

Figure S1 clinical features of patients. (A) and (B), Deformity of hands and feet of proband. **(C)** Unclose eye of proband. **(D) and (E),** Deformity of hands and feet of proband's brother. **(F),** Unclose eye of proband's brother.

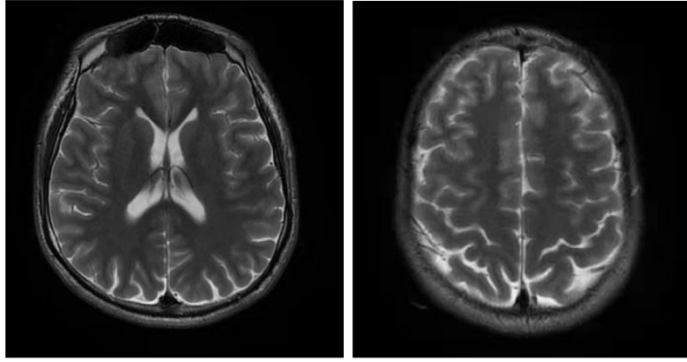


Figure S2 MRI images of proband's brother showed no abnormal sign.

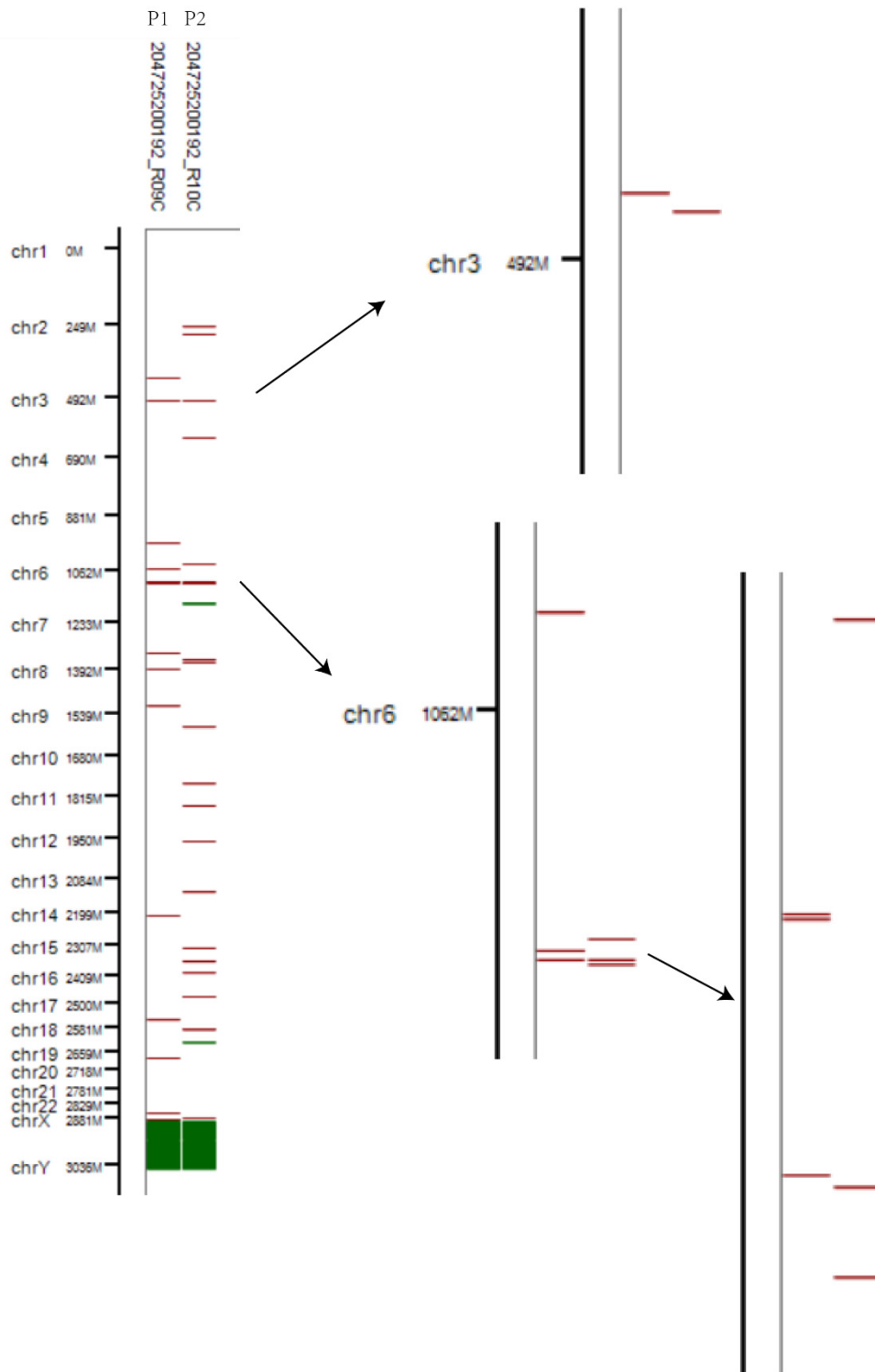


Figure S3 CNV analysis of patients. Both patients from JALS family (P1 and P2) showed no consensus CNVs. Red indicated copy number 0-0.5, green indicated copy number 1.5-2.5.

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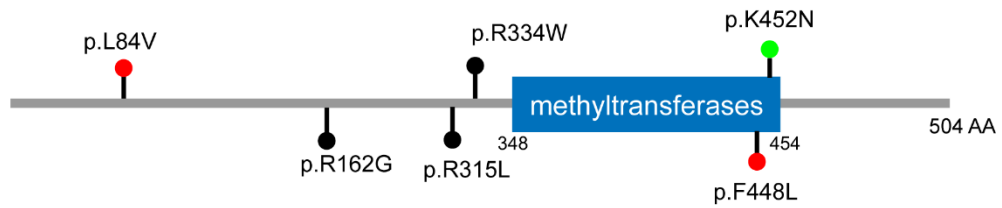


