Supplemental Materials

MPEG1/ Perforin-2 mutations in human pulmonary nontuberculous mycobacterial infections

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Supplemental Table 1: Clinical features of patients with *MPEG1* variants

Patient Code:	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**
***MPEG1* mutation (nucleotide):**	c.217A>G	c. 946C>T	c.1192C>T	c.1213C>A
***MPEG1* mutation (amino acid):**	p.T73A	p.P316S	p.Q398X	p.P405T
***MPEG1* mutation allele:**	heterozygous	heterozygous	heterozygous	heterozygous
Year of birth	1945	1929	1956	1951
Sex	Female	Female	Female	Female
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian
Deceased	N	N	N	Y, 2007
Cause of Death	N/A	N/A	N/A	progressive lung disease
Main infectious diagnosis	PNTM	PNTM	PNTM	PNTM
PNTM Organism(s)	*M. avium, M. intracellulare*	*M. avium complex, M. gordonae, M. immunogenum*	*M. avium, M. intracellulare*	*M. avium complex*
Main Additional pulmonary organisms	*Aspergillus* species, *Pseudomonas aeruginosa, Achromobacter* species	*Pseudomonas aeruginosa, Stenotrophomonas maltophilia*	*Aspergillus* species, *Pseudomonas* species, *Achromobacter* species	*Aspergillus, Pneumocystis jiroveci, Bordetella petrii*
Age at PNTM diagnosis (years)	44	55	40	49
Age at first symptoms (years)	ND	ND	21	41
Bronchiectasis	Y	Y	Y	Y
Additional Lung Disease	Left lower lobe lung resection			Right side pneumatocele; Chronic obstructive pulmonary disease
Additional Infection History	Right fungal otitis externa - *Scedosporium apiospermum,* C*andida parapsilosis* (consistent with skin flora)	Panuveitis - *Brevundimonas* species; Empyema necessitans - *P. aeruginosa;* Culture-negative endocarditis	Oral candidiasis (medication related?); Multiple pneumonias and upper respiratory infections since her 20s	*B. petrii* grown from autopsy spleen culture

Scoliosis	N	Y - thoracolumbar, convex right; 30 degrees T12-L3	N	ND
Pectus Excavatum	N	Y	N	ND
Body Mass Index	20.5	17	24	18
Ciliary Beat Frequency	8.0 Hz	5.6 Hz	8.4 Hz	ND
Smoking History	Y - 4 years, quit	N	N	Y - 7 years, quit
Other medical history	Breast cancer s/p lumpectomy; Type 2 Diabetes Mellitus; Osteopenia; Allergic Rhinitis	Chronic Lymphocytic Leukemia treated with chemotherapy and splenectomy, in remission; IgG deficiency, on IVIG	Lupus nephritis and cerebritis; Osteopenia; Vitiligo	Osteopenia
Additional Mutations	*CLEC4D* heterozygous; c.62C>T, p.S21L	None notable	*CFTR* heterozygous; c.509C>A, p.R170H and intronic c.4243-36delT	ND
Sample Code (1)	CNML012713B	CJF_20	CNML012629A	n/a

N = no, Y = yes, PNTM = pulmonary nontuberculous mycobacteria, ND = no data.

Ciliary beat frequency measured in hertz (Hz), reported in Fowler et al (2)

Supplemental Table 2: *MPEG1* mutations identified in PNTM patient cohort

Patient	1	2	3	4
Nucleotide Change	c.217A>G	c.946C>T	c.1192C>T	c.1213C>A
Amino Acid Change	p.T73A	p.P316S	p.Q398X	p.P405T
EUR allele count/total alleles	220/73354	1107/72774	5/73354	193/73350
EUR frequency	0.00299	0.01521	0.00006	0.00263
EUR p-value	0.9028	0.6644	8.53 e-16	0.8297
ALL frequency	0.00234	0.02189	0.00005	0.00167
ALL homozygotes	2/60385	65/59840	0/60382	1/60377
GERP	5.41	5.53	5.73	5.73
Polyphen-2	probably damaging	benign	NA	probably damaging
SIFT	deleterious	tolerated	NA	deleterious
CADD PHRED	20.1	15.17	20.9	21.1

