Supplementary Information

Disruptive *de novo* mutations of *DYRK1A* lead to a syndromic form of autism and ID

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Supplementary Note

Clinical Reports

UMCN1

This female was born after a normal pregnancy. Birth was complicated by prolonged rupture of membranes. She had a low birth weight of 2000 g (<P3). At the age of three years a significant global developmental delay was noticed. In addition, she had epilepsy and showed signs of spasticity. She did not learn to speak. When she was six years old she went to a school for children with developmental problems. At the age of 15 years she moved to a sheltered home. At the age of 16 years a cardiac murmur was noticed and she was diagnosed with an aortic valve stenosis, for which medical treatment was unnecessary. At the age of 17 years she was treated with a plaster cast and a brace because of severe thoracic kyphosis. At adult age she had a profound to severe ID without any speech development. Seizures (atonic attacks and absences) were well controlled using Depakine. At 59 years of age she was unilaterally blind due to ablatio retina, had unilateral hearing loss and was under treatment for chronic heart failure. Diabetes had been excluded. Secondary to her spasticity she had developed several joint contractures and bilateral pes excavates. Despite some feeding difficulties she remains at normal weight. Developmental age has been described between 0 and 2 years of age with a harmonious social-emotional profile. She has a quiet personality and is able to make non-verbal contact with familiar persons. Communication skills, including signs of autism, have never been formally assessed. Sleeping pattern was normal.

Upon physical examination at the age of 59 years she had a height of 143 cm (< -2.5 SD), weight of 49 kg (+2 SD) and an OFC of 50,2 cm (< -2.5 SD). Facial features included upslanting palpebral fissures, a long nose with a high nasal bridge, short philtrum, broad mouth with thin lips, widely spaced teeth and a prominent chin. She used a wheeled walker to move around.

UMCN2

This patient was born at term after a normal pregnancy with a birth weight of 2600 grams (<-2.5 SD) and skull circumference of 32.6 cm (-2.5 SD)

He showed psychomotor delay and was hypertonic from the start. He achieved independent walking at the age of 21 months and spoke his first words after that age. At 17 months of age cerebral CT, EEG, endocrinologic and metabolic screen were normal. At that time nine months delay was noted.

He showed feeding problems in infancy, mainly after the introduction of solid food, which resolved gradually

At the age of 16 years he had an IQ of 35-50 and was in good general health, without epilepsy. He could recognize and remember people and events. He would not make an initiative to get into contact with other people, but did show interest in other peoples' activities. He could not read or write but was able to communicate using several words. He showed autistic and stereotype behavior for which he received Risperdal. His height was 166 cm (-1.8 SD) and OFC was 50.5 cm (<-2.5 SD). He had microcephaly, deep-set eyes and a large coarse nose. He visited the dentist four times a year due to extreme calculus teeth. He showed a hypertonic posture, increasing during excitement.

Troina ID1818

This male was born, after a pregnancy complicated by intra uterine growth retardation and oligohydramnios, by caesarian section at 36 weeks of gestation. His birth weight was 1,980 g (<-2.5 SD).

Feeding problems were present in the postnatal weeks, leading to gavage feeding and bronchopneumonia by food ingestion at one month. Two febrile seizures have been reported at 10 and 24 months.

His morphological phenotype was characterized by microdolichocephaly, prominent metopic suture, horizontal palpebral fissures with long eyelashes, small and backward rotated ears, flat philtrum, reverse V-shaped upper lip, open bite, microdontia, micrognathia, interdigital webbing on the hands, and cutaneous syndactyly on 2^{nd}, 3^{rd} and 4^{th} toes. He had a hypotonic general appearance with sloping shoulders and mild pectus excavatum. His skin was xerotic. Ophthalmologic evaluation showed mild myopic astigmatism and right eye exotropia. ECG, echocardiogram and EEG were normal. Brain MRI, showing slight lateral ventricles dilation and several small spots on the back periventricular white matter, was compatible with a mild myelination delay and mild brain atrophy. Psychometric evaluation, performed with Griffiths Mental Development Scales (MDS) and Vineland Adaptive Behavior Scales (VABS), showed Intellectual Disability. Expressive speech was more severely affected compared to receptive speech. The Learning Accomplishment Profile (LAP) test scored 12 months for expressive speech and 26 months for receptive speech. Speech was characterized by single disyllabic words. Behavior was characterized by some avoidance reactions in non familiar environments.