Figure S4 *TRMT2B* variations identified in ALS patients. Green bottom displayed the variation we identified in JALS family, red bottom displayed variations identified within 910 sporadic cases, black bottom displayed variations identified in ALSdb; the methyltransferase domain of TRMT2B ranging from 348V to 454R amino acid in the full-length protein.

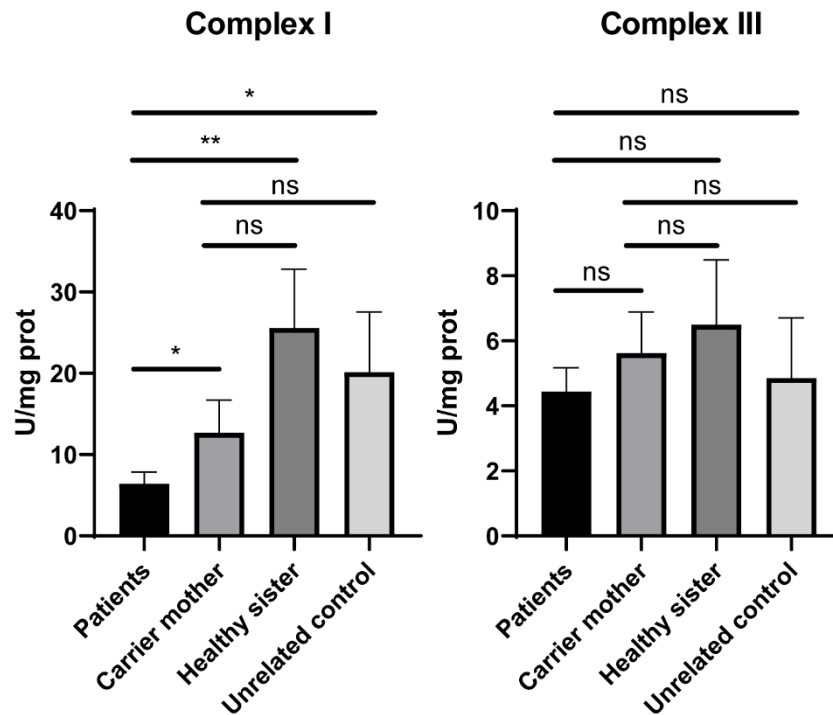


Figure S5 Mitochondrial complex activities among patients and controls. The results showed decreased mitochondrial complex I activities in Patients (XY003.P1 and P2), comparing with Carrier mother (XY003.Mo), Healthy sister (XY003.S1) and unrelated control. There was no difference among individuals in mitochondrial complex III activities

