

TBCE Mutations Cause Early-Onset Progressive Encephalopathy with Distal Spinal Muscular Atrophy

Antonella Sferra, Gilbert Baillat, Teresa Rizza, Sabina Barresi, Elisabetta Flex, Giorgio Tasca, Adele D'amico, Emanuele Bellacchio, Andrea Ciolfi, Viviana Caputo, et al.

▶ To cite this version:

Antonella Sferra, Gilbert Baillat, Teresa Rizza, Sabina Barresi, Elisabetta Flex, et al. TBCE Mutations Cause Early-Onset Progressive Encephalopathy with Distal Spinal Muscular Atrophy. American Journal of Human Genetics, 2006, 99 (4), pp.974 - 983. 10.1016/j.ajhg.2016.08.006 . hal-01463875

HAL Id: hal-01463875 https://amu.hal.science/hal-01463875

Submitted on 6 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 TBCE mutations cause early-onset progressive encephalopathy with distal spinal

2 muscularatrophy

- 3 AntonellaSferra^{1,#},GilbertBaillat^{2,#},TeresaRizza¹,SabinaBarresi¹,ElisabettaFlex³,
- 4 GiorgioTasca⁴, AdeleD'Amico¹, EmanueleBellacchio¹, AndreaCiolfi^{1,5}, VivianaCaputo7,
- 5 SerenaCecchetti⁶, AnnalauraTorella7, 8, GinevraZanni¹, DariaDiodato¹, EmanuelaPiermarini¹,
- 6 MarcelloNiceta¹,AntoniettaCoppola9,EnricoTedeschi10,,DiegoMartinelli¹,CarloDionisi-Vici¹,
- 7 VincenzoNigro7,BrunoDallapiccola, ClaudiaCompagnucci¹MarcoTartaglia^{1*},GeorgHaase^{2*},
- 8 EnricoBertini^{1*}
- 9
- ¹GeneticsandRareDiseasesResearchDivision,OspedalePediatricoBambinoGesù,
- 11 00146Rome, Italy;²InstitutdeNeurosciencesdelaTimone, UMR7289CNRSAix-
- 12 MarseilleUniversity,Marseille,France;³DepartmentofHematology,Oncologyand
- 13 MolecularMedicine,IstitutoSuperiorediSanità,00161Rome,Italy.⁴DonCarloGnocchi
- 14 ONLUSFoundation, Rome00135, Italy.⁵CentrodiRicercaperglialimentielanutrizione,
- 15 CREA,00178,Rome,Italy.⁶DepartmentofCellBiologyandNeurosciences,Istituto
- ¹⁶ SuperiorediSanità,00161Rome,Italy.⁷DepartmentofExperimentalMedicine,Università
- 17 LaSapienza,00161Rome,Italy.
- 18 [#]contributedequallytothiswork
- 19 ^{*}contributedequallyastheseniorinvestigatorsinthisproject
- 20
- 21 Correspondingauthors:
- 22 EnricoBertini,MD
- 23 UnitofNeuromuscularandNeurodegenerativeDisorders
- 24 GeneticsandRareDiseasesResearchDivision
- 25 OspedalePediatricoBambinoGesù,
- 26 00146Rome,Italy

- 27 Phone:+390668592104;
- 28 E-mail:bertini@opbg.net
- 29
- 30 GeorgHaase,PhD
- 31 InstitutdeNeurosciencesdelaTimone,
- 32 UMR7289CNRS
- 33 Aix-MarseilleUniversity,
- 34 13385Marseille,France
- 35 Phone:+33673238113;
- 36 E-mail: georg.haase@univ-amu.fr
- 37
- 38 MarcoTartaglia,PhD
- 39 UnitofMolecularGeneticsandFunctionalGenomics
- 40 GeneticsandRareDiseasesResearchDivision
- 41 OspedalePediatricoBambinoGesù,
- 42 00146Rome,Italy
- 43 Phone:+390668593742;
- 44 E-mail: marco.tartaglia@opbg.net
- 45
- 46