EUR = European (Finnish and non-Finnish) population data from ExAC (Exome Aggregation Consortium), allele count/total alleles = number of alleles with the variant / number of total alleles sequenced, ALL = population data from all ExAC cohorts, homozygotes = number of homozygotes seen per number of ExAC subjects. GERP = Genomic Evolutionary Rate Profiling, SIFT = Sifting Intolerant From Tolerant, CADD PHRED = Combined Annotation Dependent Depletion PHRED score.

Supplemental Table 3: Loss of Function variants across the *MPEG1* gene

Chr	Pos	Protein Consequence	Transcript Consequence	Annot-ation	Flags	Allele Count	Allele Number	Number of Homo-zygotes	Allele Frequency
11	58979180	p.Gln387Ter	c.1159C>T	stop gained	LoF flag	1	120768	0	0.00000828
11	58979763	p.Tyr192Ter	c.576C>A	stop gained	LoF flag	1	120766	0	0.00000828
11	58980056	p.Glu95Ter	c.283G>T	stop gained	LoF flag	1	120770	0	0.00000828
11	58980187	p.Trp51LeufsTer32	c.151dupT	frame-shift	LoF flag	1	120770	0	0.00000828
11	58979745	p.Leu199AlafsTer36	c.593_594insA	frame-shift	LoF flag	1	120758	0	0.000008281
11	58979816	p.Leu175GlyfsTer4	c.519_522dupGGAA	frame-shift	LoF flag	1	120764	0	0.000008281
11	58979857	p.Ile161SerfsTer5	c.481delA	frame-shift	LoF flag	1	120756	0	0.000008281
11	58979884	p.Val152Ter	c.454delG	frame-shift	LoF flag	1	120754	0	0.000008281
11	58979922	p.Leu139ProfsTer3	c.416delT	frame-shift	LoF flag	1	120762	0	0.000008281
11	58979971	p.Ser123GlnfsTer9	c.366_367delTT	frame-shift	LoF flag	1	120764	0	0.000008281
11	58979971	p.Ser123PhefsTer10	c.367dupT	frame-shift	LoF flag	1	120764	0	0.000008281
11	58979117	p.Gly408HisfsTer3	c.1218_1221dupCACT	frame-shift	LoF flag	1	120746	0	0.000008282
11	58979048	p.Tyr430Ter	c.1290delC	frame-shift	LoF flag	1	120714	0	0.000008284
11	58979712	p.Val209AlafsTer28	c.626delT	frame-shift	LoF flag	1	120714	0	0.000008284

11	58979030	p.Arg437Ter	c.1309C>T	stop gained	LoF flag	1	120700	0	0.000008285
11	58978979	p.Gln454Ter	c.1360C>T	stop gained	LoF flag	1	120690	0	0.000008286
11	58980271	p.Ser23Ter	c.68C>A	stop gained	LoF flag	1	120530	0	0.000008297
11	58978537	p.Leu601SerfsTer2	c.1801delC	frameshift	LoF flag	1	120384	0	0.000008307
11	58980292	p.Trp16Ter	c.47G>A	stop gained	LoF flag	1	120038	0	0.000008331
11	58978652	p.Gln563Ter	c.1687C>T	stop gained	LoF flag	1	119358	0	0.000008378
11	58980068	p.Gln91Ter	c.271C>T	stop gained	LoF flag	2	120770	0	0.00001656
11	58979091	p.Tyr416Ter	c.1248C>G	stop gained	LoF flag	2	120736	0	0.00001657
11	58978552	p.Pro594HisfsTer20	c.1779_1786delCCCACCTT	frameshift	LoF flag	2	120314	0	0.00001662
11	58980158	p.Arg61Ter	c.181C>T	stop gained	LoF flag	3	120770	0	0.00002484
11	58978719	p.Leu540ArgfsTer2	c.1619delT	frameshift	LoF flag	3	118930	0	0.00002522
11	58979736	p.Tyr201Ter	c.603T>G	stop gained	LoF flag	4	120748	0	0.00003313
11	58979950	p.Ser130ProfsTer7	c.388delT	frameshift	LoF flag	5	120770	0	0.0000414
11	58979147	p.Gln398Ter	c.1192C>T	stop gained	LoF flag	6	120764	0	0.00004968
11	58979221	p.Met373Ter	c.1117delA	frameshift	LoF flag	9	120770	0	0.00007452
11	58978659	p.Gly560AlafsTer43	c.1679delG	frameshift	LoF flag	11	119014	0	0.00009243