Subsequent check-up at the age of 12 years has defined a severe intellectual disability, a normal weight and height and showed the presence of bilateral tibial osteochondrosis and exostoses. The behavior was highlighting an anxiety disorder. Further check-up at age 13 years and seven months showed a new exostosis on the right knee leading to lameness, with a consequent diagnosis of Osgood-Schlatter syndrome. A new brain MRI documented a thinning of corpus callosum. Subsequent check-up at age 13 years and 11 months showed mild muscular hypertonia of lower limbs, muscular hypotrophy of the right leg, four limbs increased tendon reflexes, right pes cavum and an awkward gait. This led to a muscle biopsy, which showed a mild muscular impairment and suspicion of a deficit of COX. Our last check-up at age 15 years and 8 months did not add new features to the previous phenotype.

GF2852

This male was born at term after a pregnancy complicated by a small amount of bleeding at 13 weeks. He had a birth weight of 2270 g (<-2.5 SD). Placenta appeared normal. He had feeding difficulties due to poor suck. At birth, small size, microcephaly, bilateral transverse palmar creases and overfolded ears were noted. At two weeks of age he had an OFC of 31.5 cm (<-2.5 SD). An innocent heart murmur was noted and required no further action. He was treated for gastro-oesophageal reflux disease from 8 weeks of age. At 12 months of age he had an OFC of 40.3 cm (<-2.5 SD) and at three years of age 43 cm (<-2.5 SD). Development was considered to be within the normal range at 12 months of age. He started to walk without support at 16 months of age and babbled at 12 months of age. Developmental delay was noted at 22 months of age, when he had not developed speech and babbling had been lost. Hyperreflexia and hypertonia were recorded at this age. CT-scan of the brain was normal. At three years of age he was diagnosed with mild global developmental delay with

severe expressive speech delay. He went to a special class in a normal school during his early school years and went to a special school focusing on life skills from nine years of age. At 20 months of age he had generalised febrile convulsions. Primary dentition was delayed. At four years epilepsy was diagnosed. Current medication is sodium valproate. During infancy and childhood he was fascinated with spinning objects and showed some repetitive behaviors. At 10 years of age his nose was broken and not reset. At 14 years of age he was diagnosed with autism. At that age he had a height of 152 cm (-1.8 SD) and weight of 37 kg (-2.5 SD). In young adulthood he had an isolated psychotic episode, which lasted 2-3 days and was treated with olanzapine. Appetite and weight improved using olanzapine. Currently, he lives in supported accomodation and participates in day activities. He has poor social skills and does not understand body language. He is verbal but finds it difficult to maintain a conversation. He is fascinated by machinery and obsessive about Lego. He shows a tendency to repeat the same activities. He shows anxious behavior and has diffculties coping with change. He needs minor help with daily activities such as dressing, toileting and showering and has little understanding of money. He has a sense of humour. Eating is characterized by a preference for soft and cooked food.
He has sparse facial hair, synophrys, upslanting palpebral fissures, deep-set eyes, a prominent nose and chin, narrow shoulders and chest, small hands and feet with a high instep and his right great toe is significantly larger than his left. He has an awkward gait characterized by feet that drop while walking and heel-toe walking. He uses glasses for astigmatism and was diagnosed with a degree of albinism. Therefore, he is very sensitive to sunlight.

SSC13890

This Caucasian female was born at 40 weeks gestation following a labor that was augmented by amniotomy. Pregnancy had been complicated by oligohydramnios and Rh incompatibility for which RhoGAM was used. At two months she had a length of 53.3 cm (-2 SD), a weight of 4500 g (-1.1 SD) and an OFC of 36.2 cm (-0.2 SD). Abnormalities in her development were first noted at three months of age and she was floppy as an infant. She had a history of constipation and projectile vomiting through age four years. She showed historically small OFC measurements. OFC was within the average range at 2 months of age, subsequently decreased to below -3 SD at one year of age and normalised during childhood (see supl table 1 for parameter values).
She was diagnosed with autism spectrum disorder at age five years (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria) . She primarily used single words and sign language/gestures to communicate. She showed significant autism-related impairments in social communication such as poor eye contact, an odd cry, minimal social smiling, lack of shared attention, use of hands as a tool, few social overtures overall and repetitive behaviors. In addition, she had a profound intellectual disability (Verbal IQ = 16, Nonverbal IQ = 32, Full Scale IQ = 27, and Adaptive Composite = 50). CT scan at six years of age was normal. She first used single words at 7.5 years of age, and had not developed phrase speech at 17 years of age. Although she had a low receptive language (PPVT Standard Score = 62), her receptive vocabulary was significantly higher than her expressive vocabulary. She showed below average to significantly impaired fine motor coordination (Purdue Pegboard Dominant Hand T scores = 19, Nondominant Hand T score = 11, and Both Hands T score = -5). She did not have behavior problems.