47 Abstract

- 48 Wereportonanearly-onsetneurodegenerativeencephalopathywithdistalspinal
- 49 muscularatrophycausedbyTBCEmutations,withIle155Asnoccurringatthe
- 50 heterozygous/homozygousstateinallaffectedsubjects. Clinicalfeaturesresemblethe
- 51 phenotypeof*pmn/pmn*mice(Tbce^{Trp524Gly})andareassociatedwithreducedTBCElevels
- 52 and altered tubulin polymerization. While truncating *TBCE* mutations have been described
- 53 toperturbdevelopmentalprocesses, we documental tered TBCE function as a cause of
- 54 neurodegenerationinhumans.
- 55

57 Tubulinopathiesincludeafamilyofsevereneurodevelopmentaldisorderscausedby mutationsinmultiplegenesencodingtubulinisoforms(*i.e.*, TUBA1A, TUBA8, TUBB2B, 58 59 TUBB3 and TUBB5), with defective neuronal migration and malformation of the cerebral cortexrepresentingmajorfeatures¹. Truncatingmutations in TBCE(tubulin-specific 60 chaperoneE), one of the five tubulin binding co-factors involved in tubulin folding and 61 polymerization, areknown to cause two rareneurodevelopmental diseases, 62 hypoparathyroidism-retardation-dysmorphism(HRD)andKenny-Caffeysyndrome(KCS)². 63 Morerecently, microtubule-disrupting mutations intubuling eneshave been shown to 64 triggerneurodegeneration, asillustrated by TUBA4A and TUBB4A mutations underlying 65 amyotrophiclateralsclerosis³ and hypomyelinatingleukodystrophyassociated with 66 progressivespasticdystonictetraplegia⁴, respectively. Similarly, amissensemutationin 67 *Tbce*hasbeenassociatedwithprogressivedistalmotorneuronopathyinmice⁵, butno 68 69 similarphenotypehassofarbeenreportedinhumans. 70 Weusedatrio-basedwholeexomesequencing(WES)strategytodelineatethe molecularbasisofanovelrecessiveencephalopathyaffectingtwoapparentlyunrelated 71 72 youngsubjectsoriginatingfromtheislandoflschia, SouthernItaly(Fig.1a). Both 73 individualsshoweddevelopmentaldelaywithoutdysmorphiaorgrowthdefects, and 74 manifestedearly-onset, slowlyprogressived istal motorneuropathyresembling distal 75 spinalmuscularatrophy, with bilateral footdrop associated to spasticity and cerebellar 76 ataxia.WESdatawereanalyzedtoidentifygenessharedbytheprobandshaving 77 functionallyrelevantrare/privatevariants(**On-linemethods**),takingintoaccount 78 recessiveinheritancemodels(SupplementaryTableS1). Weidentifiedonlyonegene, 79 TBCE, satisfying these filtering criteria. Subject 1544334 (Pt1) washom ozygous for the c.464T>Amissensechange(p.Ile155Asn),whichwasinlinewiththedocumented 80 consanguinityascertainedinthefamily;subject2518864(Pt2)wasacompound 81 82 heterozygoteforthesameaminoacidsubstitutionandthetruncatingc.1076delCchange

