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Supplemental Data

A Syndromic Intellectual Disability Disorder Caused by Variants in *TELO2*, a Gene Encoding a Component of the TTT Complex

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Supplemental Materials

Case Reports

Family 1: Individuals II-2 and II-3 (17 year old, male and female, respectively) are fraternal twins (see Figure 1) born to a 21 year old mother by vaginal delivery following a 35 week pregnancy complicated by preeclampsia. Against normative birth parameters for twin gestation, birth weights were 1.9 kg (10-25%) and 1.7 kg (5-10%), respectively, and birth lengths were 43.8 cm (25%) and 38.7 cm (<3%). Individual II-2 had 'low' Apgar scores and remained hospitalized for 2-1/2 weeks due to poor feeding and apnea. Individual II-3 had poor respiratory effort at birth and required resuscitation for 15 minutes with 'low' Apgar scores. A cleft palate and shrill cry were noted after her resuscitation and she remained hospitalized for 1.5 months with nasogastric tube feeding. Individual II-4 was born at term with birth weight of 2.8 kg (10-25%) and birth length 47.0 cm (25%) and discharged at 1 week with difficulty feeding and hypotonia. He had 1^0 hypospadias and failed his newborn hearing screen.

From infancy, individual II-2 had symmetrical poor growth and delayed development. He sat independently at 2 years, crawled at 3 years and stood at 4 years, but was wheelchair-dependent by mid-childhood due to impaired balance that has become more pronounced with age. At 17 years, he can hold a pencil or utensil but is unable to write or feed himself independently. He occasionally uses single words, rarely makes eye contact, and is described as 'distant and withdrawn' except in the evenings, when his behavior changes dramatically to uncontrollable laughing and hugging. While awake,

his body is constantly moving without purpose but without overt tremor or chorea. There has been slow acquisition but no regression of skills. He was born with a patent ductus arteriosus (PDA) and a ventricular septal defect (VSD). Both closed spontaneously, but he also was found to have a double aortic arch with vascular ring and cleft mitral valve with mitral regurgitation. Thus far, no cardiac surgery has been required. He has bilateral hearing loss and cortical visual impairment (CVI). A comprehensive, dilated ophthalmologic exam at 17 years of age revealed grossly normal eye structure, but visual acuity could not be assessed due to poor tracking/jerky pursuits and no compliance. He was diagnosed with autism in late childhood, based on no language or communication and repetitive, self-stimulatory movements. Hypothyroidism was diagnosed at 15 years. Upon examination at age 17 years, weight was 28.7 kg (-7.6 SD), height 130.4 cm (-5.6 SD) and OFC 48.0 cm (<<3%; 50% for 1.5 years). He has brittle hair, blue sclera, dark circles under his eyes, an intact palate, pectus carinatum, decreased elbow extension but otherwise joint laxity, broad great toes with 4/5 toe syndactyly, eczema, and generalized hypotonia with spasticity. He has sparse facial and pubic hair. Prior to enrollment for research WES, he had several normal tests and studies including: brain MRI (2 years of age), comparative genomic hybridization (10 years), EEG (12 years), serum uric acid, and molecular testing for fragile X.

Individual II-3 also experienced symmetric poor overall growth after birth associated with delayed development without regression. She sat independently at 5 years of age

and has never walked, but can stand with support. She has no words but makes sounds to communicate. She is the most attentive to her environment of the three affected children in Family 1. She often exhibits hand flapping when she is frustrated, throws herself back into her wheelchair when she is angry, and has an affinity for water. Her cleft palate was repaired at 14 months and she required a frenulectomy for ankyloglossia at 3 years of age. She required multiple sets of typanostomy tubes for otitis media. In early childhood, she was found to have a double aortic arch with an atretic left arch creating an incomplete vascular ring impinging on the upper esophagus but not the trachea. Her medical course was complicated by severe gastrointestinal reflux disease and recurrent episodes of aspiration pneumonia that diminished following G-tube placement at age 12 years. She has bilateral hearing loss and CVI. On exam at 12 and 1/3 years, her height was 108.5 cm (-6.1 SD), weight 18.1 kg (-6.6 SD), and OFC 47.0 cm (<<3%, 50% for 1.5 years). She had synophrys, blue sclera, up-slanting palpebral fissures, downturned mouth, large tongue with tethered sublingual frenulum, torticollis, pectus excavatum, anteriorly placed rectum, brachydactyly (middle digit << 3%ile), 4/5 toe syndactyly, joint laxity, and generalized hypotonia. She, too, is in constant motion while awake with spasticity in her lower extremities. She has rotatory nystagmus. Prior to enrollment in this research, she had multiple normal clinical studies including: karyotype, subtelomere and 22q11 FISH, 7 dehydrocholesterol levels, and BAC array (4,000 probes).

