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Supplemental Information

Germline mutation in *POLR2A*: a heterogeneous, multi-systemic developmental disorder characterized by transcriptional dysregulation

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Supplemental Note: Case Reports

Individual 4

Individual 4 presented as a ten-year-old boy who was originally seen in the genetics clinic for global developmental delay, seizure disorder, and spastic cerebral palsy (CP). His parents first became concerned prenatally when he was found to have a two-vessel umbilical cord. He was noted to have an enlarged liver at birth, but that self-resolved. He was also noted to have a heart murmur and was found to have an atrial septal defect. At a few months old, he started having cyanotic spells and went into congestive heart failure. At 4-6 months old, he was noted to have delays–specifically not cooing and not rolling over. He started therapies around that time but was still very delayed. He crawled at 13 months and started walking at 4.5 years. At 5 years, he started saying "mama" and other sounds. He had his first seizure at 3 years of age and was trialed on multiple different anti-epileptic drugs over the course of three months including phenobarbital and levetiracetam but these were discontinued due to adverse reactions including hyperactivity and angioedema, respectively. He was then seizure free until age nine, when they became intractable without a known trigger; however, he has tremors at baseline as well. His seizure types are generalized tonic-clonic (GTC) and absence. He is hypertonic and was diagnosed with CP. His parents have also reported a decline in his motor skills. He was never very steady on his feet, but now his hips are "tighter" and his coordination is much worse than before. It remains unclear whether he is having true developmental regression, periodic regression marked by temporary phases of reduced function, or if he is limited by muscle/tendon problems. Additionally, the patient has self-injurious behaviors, including biting himself, hitting himself, and grabbing his ears. He also hits other people. Furthermore, he has a history of frequent fevers with increased white blood cell count of unknown etiology. This used to occur to him every 1-2 months and sometimes also following a seizure episode (parents note that he is afebrile prior to seizures starting, so they do not believe they are febrile seizures). He has developed some urinary retention. Parents report that he tested negative for Down syndrome, and methylation analyses for Prader-Willi

and Angelman syndromes were negative. He had trio ES in at age seven which was originally negative. At age eleven, he was admitted to the hospital for weakness of all four extremities–which began proximally and progressed to involve distal musculature–and altered mental status several days after a therapeutic botulinum toxin injection. The etiology of this episode was unclear. While he was hospitalized, the inpatient genetics consult team reviewed him and recommended a re-analysis of his prior ES, which revealed a likely pathogenic variant in *POLR2A*–NC_000017.10:g.7412890A>G, NM_000937.4:c.3752A>G (p.Asn1251Ser).

Individual 5

Individual 5, harboring the same genomic variant as Individual 4, is a 21-year-old woman, one of three children born to healthy unrelated parents. She was born after a pregnancy complicated by maternal hypertension, by normal vaginal delivery, with birth weight 3.5kg, at 39 weeks gestation. She had neonatal apnea that was thought to have possibly been seizure-related and had severe feeding problems and gastro-esophageal reflux. She had marked hypotonia and was a placid baby. Epilepsy was diagnosed at 22 months, and seizure types included febrile seizures, tonic-clonic, myoclonic and absence seizures. She was treated with and responded well to valproate and has had no seizures beyond early childhood. Valproate treatment was ceased at age 18. She had poor sleep and some behavioral traits including hand-flapping and a love of water. Development was severely delayed, and she walked at age 3.5 years. She developed single words in her teens but no further speech and communicates using sign language. She was toilet trained. She was an affectionate person with good eye contact. She had strabismus that resolved. There were no concerns regarding hearing. Around the age of sixteen years, she developed significant behavioral disturbance with some aggressive behavior that has been attributed to anxiety and some regression of skills including incontinence. The behavioral problems have shown some improvement with behavioral management strategies, but moderate to severe anxiety persists. She has some ongoing sleep disturbance. At last review she was not on any medication. Examination at last assessment at age 17 years showed a

height of 159cm (25th centile) and head circumference of 53.5cm (25th centile). She has a fair complexion, deep-set eyes, prominent supra-orbital ridges, marked pes planus and a crouched, ataxic, high-stepping gait. Investigations included SNP array, Angelman methylation testing, *UBE3A* sequencing and MLPA, all of which returned a negative result. Brain MRI at one year of age showed prominent third and lateral ventricles. Her EEG showed an excess of high amplitude slow activity during drowsiness and sleep. Trio exome showed a *de novo* missense variant in *POLR2A* (p.Asn1251Ser).

