTTCTAAAGATATATTTTATCTTTTATTGCTTTAG <mark>GTGCTTCTTGG</mark> ACAGC						
	_	Exc	on 7							
93361	TGAAAAAGCTAT	IGCACAACTGA	ATTCTAAATATGGGAAAAAAGTCC:	FTATTAATTTGTTTGTTGGCACTGATGATAAGAATTCTGTGAATCATGTAATT						
93461	CAT GTAAGTTTG	IGTGTCAAATA	ACTAAAGTTACCTTTAAATTGTAA:	PAAAACTTCCACATACATTGTTGTTATTGATATTTAGAAATAATGCTATTTTC						
				◆ E7R						
93561	AATTGAATTGAA	FAAGCAAGGAG	GTAGTATATCCATGCTCTGTCCCTA	AATACCTGGAGTTCCAATTTTGAAAGTATTTTCTTAATGCTGTTTTCGACATT						
00664										
93661	CACCCCTTGCTT	CTGCTACCTCT	GTTTTAGATACCTCTCCCTTCACAA	AAACAAATGATAAGAAGGGCTCCAGGCCCCTCCTATTCAGAAAAAGTTCCTAA						
Primer r	name Dire	ection Pr	imer sequence							
Primers	situated outside th	e AT-repeat								
F1	5′	G	GGAGGAAATGAATGTACCTCCAGA	Standard primare at Talamark Hospital Trust						
R1	3'	G	CTGTCCAAGAAGCACCTAAAGCA	Standard primers at Telemark Hospital Trust						
E5F	5′	Α٦	AATCTGGTAAGGGGTTTGGT	Standard primers at the Medical University of Vienna. For more						
E7R	3'	TO	GTTTTCGACATTCACCCC	information see also Auer-Grumbach et al., The American Journal of Human Genetics 2016, 99:607–623						
Primer situated inside the AT-repeat										
F2	5,	•	CAAAAGCTGCTATTGATAGC							
R2	3'		CAAGAAGCACCTAAAGCAATA							

Supplemental material

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

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

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

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

Fragment Analysis (FLA)

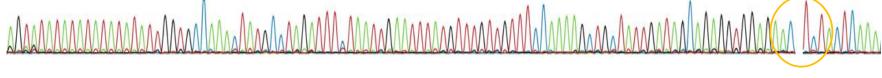
Electrophoretic length separation of the PCR products was performed on 3130XL Genetic Analyzer (Life Technologies). Data were analyzed by GeneMarker v. 1.85 (SoftGenetics LLC).

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

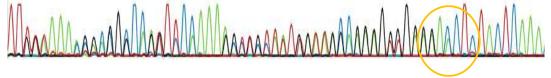
Sequence traces for MME: c.467del

Individual with one short and one long AT-repeat

False homozygous deletion with primers outside AT-repeat

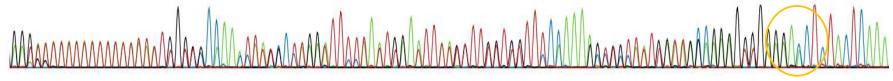


Correct heterozygous deletion with primers inside AT-repeat

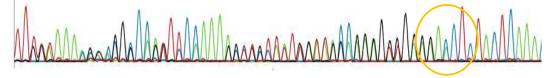


Individual with two short AT-repeats

Correct heterozygous deletion with primers outside AT-repeat



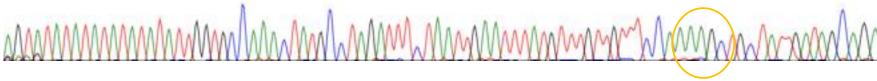
Correct heterozygous deletion with primers inside AT-repeat



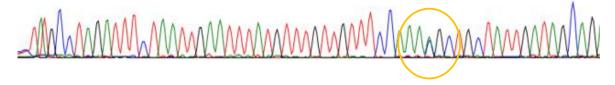
Sequence traces for *MME***: c.440-2A>**C

Individual with one short and one long AT-repeat

False wildtype with primers outside AT-repeat

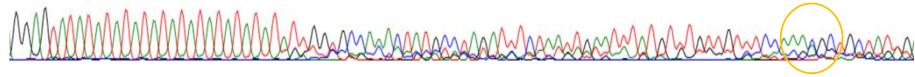


Correct heterozygous missense variant with primers inside AT-repeat



Individual with two long AT-repeats

Correct heterozygous with primers outside AT-repeat



Correct heterozygous with primers inside AT-repeat