Following birth, individual II-4 remained in hospital for difficulty feeding and hypotonia until age 1 week. His exam was notable for hypospadias. He sat at 15 months but has never ambulated. He failed his newborn hearing screen and all subsequent hearing tests, and has no speech. Based on his behavior, it is thought that he also has CVI. He was noted to have an accessory oral frenuli and coarctation of the aorta. On exam at age 9 years, he was symmetrically small with height 100.4 cm (-5.9 SD), weight 15.1 kg (-6.3 SD) and OFC 46.5 cm (<<3% for 1 year). He had blue sclera, a large mouth with thin upper lip, loose joints, hypotonia, brachydactyly and 5th finger clinodactyly. He exhibited self-injurious behavior during the exam with constant motor movement and no words. He had 3+ patellar reflexes but no spasticity.

Family 2:

Individual II-2 is a 5 year old white female who was born at term after an uncomplicated pregnancy. She was delivered vaginally to a 30 year old, gravida 2, para 1 woman. Her birth weight was 3.71 kg (50%); length 50.8 cm (60%); and OFC 35 cm (60%). Concerns regarding delayed development began at 2 months. She rolled over at 5 months, sat alone at 8 months, and walked at 14 months. At 20 months she knew about 20 words, but her parents felt her verbal skills regressed. A hearing test at 2 years of age was normal. At age 30 months, she had formal testing using the Developmental Assessment of Young Children (DAYC-2) test with a developmental quotient (DQ) of 55 in expressive and language skills, 40 in fine and gross motor skills, 40 in social skills and 37 in cognition. She was re-tested at age 60 months and scored <50 in all domains.

She had a normal cranial MRI at age 2 and strabismus surgery at age 3.5 years for intermittent esotropia. She has had a chronic sleep disturbance with difficulty falling asleep and often stayed awake screaming during the night. An EEG at age 3 and 10/12 showed slightly slow background activity with 2 brief bursts of bifrontal epileptiform activity. There have been no clinically detectable seizures.

Physical exam at age 4 and 9/12 revealed a height of 106 cm (50%), a weight of 16.8 kg (45%), and a head circumference of 47.5 cm (<3%, 50% for 18 months). She was non-dysmorphic and ambulatory with intermittent abnormal truncal movements. Examination of her chest, spine, heart and abdomen was normal as were her deep tendon reflexes.

Pertinent laboratory testing included normal chemistries; normal 15q methylation testing for Prader Willi syndrome and Angelman syndrome; normal *MECP2* sequencing and deletion/duplication testing; normal transferrin isoelectric focusing for carbohydrate deficient transferrin; normal creatine disorders panel; and normal urine purines and pyrimidines.

Family 3:

Individual II-1 is a 17 year old female who was born at 37.5 weeks of gestation after an uncomplicated pregnancy. She was delivered with vacuum and forceps assistance due

to unspecified dystocia. Birth weight is unknown and Apgar scores were reported as "low". She had poor respiratory effort and was observed in the NICU for several hours but discharged at 2 days of age. Her parents noted slow acquisition of milestones. She sat independently at 2 years, never developed words to communicate, can assist with transfers from chair to floor but cannot stand or walk independently. She has not had formal psychometrics but currently (age 17) she is non-ambulatory, non-verbal, and incontinent and performs no activities of daily living. Formal audiology assessment in early childhood was normal. She is in special school 4 hours a day and has an irregular sleep pattern. On occasions, she does not sleep at night for several days. She has nighttime outbursts of laughing if she is awake for prolonged periods.

Seizures began in early childhood and, currently, she experiences absence, partial complex and generalized tonic clonic seizures. At times the frequency has been as high as 20 or 30 per day, but currently she has ~6 per month on Trileptal and clonazepam. She has kyphoscoliosis with progressive vertebral deformation, which began in mid-childhood. Her kyphoscoliosis worsened in puberty in association with a linear growth spurt.