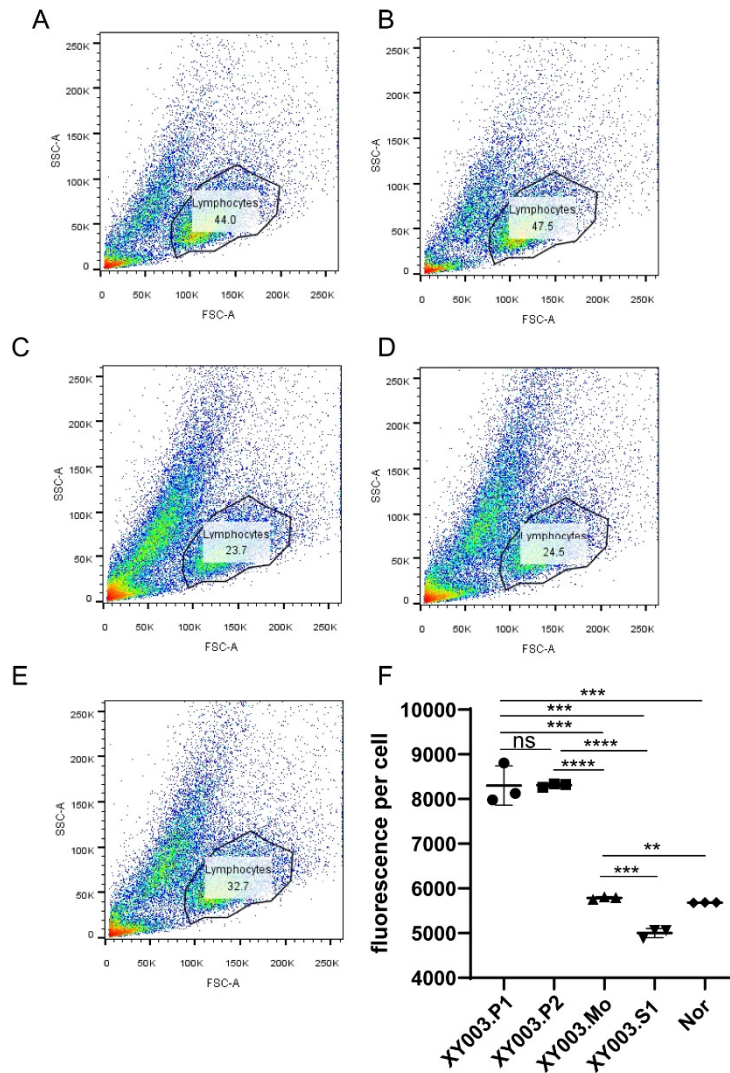


Figure S6 ROS levels tested by flow cytometry. (A)-(E) showed Gating strategy used in the analysis of patients (XY003.P1 and P2), comparing with Carrier mother (XY003.Mo), Healthy sister (XY003.S1) and unrelated control, respectively. **(F)** showed the statistical analysis among diverse data among them.

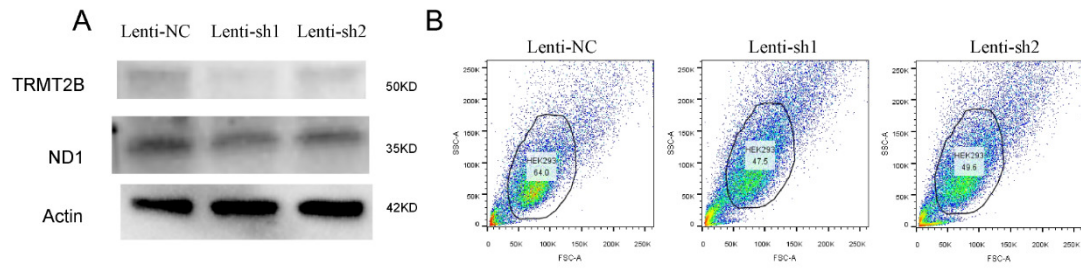


Figure S7 Interfering the expression of TRMT2B in HEK293

(A) By interfering the expression of TRMT2B in HEK293, the expression of ND1 down regulated; (B) showed Gating strategy used in the analysis of NC and Lentivirus interfered cells.