83	(p.Ser359fs*1).Sangersequencingconfirmedbothmutationsandsegregationinboth
84	families(SupplementaryFig.S1).Thesharedgenomicregionencompassingthe
85	c.464T>Achangecovered2.5Mb(chr1:234529552-237060433).Mutationscanofthe
86	TBCEcodingsequenceperformedon14subjectswithsimilarclinicalfeaturesallowedto
87	identifyathirdfamilyfromthesamegeographicalareahavingtwomonozygotictwins
88	homozygousforthec.464T>Achange.Allsubjectsexhibitedearly-onset,slowly
89	progressivespasticataxia, distalspinalmuscularatrophy, cerebellaratrophy, and
90	developmentaldelay(SupplementaryTableS2). Thisphenotypeisreminiscentof the
91	mutantpmn/pmnmousewithprogressivemotorneuronopathy,hindlimbatrophyand
92	reducedbrainsize ⁵ .Inthesesubjects,theneurodegenerativesigns,normal
93	calcium/phosphatemetabolism, and absence of repeated infections (Supplementary
94	Information)clearlydifferfromHRDandKCS.
95	TCBEischaracterizedbyaCAP-GlydomainattheN-terminus,followedbynine
96	tandemlyarrangedleucine-richrepeats(LRRs), and a C-terminal ubiquitin-likedomain
97	(SupplementaryFig.S1).Ile ¹⁵⁵ is a highly conserved residue acrossor thologs
98	$({\bf Supplementary Fig. S1}) located within the LRR domain. A homology model of the TBCE$
99	regionspanningthenineLRRs(residues123-
100	$348) was generated, which fitted well with the established structure of the semotifs {}^{6}, and placed lle {}^{155}i$
	ntothehydrophobiccore
101	stabilizingthesecondLRRmotif(Fig.1b).Theintroductionofthehydrophilicasparagine
102	ispredictedtoperturbdramaticallythestructureofthemotifandtheoverallfoldingofthe
103	LRRdomain.SinceLRRdomainsarefunctionalmodulesthatmediateprotein-protein
104	$interaction, this a minoacid substitution is expected to affect {\sf TBCE} function or the overall {\sf States} and {\sf States} a$
105	${\sf TBCE} structure and protein stability. To verify the latter hypothesis, the level of the {\sf TBCE}$
106	proteinwasevaluatedinprimaryfibroblastsobtainedfromPt1andPt2.Westernblot
107	analysisdocumentedastronglyreducedamountofTBCEcomparedtocontrolcells(Fig.
108	1c),suggestiveofrapiddegradationoftheTBCEmutant.Ofnote,reductioninTBCE

109	levelswasmorepronouncedinfibroblastsfromPt2,whowascompoundheterozygotefor
110	thelle155AsnandSer359fs*1changes.Inthesecells, TBCEmRNApoolwasreducedto
111	halfofnormal(asshownbyreal-timeqRT-PCR)(Fig.1d),andthemutantallelecarrying
112	theframeshiftvariantwasundetectablebySangersequencingofretro-transcribedRNA
113	(datanotshown), indicating nonsense-mediated mRNA decay. A
114	$similar reduction in Tbcelevels was documented infibro blasts ({\bf Fig.1e}) and moton eurons ^7 of the {\it pmn/p}$
115	mnmouse.Remarkably,TBCElevelshadbeenreportedtobevirtuallyabsentinHRDandKCS ⁸ .
116	$\label{eq:transform} \textbf{TBCE} is required for the folding of α-tubulin, α/β-tubulind imerization and α-tubulin, α/β-tubulind imerization and α-tubulind imerization and α-tub$
117	subsequentheterodimerpolymerization into microtubules, which are major components of the neur
118	onalcytoskeleton9.ToexploretheimpactofthetwoTBCEmutationson
119	$\label{eq:constraint} microtubulepolymerization, we first determined the levels of total, soluble and polymerized$
120	α -tubulininpatientandcontrolfibroblasts, using biochemical fractionation. The level of
121	total α -tubulinwas not affected. By contrast, the amount of polymerized α -tubulinwas
122	significantlyreducedcomparedtocontrolcells(Fig.2a), inline with what occurred intail
123	fibroblastsofpmn/pmnmice(Fig.2b). Wethendeterminedthenucleationofmicrotubules
124	atthecentrosomeandatextracentrosomalsites. Centrosomesorganizethespindle
125	microtubulesduringmitosis, and confocal microscopy analysis of synchronized fibroblasts
126	frompatientswithmutated TBCE alleles documented abnormalmitotic morphology and
127	disorganizedmitoticmicrotubules;spindlesappearedasymmetricandunrefined,with
128	irregularasterstructure(SupplementaryFig.S2). Defectivenucleationof
129	extracentrosomalmicrotubuleswasalsonoticed. Specifically,weobservedsignificantly
130	reduced levels of Golgi-associated detyros in a ted α -tubulin in patient fibroblasts (Fig.2c),
131	inlinewiththereportedlossofGolgi-derivedmicrotubulesinmotorneuronsofpmn/pmn
132	mice ⁷ .
133	Todemonstratealteredmicrotubuledynamics, wetreated the fibroblast cultures with