At age 15, she had a routine echocardiogram and lab work in preparation for possible spine surgery. Her parents were told her cardiac function was sufficient to tolerate the surgery but are unaware if any structural anomalies were noted. There were no specific pre-operative laboratory abnormalities other than low vitamin D and anemia. The

former has been treated with supplemental oral vitamin D (50,000 IU weekly) and the latter with oral iron, though the anemia was reported to be minimally responsive.

On exam at age 17 years of age, her head circumstance is 51cm (<3%, 50% for 7 years old). Her face is expressionless with slightly elongated palpebral fissures and she holds her tongue outside her mouth. Her neck and thorax are grossly normal with normal breast development. She has small hands with wasting of her peripheral muscles. Her hearing is grossly normal. She tracks people moving around her and can 'spot' small toys or objects. She avoids eye contact and has difficulty reaching objects with a shaky, poor grasp.

Family 4:

Individual II-1 is an 8 year old white male who was the 45.7 cm (<3%) and 3.09 kg (25%) product of a 38 week, uncomplicated pregnancy and delivery. Apgar scores were 9 at 1 and 5 minutes. He required phototherapy for hyperbilirubinemia but was discharged at age 5 days. Development was slow: he sat at 12 months, crawled at 3 years, and walked at 4 years. There has been no regression. He has dysphagia with difficulty swallowing thin liquids and chronic respiratory congestion. He required a frenulectomy, multiple typanostomy tubes for chronic otitis media and unilateral nasolacrimal duct probing for an obstructed tear duct. He developed kyphoscoliosis in childhood and is non-verbal. He has CVI and a movement disorder diagnosed as ataxia by consulting neurologists. Multiple cranial MRIs have been read as normal except for small brain size. He has not

had seizures and an EEG at age 7.5 was negative. His physical exam at age 7 years 9 months was remarkable for short stature (height 113.5cm, 1 %), small size (weight 20.6kg, 8%) and OFC 47cm (<<3%, 50% of 1 year). HEENT exam was remarkable for downslanting palpebral fissures; a scar on his right upper eyelid from surgical repair of trauma; short, smooth philtrum; broad nasal bridge; and a chronically open mouth. Chest, cardiovascular and abdominal exams were normal. He had a small penis with mild chordee. His tone was mildly increased in his lower extremities with normal deep tendon reflexes and abnormal coordination with ataxia. An echocardiogram at 8 years of age was normal with no abnormalities of the great vessels.

Pertinent laboratory testing included normal chemistries and plasma amino acids; normal 15q methylation testing for Prader Willi syndrome and *UBE3A* sequencing for Angelman syndrome; normal *NIPBL* sequencing for Cornelia de Lange syndrome 1; normal *MECP2* sequencing; normal 7-dehydrocholesterol and uric acid levels; normal transferrin isoelectric focusing for carbohydrate deficient transferrin; and normal Fragile X testing. His karyotype was 46, XY and a chromosomal microarray revealed a heterozygous 40kb deletion at 22q12.3 that removed segments of *C22orf42* and *RFLP2.* His asymptomatic mother has the same copy number variant.