Supplemental Material

Methods

Phenotyping

Phenotypes for Individuals 1, 6, 8-12 were initially obtained through clinical ES requisitions at BG. Attempts were made to establish contact and research consent with each of the patients. Phenotypes for BG samples consenting to participate in research (protocol H-29697 approved by the institutional review board at Baylor College of Medicine) were updated and expanded via a combination of chart review and self-reported data obtained through conversations and questionnaires. Phenotypes for Individuals 2-5 and Individual 7 were obtained after informed research consent via a combination of chart review and self-reported data obtained through conversations and questionnaires.

Functional Evaluation of Variants in Yeast

One missense variant–p.(Gly1418Arg)–affected the homologous amino acid residue to a known yeast variant–p.Gly1388Val–so it was included in addition to p.Gly1388Arg.[1] As annotated by VEP, the protein consequence for the variant observed in Individual 1 (NC_000017.10:g.7401508_7401513del, VCF description hg19:17:7401503:GACCTTC:G) was originally reported as p.(Asp437_Leu438del). After the completion of the yeast experiments, a reexamination of the human genomic data revealed an apparent VEP annotation error, as the true protein consequence of the genetic variant is p.(His439_Leu440del) (equivalent to the meningioma-causing p.Leu438_His439del described by Clark et al).[2] Therefore, the yeast variants corresponding to p.(Asp437_Leu438del)–including Asp423del and Ile424del–were studied in lieu of those corresponding to p.(Leu438_His439del) or p.(His439_Leu440del). Thus, fifteen variants were studied in addition to the wild type allele.

Yeast strains used in this study were derived from S288C (**Table S1**).[3] Phenotyping assays for *rpb1* (yeast homolog of *POLR2A*) mutants were performed on standard media as described by Amberg et al., 2005, with previously noted modifications.[4,5] Mutations in *RPB1* were generated by site directed mutagenesis using the QuikChange strategy (Stratagene/Agilent) in a pBS KS+ plasmid, followed by transfer into pRS315-based *RPB1* expression vector (expressed from native promoter). Mutant plasmids were transformed by methodology described by Gietz and Woods[6] into each of two yeast strains in **Table S1**, with plasmid shuffling performed as previously described[4,7] to remove the wild type (WT) copy of *RPB1* present on a *URA3* plasmid (**Table S2**). Phenotypes were determined by spotting 10-fold serial dilutions of saturated yeast cultures on control or experimental media, as previously described.[4] General growth was determined in strains derived from CKY283 on rich medium YPD (1% yeast extract, 2% peptone, 2% dextrose w/v) at 30˚C or synthetic complete lacking leucine (SC-Leu) at 30˚C. Conditional phenotypes were determined on the following media: YPD incubated at 37˚C (temperature sensitive Ts- phenotype); YPD supplemented with 3% formamide;[8] synthetic complete lacking lysine (SC-Lys) for determination of suppression of *lys2-128∂* (Spt$^-$ phenotype derived from altered transcription at *lys2-128∂)*;[9] SC-Leu supplemented with 20 µg/ml mycophenolic acid (MPA) (detects initiation defects at the *IMD2* gene);[10–12] and YP supplemented with 2% raffinose, 1% galactose and 1µg/ml antimycin A (YPRaf/Gal), for determination of suppression of *gal10Δ56*. *gal10Δ56* confers galactose sensitivity due to altered termination at *GAL10* causing transcription interference at downstream *GAL7*. Suppression of these defects in certain transcription mutants, including Pol II, confers resistance to galactose for *gal10Δ56* strains.[13–15] Constitutive induction of *IMD2*, which can be caused by decreased Pol II initiation efficiency or decreased catalytic activity allowing a normally inducible TSS to be used all the time, was assayed in strains derived from CKY865, containing an *imd2Δ::HIS3* reporter.[4,12] Here, observation of a His$^+$ phenotype would indicate abnormal, constitutive transcription from the *IMD2* promoter, which drives *HIS3* expression in *imd2Δ::HIS3*.