themicrotubule-disruptingdrugnocodazole, and analyzed microtubule regrow thafter drug

135	washout.Incontrolfibroblasts,microtubulebegantopolymerizeefficientlyasearlyas10
136	minutesafternocodazolewashout, and extended polymerized structures were well-formed
137	at30min(Fig.2d).Differently,microtubulere-polymerizationappearedmarkedlydelayed
138	inpatientfibroblasts;microtubuleswerealsolessabundantandstronglydisorganizedin
139	bothearlyandlatestagesofre-polymerization.Suchaltereddynamicsappeared
140	particular ly pronounce dinfibro blasts with compound heterozygosity for the lle155 Asn and the standard structure of the standard structure of the standard structure of the
141	Ser359fs*1changes, consistent with the more profound reduction of TBCE expression
142	levels.
143	Overall, we describe a new distal neuron opathy and spasticat axia caused by biallelic
144	mutationsin TBCE overlapping the phenotype of pmn/pmn mouse, a well-known model of
145	motorneurondisease.Onthebasisofthepreviouslydocumentedimpactofabolished
146	TBCEfunctionondevelopmentalprocessesandgrowth,ourfindingsdemonstratea
147	$stringentrequirement of {\sf TBCE} function {\sf specifically} for neuronal survival and function.$
148	
149	URLs
150	GenomeAnalysisToolkit,https://www.broadinstitute.org/gatk/;Gene,
151	http://www.ncbi.nlm.nih.gov/gene/;RCSBProteinDataBank,
152	http://www.rcsb.org/pdb/home/home.do;Gromacs,http://www.gromacs.org;SWISS-
153	MODEL,http://swissmodel.expasy.org;vmd,http://www.ks.uiuc.edu/Research/vmd/;
154	MODELLER,https://salilab.org/modeller/.

155

156 Acknowledgments

- 157 Wearegratefultotheparticipatingpatientsandtheirfamilies.Thisworkwassupportedin
- 158 partbygrantsfromFondazioneBambinoGesù(ViteCoraggiosetoM.T.),Bulgari
- 159 (GeneRaretoB.D.)andMinisterodellaSalute(RC2015andRC2016,toA.F.,M.N.,G.Z.,
- 160 E.B.andM.T.).

162	Authorcontributions						
163	A.S.,G.B.,T.R.andE.P.designedandperformedthebiochemicalandfunctionalstudies,						
164	analyzedthedata;S.B.performedmutationanalyses;E.F.,C.C.,andS.C.carriedoutthe						
165	confocal lasers canning microscopy analysis of cultured human and micefibroblasts; A.C.,						
166	M.N.andV.C.performedWESdataprocessing, analysis and validation.E.Bell.performed						
167	thestructuralanalyses.G.T,A.D'A.,B.D.,G.Z.,D.D.,D.M.andC.DV.,clinically						
168	characterizedtheaffectedsubjects, collectedbiologicalspecimensandstudied						
169	histopathologyofmusclebiopsies.M.T.,G.H.andE.B.conceivedtheproject,designed						
170	and supervised the experiments, analyzed and interpreted the data, and wrote the						
171	manuscript.Allauthorscontributedtothefinalmanuscript.						
172							
173	Competingfinancialintereststatement						
174	Allauthorsstatethattheyhavenocompetingfinancialinterests.						
175							
176	References						
177	1.Ba hi-BuissonN., <i>etal.Brain</i> 137 ,1676-1700(2014).						
178	2.Pa rvariR., etal. Nat. Genet. 32 , 448-452(2002).						
179	3.Smi thB.N., etal. Neuron 84, 324-331 (2014).						
180	4.Si monsC., etal. Am. J. Hum. Genet. 92, 767-773 (2013).						
181	5.Mart inN., etal. Nat. Genet. 32 , 443-447 (2002).						
182	6.Be IlaJ., etal. Cell. Mol. LifeSci. 65, 2307-2333 (2008).						
183	7.Be IlouzeS., etal. Hum. Mol. Genet. 23, 5961-5975 (2014).						
184	8. Co urtensW., etal. Am. J. Med. Genet. A140, 611-617 (2006)						
185	9. KapiteinL.C.andHoogenraadC.C.Neuron87,492-506(2015).						