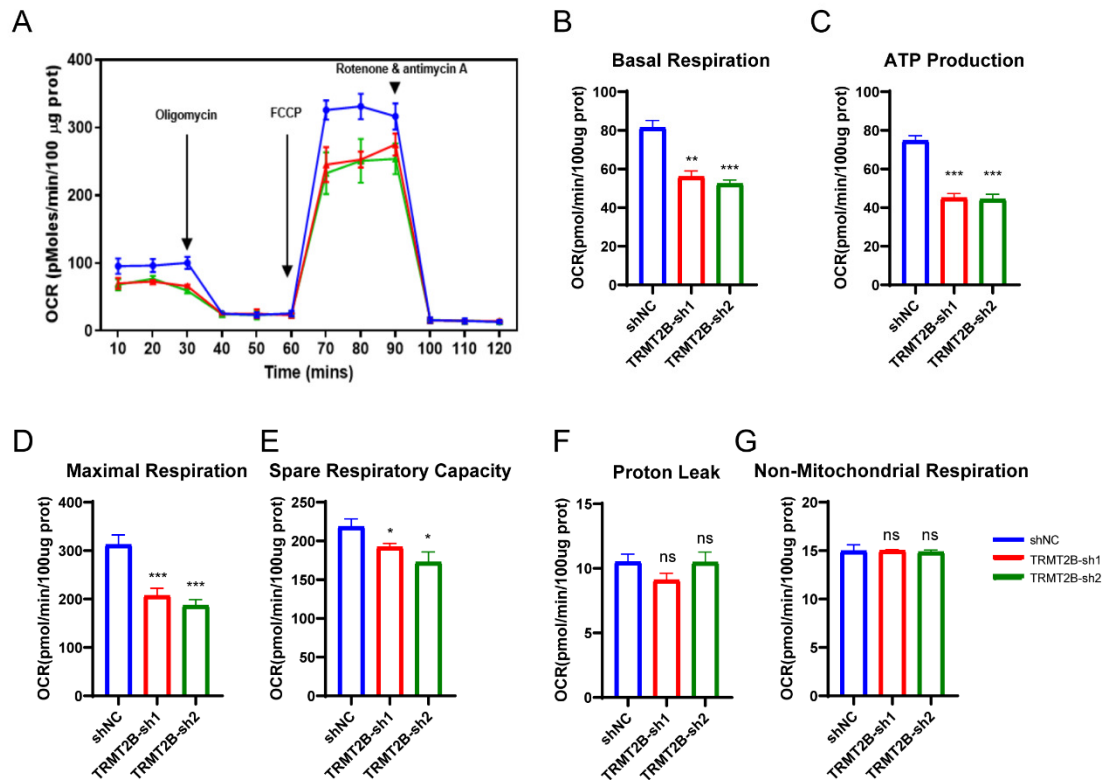


Figure S8 Mitochondrial aerobic respiration

(A) oxygen consumption rate (OCR) of *TRMT2B* interfering cell lines decreased; (B)-(G), after interfering with the expression of *TRMT2B*, the levels of Basal Respiration (B), ATP production (C), Maximum Respiration (D), and Spare Respiration Capacity (E) decreased significantly, and there was no significant difference between the levels of proton leak (F) and non-mitochondrial respiration (G) among groups, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

