

## Supplementary Material

### A family with autism and rare copy number variants disrupting the Duchenne/Becker muscular dystrophy gene *DMD* and *TRPM3*

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#### Methods

##### Thermocycling conditions for quantitative PCR

The qPCR cycles were as follows: 95°C 3 minutes; 45 cycles of 95°C 30 seconds, 56°C 30 seconds, 72°C 30 seconds; 95°C 1 minute; 55°C 1 minute; 81 cycles of 55-95°C for 10 seconds with set point temperature increased by 0.5°C after cycle 2.

##### Thermocycling conditions for long-range PCR

BIO-X-ACT thermocycling conditions were: 94°C 2 minutes; 10 cycles of 94°C 10 seconds, 60°C 30 seconds, 68°C 8 minutes; 25 cycles of 94°C 10 seconds, 60°C 30 seconds, 68°C 8 minutes + 10 seconds/cycle; 68°C 10 minutes.

SequalPrepTm Long thermocycling conditions were: 94°C 2 minutes; 10 cycles of 94°C 10 seconds, 60°C 30 seconds, 68°C 8 minutes; 25 cycles of 94°C 10 seconds, 60°C 30 seconds, 68°C 15 minutes + 20 seconds/cycle; 72°C 5 minutes.

##### Fluorescence *in situ* Hybridization methods

Chromosome slides for metaphase and interphase FISH analysis were obtained from EBV transformed B-lymphoblastoid cell lines. The cells were cultured in RPMI-1640 supplemented with 10% foetal bovine serum (FBS) and 1% L-Glutamine (Sigma) at 37°C in a 5% CO<sub>2</sub> incubator. One hour before harvesting they were treated with Colcemid (Gibco BRL) at a final concentration of 0.2 µg/ml. They were then resuspended in hypotonic solution (0.0075 M KCl) at 37°C for 5 minutes and fixed in three changes of 3:1 methanol: acetic acid. For fibre-FISH analysis, the cells were resuspended to a density of 2 x 10<sup>6</sup> cells/ ml in 1 X PBS. Ten µl of this solution was dried on a microscope slide. The slides were assembled in a cover-plate holder (Shandon), and the cells were lysed using a sodium hydroxide solution (5 volumes 0.07M NaOH with 2 volumes ethanol) and then fixed with methanol.

The hybridization procedure followed a standard protocol. BAC and fosmid probes were obtained courtesy of Wellcome Trust Sanger Institute and labeled by nick-translation (Abbott Molecular). Following labeling, the probes were ethanol precipitated in a mix of Salmon testis DNA (Gibco BRL), Escherichia coli tRNA (Boehringer) and 3M sodium acetate. They were then dried on a heating block at 60°C with a 50X excess of Human Cot-1 DNA (Invitrogen) and resuspended at 20 ng/μl in hybridization solution (50% formamide, 10% dextran sulphate, 2x SSC). The probes were denatured at 72°C for 5 minutes and pre-annealed at 37°C for 30 minutes, before being applied to the denatured slides. The slides were denatured in 70% formamide at 70°C for 2 minutes, quenched in 2X SSC at 4°C and then dehydrated in an ethanol series. Following hybridization, the slides were washed in 50% formamide at 42°C for 10 minutes and 2X SSC at 42°C for 5 minutes.

Probes (genomic locations indicated in Supplemental Fig. 1) were labelled with either with Biotin-16-dUTP (Roche) or Digoxigenin-11-dUTP (Roche) biotin or digoxigenin and detected with Texas Red-conjugated Streptavidin (anti-biotin) (Molecular Probes) or Fluorescein anti-Digoxigenin antibody (Roche), respectively. The slides were mounted with Vectashield (Vector Laboratories) containing 4', 6-diamidino-2-phenylindole (DAPI) for chromosome counterstaining. Image capture and analysis were carried out on a CytoVysion system (Genetix) consisting of an Olympus BX-51 epifluorescence microscope coupled to a JAI CVM4+ CCD camera.

**Supplemental Table 1:** Sequences of PCR primers used for validation of *DMD* duplication and *TRPM3* deletion in AGP family 3019.

DMD33-F	CTTCATCAAGTTCCTTGGGATT
DMD33-R	AGTGAAGTCTGAAGTGAAATGG
DMD32-F	TGTTCCACACTCTTGTTCCTCA
DMD32-R	CCATGAAGTTTCGATTATTCCAG
DMD31-F*+	TATACCTGTGCAACATCAATCTG
DMD31-R	GACAAGTCATGAGATCAGTTTAG
DMD30-F	GCAATATAAGCTGCCAACTGC
DMD30-R	GCTTGAACAGAGCATCCAGTC
DMD44-F	CCCAATTCTCAGGAATTTGTGT
DMD44-R	CTTTTACCTGCAGGGGATTT
ASS-F	CACTCAGACACACTGCCTACC
ASS-R	CTTCACACTAGCAATGGCAAAG
intron_44A-FCATCTGTTAATACGGCTGACACA	
intron_44A-R*	TCCCTGTTTCATAATGTGAGGTG
intron_44B-FCAGAAGCACAAAGCATTCAT	
intron_44B-R**	TGTGAATTGGGCGATAAATAAA
intron_44C-F	ATCCCTCAGCCTCTCATAACAGA
intron_44C-R	TTTGATACAGCAAGAACATAAGGA
TRPM3-F#	GAGGTAGTAATAAGAGGCAC
TRPM3-R#	GCAACCTCATCTATTAAGCTG
TRPM3-seq+	GTACAATTGTAATGGAAGATGG

Primers used for qPCR unless otherwise stated. \*, used for qPCR and LR-PCR; #, used for LR-PCR but not qPCR; +, used for Sanger sequencing of junction fragment; ASS, alternative start site.