Further medical history revealed two foot surgeries to address abnormal features of feet/toes and extractions of supernumeray teeth. She had irregular menses, occurring every 6 – 8 weeks. There were no sleep disturbances. She wore corrective lenses. Parent report about her social responsiveness on the SRS suggests severely impacted social communication, social awareness, social cognition, social motivation, and autistic mannerisms. At 17 years of age she had a height of 164.5 cm (0.2 SD), a weight of 105 kg (+2.3 SD) and an OFC of 54.8 cm (-0.2 SD). Physical examination revealed obesity, small jaw, pointed nose, deep-set eyes, and large feet.

SSC12099

This Caucasian male was born at 41 weeks of gestation via planned caesarian section with a length of 49.9 cm (-0.1 SD) and a weight of 2900 g (-1.1 SD). Birth was complicated by meconium staining. As an infant he was floppy, lethargic and had a poor suck. Developmental abnormalities were reported at 12 months of age. He first used single words at three years and six months of age and spoke his first phrases at four years and six months. He experienced multiple febrile seizures in early childhood. At eight years of age he had a height of 119.1 cm (-1.6 SD), a weight of 22.4 kg (-1 SD) and showed microcephaly with an OFC of 47.6 cm (-3.6 SD). At 12 years he had a height of 154.4 cm (-0.2 SD), a weight of 40.1 kg (-0.7 SD) and an OFC of 50.4 cm (-2.6 SD). In addition, he had a small chin, deep-set eyes and slender posture. He had been diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria) and mild intellectual disability (Verbal IQ = 52, Nonverbal IQ = 53, Full Scale IQ = 52 and Adaptive Composite = 59) and below average receptive and expressive vocabulary (PPVT Standard Score = 76, EVT Standard Score = 72). He primarily spoke in phrases, with occasional complex language. He showed clear autism-related impairments in social communication such as unusual intonation and rhythm to speech, no participation in reciprocal conversation or social interaction, poor eye contact, repetitive/scripted speech, echolalia, sensory interests, complex motor mannerisms and toe walking. He had attention problems and anxiety. He showed excessively clumsy and uncoordinated movements and was diagnosed with mild cerebral palsy at 10 years of age. Both fine and gross motor coordination were significantly impaired (Purdue Pegboard T scores all <20 and MABC Manual Dexterity Scaled Scores = 1, MABC Aiming & Catching subtest and Balance subtest Scaled Scores = 1). He had intermittent difficulty falling asleep throughout his lifetime. He wore corrective lenses for hyperopia.

SSC13552

This Caucasian male was born vaginally at 37 weeks gestation following a labor induced by pitocin due to failure to progress. He presented with feeding difficulties and poor suck, and was fed exclusively soymilk for 12 months. Abnormalities in his development were first noted at 12 months of age. He has had febrile seizures beginning at 9 months, and approximately 8 petit mal seizures (and possibly one grand mal seizure) with an onset of 2.5 years of age. He first used single words at 2 years and 10 months of age, and first phrases at 3 years and 9 months. There is a history of recurrent otitis media with myringotomy. Patient had reflux as an infant from 6 – 12 months of age.

At 6 years of age he had a height of 109.5 cm (-1.1 SD), OFC of 48 cm (-2.9 SD) and weight of 17.5 kg (-1.2 SD). Physical examination at 10 years of age showed a height of 128 cm (-1.8 SD), OFC of 50.5 cm (-2.1 SD) and a weight of 24.9 kg (-1.8 SD). He had a triangular face with a broad forehead, bilateral epicanthal folds, prominent eyebrows, high nasal bridge, a long

thin nose, widely spaced teeth and a shallow cleft in his narrow chin. In addition, he showed mild 2-3 syndactyly, single vertical plantar crease and camptodactyly of toes.
He had been treated with growth hormones, without effect. He was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria) and mild intellectual disability (Verbal IQ = 55, Nonverbal IQ = 63, Full Scale IQ = 60, and Adaptive Composite = 69). He could speak in sentences and used relatively complex speech with recurrent grammatical errors. He showed clear autism-related impairments in social communication, repetitive speech, stereotyped interests, and some sensory interests and hand mannerisms. Patient showed below average receptive and expressive vocabulary (PPVT Standard Score = 71, EVT Standard Score = 70). Patient is reportedly excessively clumsy/uncoordinated, particularly in fine motor coordination (Purdue Pegboard Dominant Hand T scores = 29, Nondominant Hand T score = 16, and Both Hands T score = 18; MABC Manual Dexterity Standard Score = 2) as well as in gross motor coordination (MABC Aiming & Catching subtest standard score = 2 and Balance subtest Standard Score = 1).
Patient is currently taking antiepileptic medication and has had normal EEG and PET/MRI/CT scans of the brain. He has a history of difficulty falling asleep and currently experiences night-time awakenings.