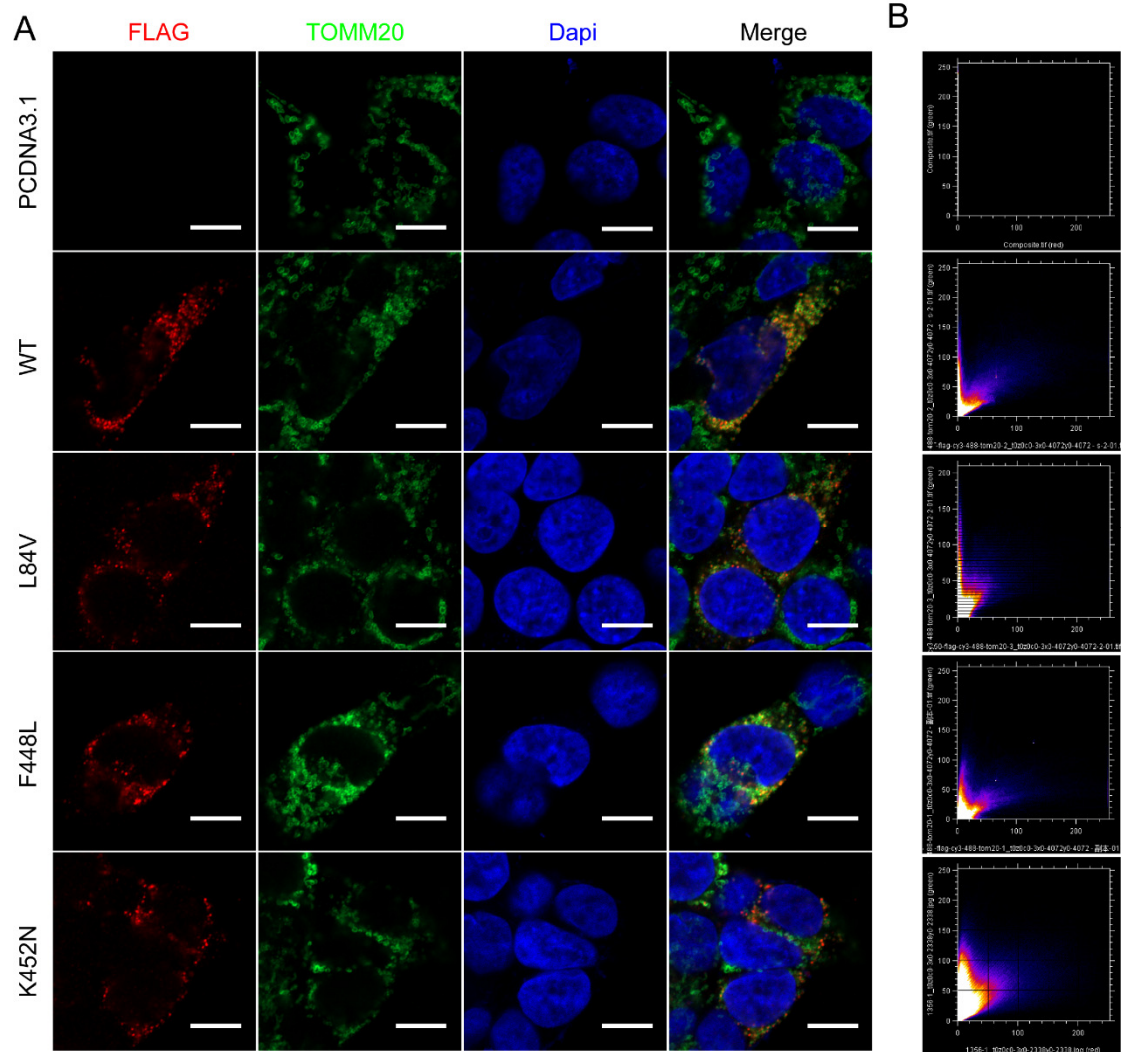


Figure S9 subcellular location of TRMT2B and mutated proteins.

(A) TRMT2B proteins (in red) were co-located within mitochondrial (in green), Dapi (in blue) showed the nucleus; (B) Co-localization analysis by ImageJ- colocalization-finder showed no difference between *TRMT2B* wild type and mutated proteins; the scale was 10um.