Supplemental Data
Figure S1

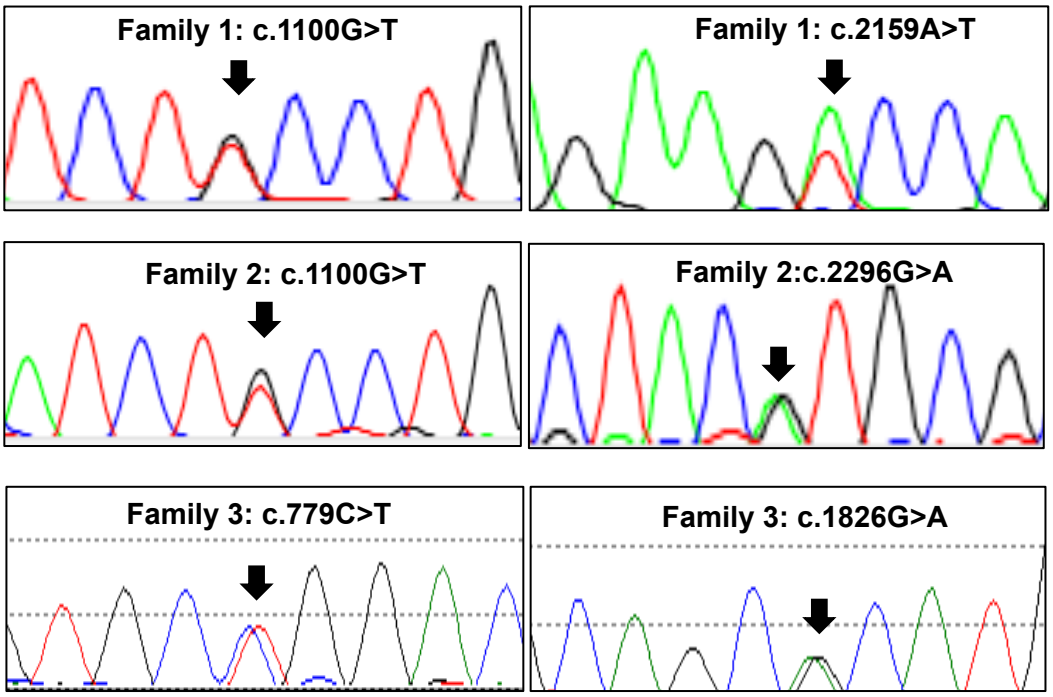

Figure S1.
Sequence of *TELO2* variants in Family 1 (II-3, II-4 and II-5), Family 2 (II-2) and Family 3 (II-1).
Family.1: *TELO2*, p.Cys367Phe (c.1100G>T) in exon 8 and p.Asp720Val (c. 2159A>T) in exon 18.
Family.2: *TELO2*, p.Cys367Phe (c.1100G>T) in exon 8 and p.Val766Met (c. 2296G>A) in exon 20.
Family.3: *TELO2*, p.Pro260Leu (c.779C>T) in exon 5 and p.Arg609His (c. 1826G>A) in exon 15.