11	58979894	p.Arg149Ter	c.445C>T	stop gained	LoF flag	13	120756	0	0.0001077
11	58978776	p.Gly521GlufsTer21	c.1562delG	frame-shift	LoF flag	27	119874	0	0.0002252

Data exported from Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/) (3), accessed 01/16/17.

Chr = chromosome, Pos = position, Annotation lists the predicted functional consequence of the variant, Allele count = number of alleles with the variant, Allele number = total number of alleles sequenced across all populations. LoF = loss of function.

Supplemental Table 4: Sequencing Primers for *MPEG1*

Primer Location	**Primer Sequence**	**Captures**
457F 3485R	5' CTCTTCAGGCCCTGTCAGCTGTGCTCCAGAC 3' 5' CCCTCTCCCCAATCCCAACCTTATCCATGTAACAG 3'	Whole gene
1056F 3485R	5' GTTTTCCGCTTCATCATCTGCTTCTGTTTTCTCCA 3' 5' CCCTCTCCCCAATCCCAACCTTATCCATGTAACAG 3'	Whole gene
284F 3445R	5' TATGTTCCTTGGCCCCTGTCTGCTGTTGATAAATC 3' 5' TTTCCTGGCCCAGAGACCTAAACAAGAAGTCACGA 3'	Whole gene
1300F 1874R	5' AGGGGGCTGGGACAATCTGCGGAATG 3' 5' CAGAGGCGGTCACGGCACTACGACTGC 3'	c.217A>G
1781F 2410R	5' GTCGACGCTGGGGCTGCTCTTATTCAGGA 3' 5' CGGGGAGTAGCCAGAGGGGCAGGAGAAAT 3'	c.946C>T c.1192C>T
2286F 2747R	5' CTTTCGGTGGGGTTTATCAGGAATGCACTCAGC 3' 5' ACCCGCCAAAGGGGACCGCAAACC 3'	c.1213C>A

Note: Numbering for “Primer Location” begins at 1000 bases downstream of the mRNA start