Supplementary table S1. Genes excluded in this study

Autosomal dominant						
<i>SOD1</i>	<i>TARDBP</i>	<i>PFN1</i>	<i>ANXA11</i>	<i>SSI8L1</i>	<i>CCNF</i>	<i>ALS2</i>
<i>SETX</i>	<i>FIG4</i>	<i>ERBB4</i>	<i>NEK1</i>	<i>GLT8D1</i>	<i>GRN</i>	<i>ATP13A2</i>
<i>FUS</i>	<i>OPTN</i>	<i>hnRNPA1</i>	<i>KIF5A</i>	<i>CHCHD10</i>	<i>TIA1</i>	<i>DJI</i>
<i>VAPB</i>	<i>VCP</i>	<i>MATR3</i>	<i>DAO</i>	<i>SQSTM1</i>	<i>DCTN1</i>	<i>ERLIN1</i>
<i>ANG</i>	<i>CHMP2B</i>	<i>TUBA4A</i>	<i>GLE1</i>	<i>TBK1</i>	<i>GARS</i>	<i>CACNA1H</i>
<i>ELP3</i>	<i>VRK1</i>	<i>TAF15</i>	<i>DNAJC7</i>	<i>NEFH</i>	<i>hnRNPA2B1</i>	<i>EWSR1</i>
<i>PRPH</i>	<i>C21orf2</i>	<i>C9orf72</i>	<i>ATXN2</i>			
Autosomal recessive						
<i>SIGMAR1</i>	<i>C19orf12</i>	<i>SPG11</i>	<i>SYNE1</i>	<i>OPTN</i>		
<i>X-linked dominant</i>						
<i>UBQLN2</i>						

Note:the *OPTN* gene with both patterns of AD and AR

Table S2 Primers used in plasmids constructions and qPCR

Primers	Sequences (5'-3')
TRMT2B-F	aagtgattcaagccattcg
TRMT2B-R	aaagaggaccagctcac
Actin-F	catcctgcgtctggacctgg
Actin-R	taatgtcacgcacgatttcc
ND1-F	ggctatatacaactacgcaaaggc
ND1-R	ggtagatgtggcgggttttagg
TRM-F	cggaattcgccaccatggcaggccttaagaga
TRM-R	atagtttagcggccgcttactgtcatcgtcatcctgtaatcgatgtc
L84V-F	cctggcaggaaagggtggctgatggttg
L84V-R	cacaacatcagccacctttcctgccagg
N150S-F	tattataccctctcctgtcatcagtggtaccgaaataagtc
N150S-R	gacttatttcggtaaccactgatgacaggagagggtataata
F448L-F	ccatccacacgctagtttggttctgcaagctc
F448L-R	gagcttcaggaaacaaaactagcgtgtggatgg
K452N-F	ctagttttggttctgcaatcctcatggtgaatccact
K452N-R	agtggattcaccatggagattgcaggaaacaaaactag
E477K-F	ctaagaagctcttaggcaagccctttgtcctgcag
E477K-R	ctgcaggacaaagggttgcctaagagcttcttag

Table S3 Antibodies used in this study

Antibodies	Manufacture	Catalog number
TOM20 Polyclonal Antibody	Proteintech	11802-1-AP
TRMT2B Polyclonal Antibody	Proteintech	20767-1-AP
Anti-LC3B Antibody	Abcam	ab192890
MTCO1 Rabbit pAb	Abclonal	A17889
MTCO2 Rabbit pAb	Abclonal	A11522
FAM Rabbit pAb	Abclonal	A1926
β -Actin	CST	3700S
DYKDDDDK Tag	CST	14793
Anti-SQSTM1 / p62	Abcam	ab109012
MT-ND1 Rabbit pAb	Abclonal	A17967
AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson Immuno Research	111005003
AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson Immuno Research	115005003
AffiniPure Goat Anti-Mouse IgG (H+L)-488	Jackson Immuno Research	115545003
AffiniPure Goat Anti-Rabbit IgG (H+L)-cy3	Jackson Immuno Research	111165003

Table S4 Two candidate genes in recessive-pattern analysis

Chr	Start	End	Ref	Alt	Genotype	Depth	HGVS_gene	Func.refGeneWithVer
chr1	146408086	146408086	A	G	hom	61	NBPF12	exonic
chr3	195506746	195506746	G	A	hom	59	MUC4	exonic