Figure S2

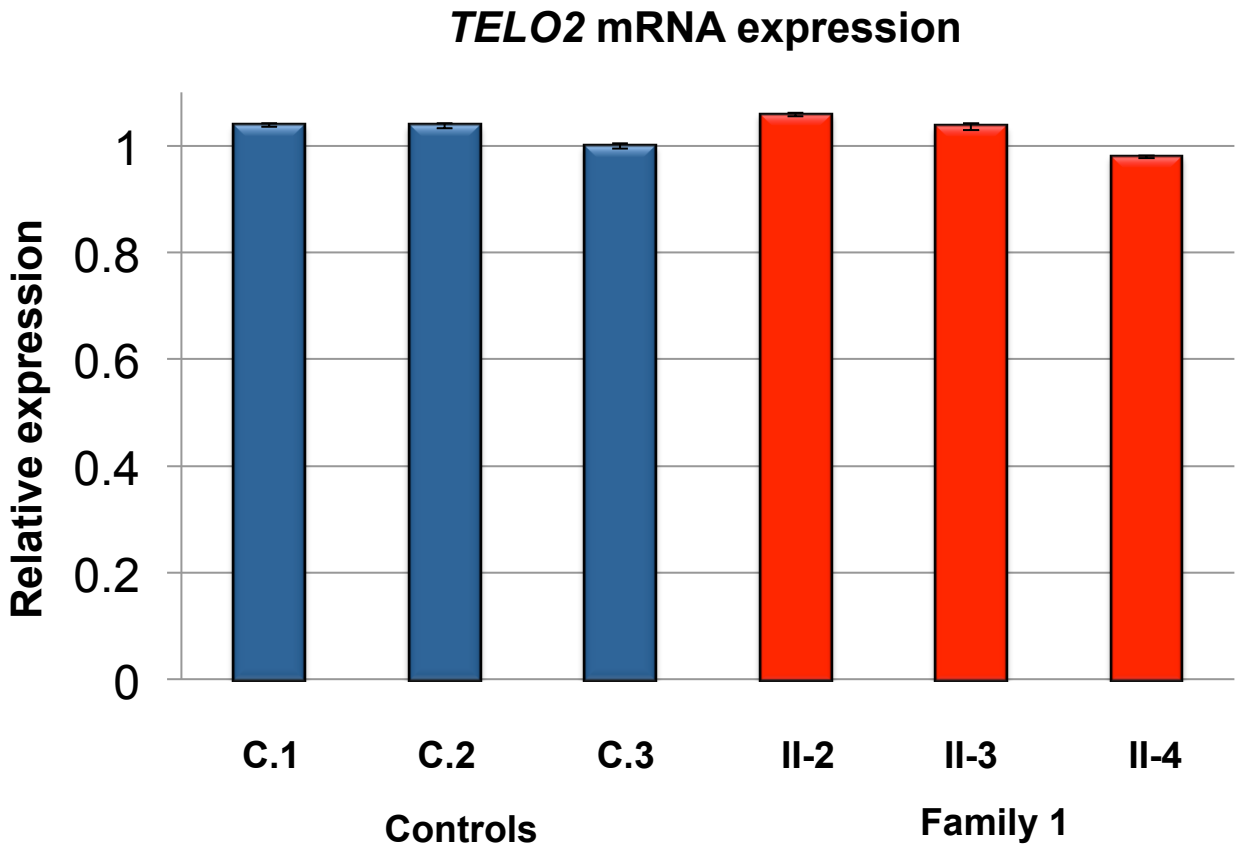

Figure S2. **Comparison of *TELO2* mRNA expression in affected individuals from Family 1 and controls**

Levels of *TELO2* mRNA are within normal range in RNA extracted from primary affected individual-derived fibroblast cell lines in Family 1 (II-2, II-3 and II-4) (n=3 experiments, performed in triplicate; error bars indicate 1 SEM, Student's t-test)

Figure S3

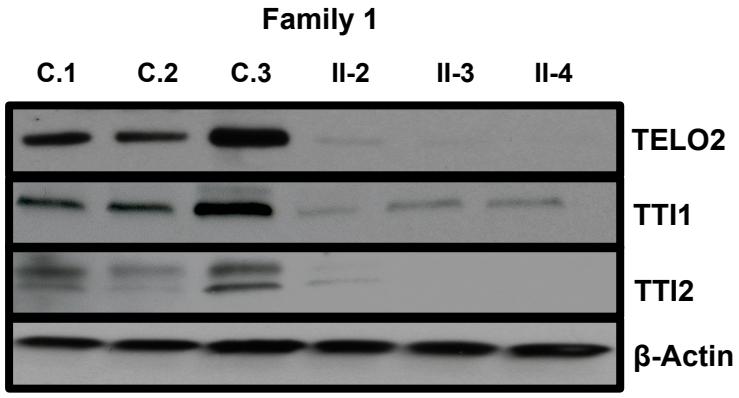

Figure S3. **Repeated western blot of steady state expression of TTT complex in fibroblasts extracts in the affected individuals of Family 1 (II-2, II-3 and II-4) and three normal controls (C.1, C.2 and C.3).**

Figure S4

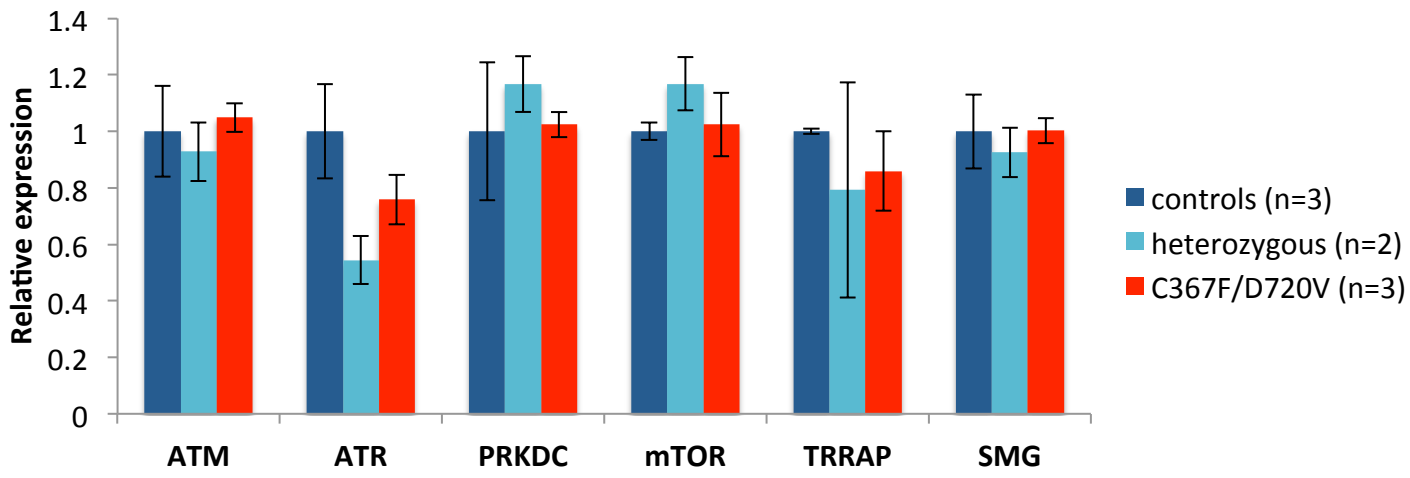

Figure S4. Steady state expression of PIKKs in LCL extracts in the affected individuals of Family 1 (II-2, II-3 and II-4), their heterozygous parents (I-1 and I-2) and three normal controls (C.1, C.2 and C.3).
There was no significant difference between control, affected individuals and heterozygous parents (Student's t-test).