Supplemental Table 5: Oligonucleotides used in CRIPSR design

Name	**Sequence**	**Application**
gRNA T73 Target 1	AGATTGTCCCAGCCCCCTCC	Vector Targeting Mpeg1 T73
gRNA T73 Target 2	AATTGACTTACTCCAACTGC	Vector Targeting Mpeg1 T73
gRNA P316 Target 1	TGGCAAGTCAGGTAGCATGT	Vector Targeting Mpeg1 P316
gRNA P316 Target 2	GAAGAAGGTGTCAAAGACAG	Vector Targeting Mpeg1 P316
gRNA P398/P405 Target 1	TGCACCGGGGAGTAGCCAGA	Vector Targeting Mpeg1 P398/405
gRNA P398/P405 Target 2	GTTATCCCAGATCCACGAGG	Vector Targeting Mpeg1 P398/405
ssDNA T73A	AGGTTGCTCTGTTTCTGGGGAATGGTGAAGATTTCATCAGGGATGATATACTGTCCATCCTCTGCTGTTCTGCAGTTGGAGTAAGTCAATTCCATAACTCGTCCCATGTCCACATTCCGC (Underline T73A)	Donor DNA Template
ssDNA P316S	ACCAGGGGGCCTCCGAAGTCAGGTAGCATGTTGGAGTTGATGAAGAAATGCAGCGGCAGGCCAGAGCGGT (Underline P316S)	Donor DNA Template
ssDNA Q398X	CAACTTCTCTTTCGGTGGGGTTTATCAGGAATGCACTCAGCTCTCAGGGAATAGGGATGTCCTCCTCTGCTAAAAGTTGGAGCAGAAGAATCCACTCACTGGTGATTTCTCCTGCACCTCTGGCTACTCCCCGGTGCACCTGTTATCCCA (Underline Q398X)	Donor DNA Template
ssDNA P405T	TTTATCAGGAATGCACTCAGCTCTCAGGGAATAGGGATGTCCTCCTCTGCCAAAAGTTGGAGCAGAAGAATACACTCACTGGTGATTTCTCCTGCCCCTCAGGATATTCGCCAGTACACCTGTTATCCCAGATCCACGAG (Underline P405T)	Donor DNA Template
Sequencing Primer T73	F - TCCAGCAGAGGCGGTCACG R – ACCATCCTCTTCTGGGCAGCG	Amplify flanking region for analysis
Sequencing Primer P316	F - CCGCCTCTGCTGGACTTG R – TGGAAAGTAGCCGGCTGG	Amplify flanking region for analysis
Sequencing Primer P398/P405	F - TGGGTGCTGGCTGAAGC R - GGAAACTGCTGTGAAGCGC	Amplify flanking region for analysis

Supplemental Table 6: Significant interactions by one-way ANOVA with Tukey post-hoc test

Figure 2A:

Hour:	**Comparison**	**Significance: (Tukey Post-Hoc Test)**
4	T73A to Age Matched #1	0.017
4	P316S to Age Matched #1	0.034
4	Q398X to Age Matched #1	0.022
8	T73A to Age Matched #1	0.001
8	T73A to Age Matched #2	0.001
8	P316S to Age Matched #1	0.006
8	P316S to Age Matched #1	0.009
8	Q398X to Age Matched #1	<0.001
8	Q398X to Age Matched #2	<0.001
8	P405T to Age Matched #1	0.001
8	P405T to Age Matched #2	0.002
12	T73A to Age Matched #1	0.007
12	T73A to Age Matched #2	0.012
12	P316S to Q398X	0.026
12	P316S to P405T	0.013
12	Q398X to Age Matched #1	<0.001
12	Q398X to Age Matched #2	0.001
12	P405T to Age Matched #1	<0.001
12	P405T to Age Matched #2	<0.001
24	T73A to Q398X	0.014
24	T73A to Age Matched #1	0.015
24	T73A to Age Matched #2	0.023
24	P316S to Q398X	<0.001
24	P316S to P405T	0.002
24	Q398X to Age Matched #1	<0.001
24	Q398X to Age Matched #2	<0.001
24	P405T to Age Matched #1	<0.001
24	P405T to Age Matched #2	<0.001

Figure 2B:

Hour:	**Comparison:**	**Significance:**
1	T73A to Young Control	0.006
2	T73A to Young Control	0.049
4	T73A to Age Matched Control	0.002
4	T73A to Young Control	0.001
6	T73A to Age Matched Control	<0.001
6	T73A to Young Control	<0.001
8	T73A to Age Matched Control	0.004
8	T73A to Young Control	0.001

Figure 2C:

Hour:	**Comparison:**	**Significance:**
4	T73A to Young Control	0.024
8	T73A to Age Matched Control	0.020
8	T73A to Young Control	<0.001
8	Young Control to Age Matched Control	0.029
12	T73A to Age Matched Control	0.020
12	T73A to Young Control	<0.001
24	T73A to Age Matched Control	0.001
24	T73A to Young Control	<0.001

Figure 3:

Hour:	**Comparison:**	**Significance:**
8	WT to Q398X	<0.001
8	WT to P405T	0.003
8	T73A to Q398X	0.003
8	Q398X to P316S	0.000
8	P405T to P316S	0.006
24	WT to Q398X	0.001
24	WT to P405T	<0.001
24	T73A to Q398X	0.005
24	T73A to P405T	0.002
24	Q398X to P316S	0.001
24	P405T to P316S	<0.001

Figure 4A:

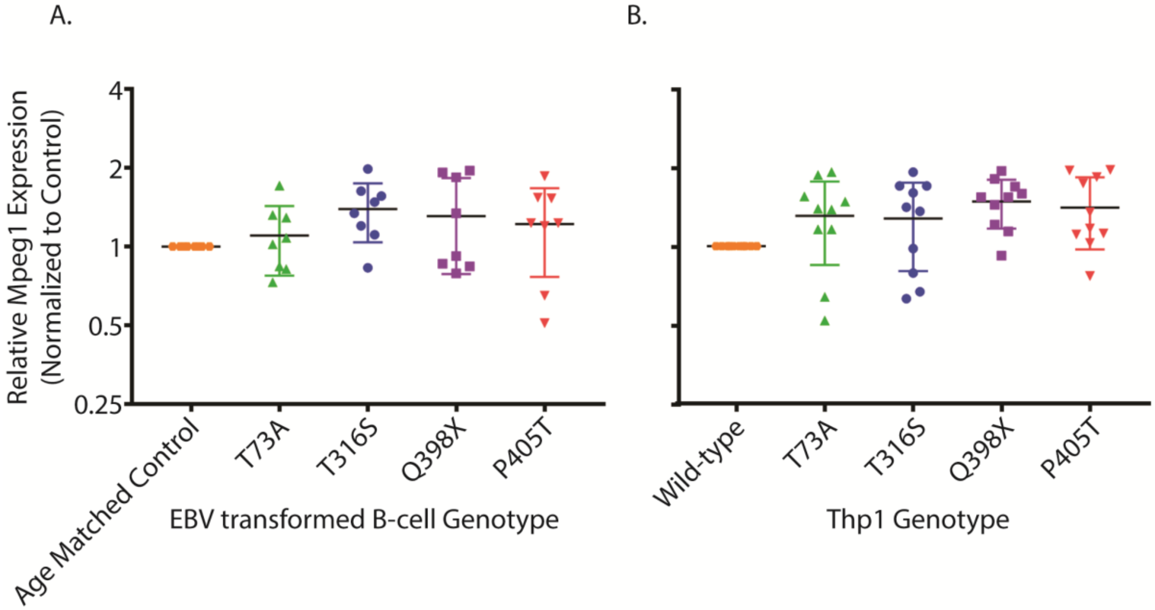
Hour:	**Comparison:**	**Significance:**
4	WT to Q398X	0.003
4	T73 to Q398X	0.011
4	P316S to Q398X	0.003
8	WT to Q398X	0.048
24	WT to Q398X	0.032
24	T73 to Q398X	0.033
24	P316S to Q398X	0.031

Figure 4B:

Hour:	**Comparison:**	**Significance:**
8	WT to Q398X	0.002
8	T73 to Q398X	0.002
8	P316S to Q398X	0.006
8	Q398X to P405T	0.016
24	WT to Q398X	<0.001
24	T73 to Q398X	<0.001
24	P316S to Q398X	<0.001
24	Q398X to P405T	0.001

Supplemental Figure 1. *MPEG1* mutation does not alter expression levels in EBV-transformed B cells and THP1 cells.

EBV transformed patient B-cells (A) and CRISPR/Cas9 mutated THP1 macrophages (B) were harvested in equal quantities while in logarithmic phase. RNA extraction followed by cDNA synthesis was performed followed by qRT-PCR analysis utilizing Mpeg1 probes or GapDH and β-actin as housekeeping genes. Eight biologic replicates were analyzed (as demonstrated by individual data points) with three technical replicates. These graphs are representative of three experimental replicates. Statistical analysis was conducted utilizing one-way ANOVA with Tukey's post-hoc test. P <0.05. No statistical differences were observed.



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