Strain Number	Genotype
CKY283	*MAT***a** *ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128∂ gal10Δ56 rpb1Δ::natMX4 RPB3::TAP::KlacTRP1 [pRP112 RPB1 CEN URA3]*
CKY865	*MAT***a** *leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128∂ RPB3::TAP::KlacTRP1 rpb1Δ::natMX4 imd2Δ::HIS3 [pRS316 RPB1 CEN URA3]*

Table S1 – Yeast strains and genotypes used in this study. S288C-derived strains[13] and their genotypes used in this study for analyzing general and transcription-associated phenotypes of *RPB1* alleles by plasmid shuffling. The *lys2-128∂* [9] and *gal10Δ56* [13–15] alleles of *LYS2* and *GAL10-GAL7* respectively allowed testing for Spt^- and Gal^Sphenotypes in CKY283, whereas the *imd2Δ::HIS3* [4,12] allele of *IMD2* allowed analyzing the His^+ phenotype in CKY865.

Mutant	Plasmid number	Plasmid genotype	Reference
RPB1 WT (*URA3*)	pRP112	*RPB1 URA3 CEN ARS*	PMID: 3304659
RPB1 WT (*URA3*)	pCK518	pRS316 *RPB1 URA3 CEN ARS* amp^r	PMID: 22511879 PMID: 23932120
RPB1 WT (*LEU2*)	pCK859	pRS315 *RPB1 LEU2 CEN ARS* amp^r	PMID: 22511879 PMID: 23932120
Empty Vector	pRS315	pRS315 *LEU2 CEN ARS* amp^r	PMID: 2659436
rpb1 Pro24Arg	pCK2742	pRS315 *rpb1* P24R *LEU2 CEN ARS* amp^r	This study
rpb1 Glu26Val	pCK2656	pRS315 *rpb1* E26V *LEU2 CEN ARS* amp^r	This study
rpb1 Glu104His	pCK2658	pRS315 *rpb1* E104H *LEU2 CEN ARS* amp^r	This study
rpb1 Glu104Leu	pCK2657	pRS315 *rpb1* E104L *LEU2 CEN ARS* amp^r	This study
rpb1 Arg134Trp	pCK2659	pRS315 *rpb1* R134W *LEU2 CEN ARS* amp^r	This study
rpb1 Arg175Trp	pCK2660	pRS315 *rpb1* R175W *LEU2 CEN ARS* amp^r	This study
rpb1 Gln313Cys	pCK2661	pRS315 *rpb1* Q313C *LEU2 CEN ARS* amp^r	This study
rpb1 Leu388Val	pCK2752	pRS315 *rpb1* L388V *LEU2 CEN ARS* amp^r	This study
rpb1 Asp423delIle424del	pCK2743	pRS315 *rpb1* D423ΔI424Δ *LEU2 CEN ARS*amp^r	This study
rpb1 Asp423Δ	pCK2744	pRS315 *rpb1* D423Δ *LEU2 CEN ARS* amp^r	This study
rpb1 Ile424del	pCK2745	pRS315 *rpb1* I424Δ *LEU2 CEN ARS* amp^r	This study
rpb1 Ala1069Val	pCK2664	pRS315 *rpb1* A1069V *LEU2 CEN ARS* amp^r	This study
rpb1 Thr1272Ala	pCK2739	pRS315 *rpb1* T1272A *LEU2 CEN ARS* amp^r	This study
rpb1 Gly1388Arg	pCK2740	pRS315 *rpb1* G1388R *LEU2 CEN ARS* amp^r	This study
rpb1 Gly1388Val	pCK2741	pRS315 *rpb1* G1388V *LEU2 CEN ARS* amp^r	This study

Table S2 – Yeast mutants and plasmid descriptions used in this study. Description of CEN plasmids used in this study for analyzing *RPB1* alleles in yeast, by plasmid shuffling.[4,7]

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