Figure S5

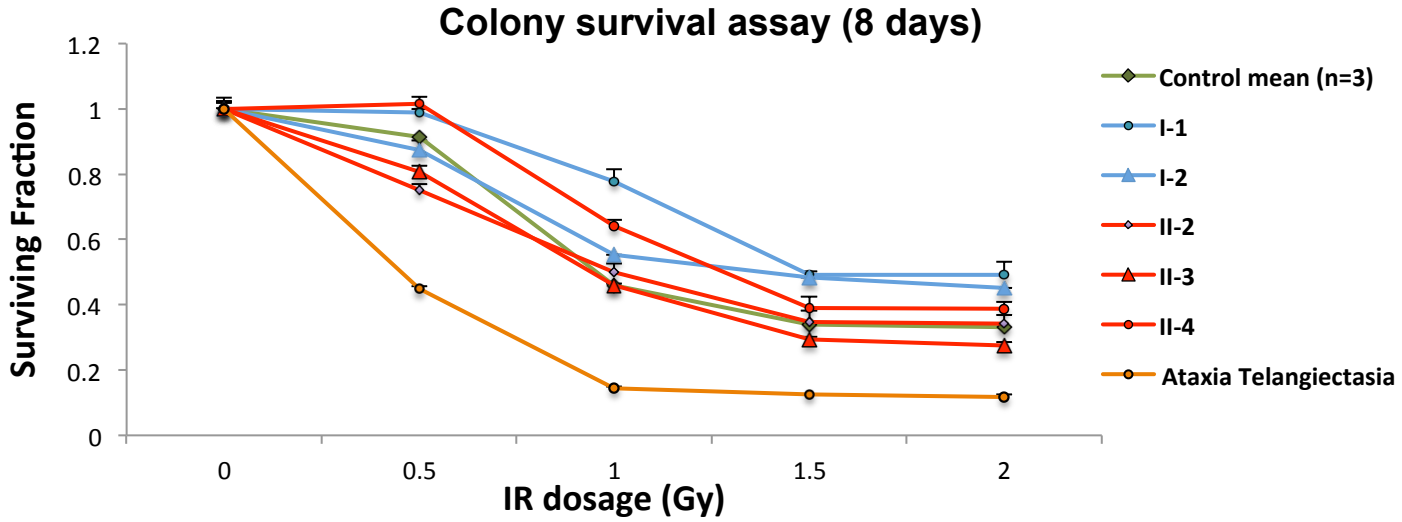

Figure S5. Colony survival assay following exposure to ionizing irradiation.
LCLs from affected individuals in Family 1 (II-2, II-3 and II-4) are labeled in red and the mean of three controls is labeled in green. LCLs from an affected individual with molecularly confirmed ataxia-telangiectasia (*ATM* nonsense variant) was used as a positive control.