**Supplemental Table 2:** *TRPM3* and *MIR204* sequencing primers and conditions.

Exon	Primer	Sequence	Annealing (°C)	[Mg <sup>2+</sup> ] (mM)
1	F	GGCTGTCCCTCTTCTTCAAA	60	1
	R	ATTTAGGCTCCCCAAAGGT		
Alt 1	F	ACAGAGCCAGTACTGCATG	60	2.5
	R	CTTTTGCGATCCTCCTGGC		
2	F	ATTGCCTCAAAGATGTCCAG	55	2.5
	R	TAGTGTTTATGTGTGAAATAATC		
3	F	AGATTCTCCAGCTGGTTGC	60	2.5
	R	TGGCAGAGTGGAGATTAGGC		
4	F	GTGATAACCAGCAACTATGG	60	2.5
	R	ATGAACTCTGTCTAATGAAGG		
5	F	CTCGGGAGAAACCATTACCA	60	2.5
	R	TTTTCCAAGATACCATTCCCTT		
6	F	TCCTCTCATTCTAAGCTCTG	60	2.5
	R	AGAAGAAGATGTAGACTGTAC		
7	F	TCTCTGTTCTTCACCACAGC	60	2.5
	R	ATCCCTTCATATGTTGCTACC		
Alt 7a	F	TGAATGTCAGAAGAGATAGAG	50	2.5
	R	TGGATGATGCTCTACATAGG		
Alt 7b	F	TTCTCTGAAGTTACCAACCTG	60	2.5
	R	ACCCAATGTGATCTCGATTGT		
8	F	TGTCTTAGTACTTAGGAGAG	55	2.5
	R	CAGAAATAACTCCTATCTAAAG		
9	F	GTAACGTGCTTACAGGAGAG	55	2.5
	R	TTCTTGTTATGTGTGCTTACC		
10	F	TTAATGGCAGAGGGATGGAG	60	1
	R	GCAATCGTTTTCTGAGCTGAC		
11	F	ACCAGATGTCAAAGGCATCC	60	1
	R	CGGGAGAAGGGAAGAGAATC		
12	F	CAAGGTCCATCTTAATGAACTGC	60	2.5
	R	GAAATGAGTGGCGCCTGATA		
13	F	GCGAGAGTATCCAGGTTTCG	60	2.5
	R	AGTTCACTCTCAGCGCAAT		
14	F	TAGGACTCTTCTTACCTTTATG	60	1.5
	R	TGCTGTGAGCAACCCTTATG		
15	F	CTGGCCTCGTTCATTTCACT	60	2.5
	R	GTCCATCCTGTGTGTGCATC		
16	F	GACCCATATGAAACACCTACA	60	2.5
	R	TCTGAGGCACAGTCAGGATTT		
17	F	CCCCACAGAGCATATAACAA	55	2
	R	TCCATGATTAACCTGGTGGA		
18	F	TCTAAATCTCTGGGCCTTG	60	2.5
	R	GAACCTTCACTCCTGTGTGTGG		
19	F	TGTCATAAAGTCTGTGTCCTGCT	55	2.5
	R	GCAAAGAAGCAGCTGTGGTA		
20	F	GCTCAGTCGGACTTTTACAGC	60	2.5
	R	ACACCTCTAGGGCCATCTCA		
21	F	CACTGGCTTCAACAGACCAA	60	2.5
	R	TGTTTCCTCCAAGATAGCAAGC		
22	F	TCAATTTGGCTTGTTTGGG	60	1
	R	GAAACTTCCAGACTTGGTTGG		
23	F	GCAAACATCTTGCTGGTCAA	60	1
	R	CCAAGCCTTCAGAGCTGTCT		
24	F	AAGGAATCTAGGCCCTTTTC	60	2.5
	R	TCTCATAAGCTCCATGATTTCTCA		
25	F1	CAACCCCTCTCAACCTGTGA	60	1
	R1	GGTGTAGGCTCAGGGACCTT		
	F2	CAACCTTAATGCCCGTATG	60	2.5
	R2	TCTGGGAGGTGGGTGTAGTC		
	F3	AACACTTGCACCCACAGACA	60	2.5
	R3	TTTGGCCTCATTCTCCTCAC		
	F4	CCTGGAGGTCTTGGAGACAA	60	2.5
	R4	GCCTTCTGTGGCGGATATTA		
F5	CCAGGAAGAGCTTCTCCATC	60	1	
R5	TCATGGCTTTCTGCTGTGAC			
MIR204	F	TGGATGAAGTTCTACTATGAC	60	2.5
	R	TGATCGTGTATCCATAGGAC		

Exon 7 amplified by 7bF and 7R, and then sequenced with those and 7bR and 7F.