187 LegendstoFigures

188

189	Figure1.TBCEmutationscauseearly	v-onsetence	phalop	ath	withdistals	pinal
±05		,				

- 190 **muscularatrophy**.(a)ClinicalfeaturesofPt1(1)andPt2(4,5,6);musclebiopsyofPt1
- 191 stainedwithATPase4.3(2)andesterase(3)showingneurogenicabnormalities;brain
- 192 MRIsofPt1(7,8,9,at2years;10,11,12,at3years)andPt2 (13,14,15,at1year;16,
- 193 17,18,at2years).Notethehypoplasiaofcorpuscallosum,andcerebellaratrophywithprominentf
- 194 olia(12,17).(**b**)HomologymodeloftheTBCELRRdomain.Ile¹⁵⁵with
- 195 surroundinghydrophobicresiduescontributetothehydrophobiccorerequiredforproper
- 196 foldingofthedomain.(c)Westernblot(WB)analysisdocumentingreducedTBCElevels
- inpatientfibroblasts.Dataarepresentedasmeans±s.d.,*indicatesP<0.05. (d)Real-
- 198 timeqRT-PCRshowingmarkedlyreducedexpressionlevelof *TBCE*mRNAinfibroblasts
- 199 fromPt2.*indicates*P*<0.05.(**e**)WBanalysisdemonstratingreducedlevelsofTbceinthe
- 200 *pmn/pmn*mouse(Tbce^{Trp524Gly});recombinantprotein(TBCE)isalsoreported.
- 201
- 202

203 Figure2.Defectivemicrotubulepolymerizationinsubjectswith

- ²⁰⁴ homozygous/heterozygousTBCEp.lle155Asnsubstitution,and*pmn/pmn*mice.(a)
- 205 Westernblot(WB) analyses showing total (T), soluble (S) and polymerized (P) α -tubulin
- $(above), detyrosinated tubulin (middle), and \beta-actin (below) in patient fibroblasts and$
- $207 \quad controlcells (Ctrl1, Ctrl2). Diagrams (right) show reduced fractions of polymerized \alpha-$
- $\label{eq:constraint} 1000 tubulin and detyrosinated tubulin in patient fibroblasts. (b) WB analyses showing reduced$
- $amount of polymerized \alpha-tubulin (P) infibroblasts from {\it pmn} mice compared to$
- ${\tt 210} \qquad {\tt fibroblasts from unaffected mice.} ({\bf c}) {\tt Immunofluorescence analysis documenting loss of}$
- $\label{eq:211} microtobules containing detyrosinated tubulin (detyr-Tub) in patient fibroblasts. Note that$
- 212 detyrosinatedmicrotubules(red)incontrolfibroblastsarecloselyassociatedwithGM130-

- stainedGolgimembranes(green).NucleiareDAPI-stained.Scalebar,10µm. (d)
- 214 Immunofluorescenceanalysesshowingreducedre-polymerizationofmicrotubules
- followingnocodazoletreatmentinfibroblastsfromPt1andPt2,andcontrolcells(Ctrl).
- 216 Scalebar,20μm.
- 217