Figure S6

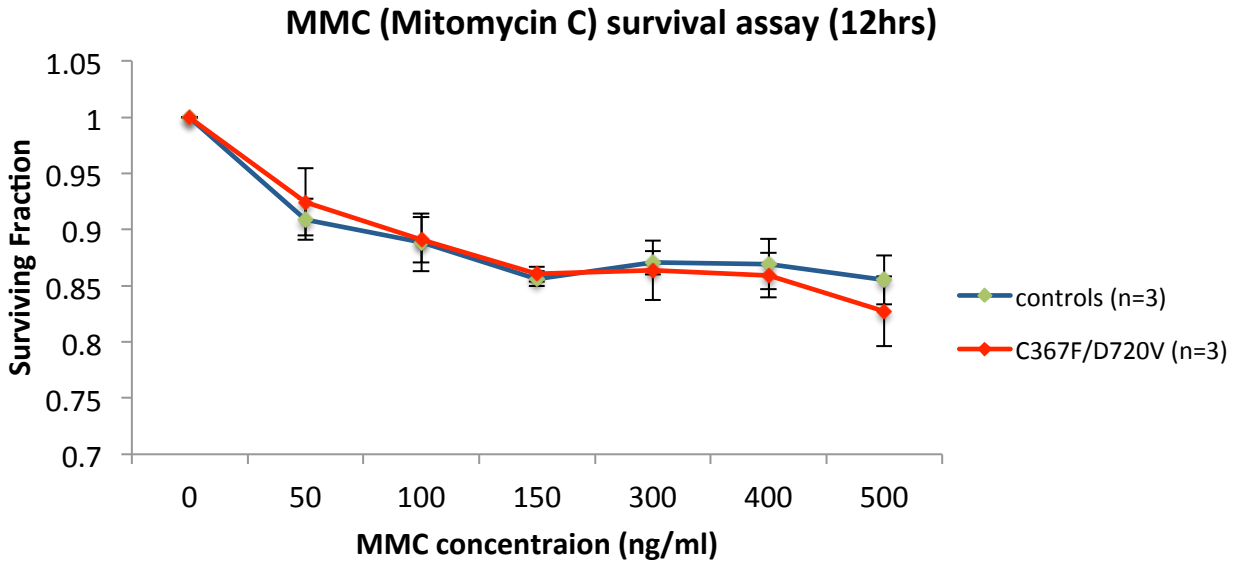

Figure S6. Mitomycin C (MMC) survival assay

Cultured skin fibroblasts from affected individuals in Family 1 (II-2, II-3 and II-4) and controls were used to measure the survival fraction following MMC treatment. The assay was performed in triplicate, and the plot shows the means of the 3 affected individuals and the 3 controls. There was no significant difference between controls and affected individuals in survival fraction to MMC treatment (Student's t-test).

Figure S7

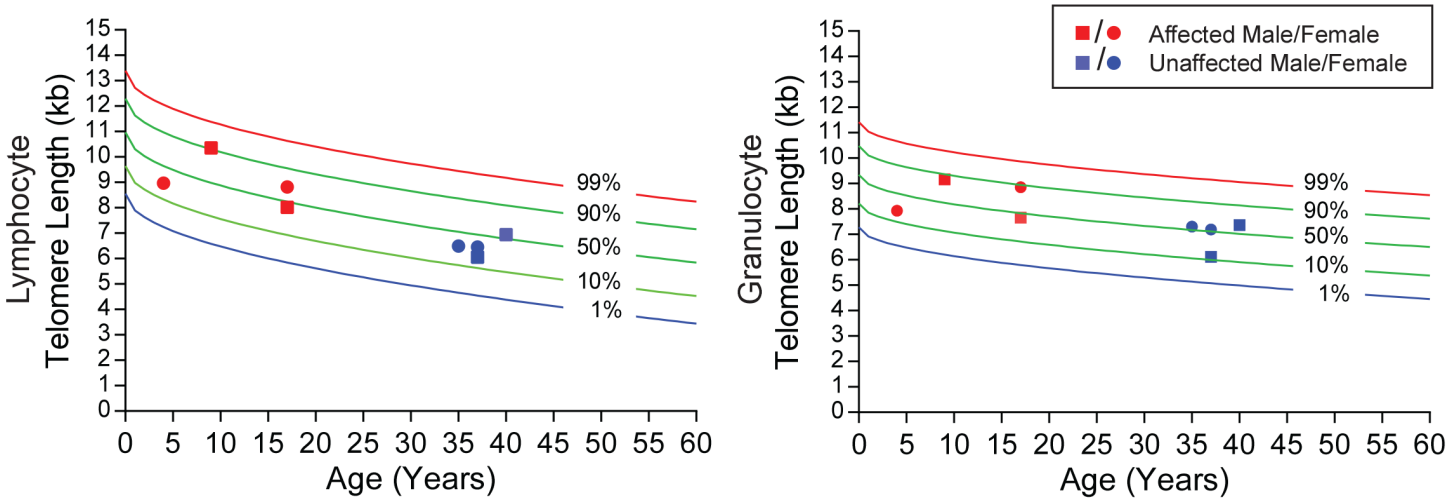

Figure S7. Telomere length measurement in affected individuals' primary lymphocytes and granulocytes.

The percentile lines are derived from 200 normal controls. Affected individuals and heterozygous parents from Families 1 and 2 were measured.