Leuven_306636
This girl was the second child of healthy non-consanguineous parents. She was born by repeat cesarean section after an uneventful pregnancy at gestational age 40 weeks. She had a birth weight of 2890g (<-2 SD), length of 48 cm (<-2 SD) and OFC of 31 cm (<-2.5 SD). There was one neonatal tooth. She had neonatal feeding difficulties. She had delayed motor milestones: sitting at age 10 months, crawling at age 16 months, first steps at age 20 months. There was delayed primary dentition starting at 18 months. At the age of two years a delay in expressive language became apparent while receptive language was fairly good. She started normal school at the age of three years but was referred to special education after a few months. She is an anxious but pleasant and happy child with a lot of stereotypic behavior. She has an abnormal gait. She has short stature, microcephaly, deep-set eyes and retrognathia.

Case report of proband with inherited variant in *DYRK1A* *(listed separately as it is unlikely that this variant results in a loss of DYRK1A)*

Murdoch CRI

This male was born after an uncomplicated pregnancy with a weight of 2830 g and length of 49 cm. In the neonatal period there were feeding problems related to reflux and dairy intolerance. There were no signs of developmental delay during infancy or childhood. He showed apparent communication problems and social difficulties. He was diagnosed with Autism Spectrum Disorder during early childhood. He received intensive speech therapy which led to much improved communication and social skills. However, he does still get distracted during complex commands, depending on attentiveness. At re-contact (18 years of age) he was only very mildly affected and did not meet the cut-off for Autism Spectrum Disorder on the Autism Diagnostic Observation Schedule (ADOS-2). He responded appropriately to questions of others, although he did not elaborate during conversations. He had a T-score (social responsiveness scale-2) of 53, which is within normal limits. (Individuals with very mild ASD may show scores in the upper end of the normal range if they are well adjusted and their adaptive functioning is relatively intact). At 18 years of age he had a height of 163 cm, weight of 71.5 kg and an OFC of 58.4 cm.

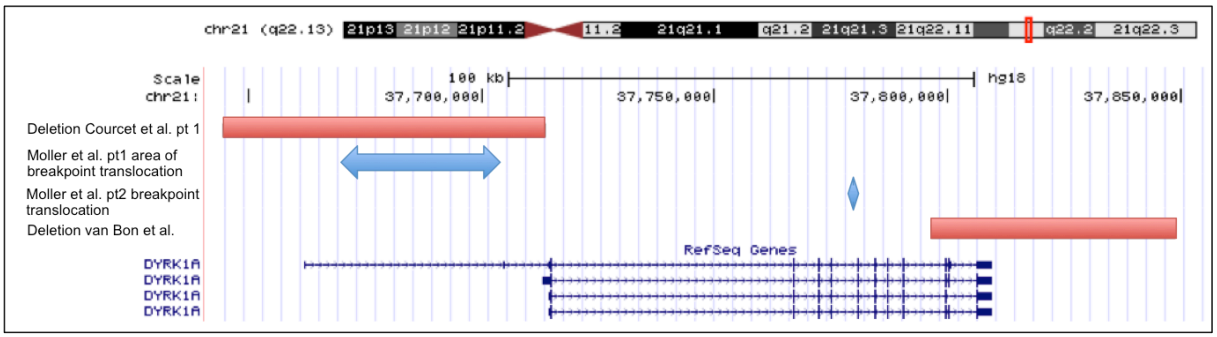
At 11 years of age he had a period of difficulties falling asleep and used melatonin for the duration of one year. Currently he has again sleep disturbances, which are likely due to stress and anxiety caused by perceived pressure of school duties. He also displays anxiety about being alone and when he was younger he was anxious around loud noises. His response to loud noises has improved, but has a 'flight' response as a natural reaction. For anxiety problems and related depression he is receiving Fluoxetine hydrocholoride (80 mg). Glucose levels were tested normal. He had a normal intelligence, is affectionate and visited a regular secondary school.

His mother was a healthy female without signs of ASD. Her T-score was 40. She had a height of 160 cm, weight of 95 kg and OFC of 57.4 cm.

Supplementary Table 1 - Predicted effects of splice-site variation.

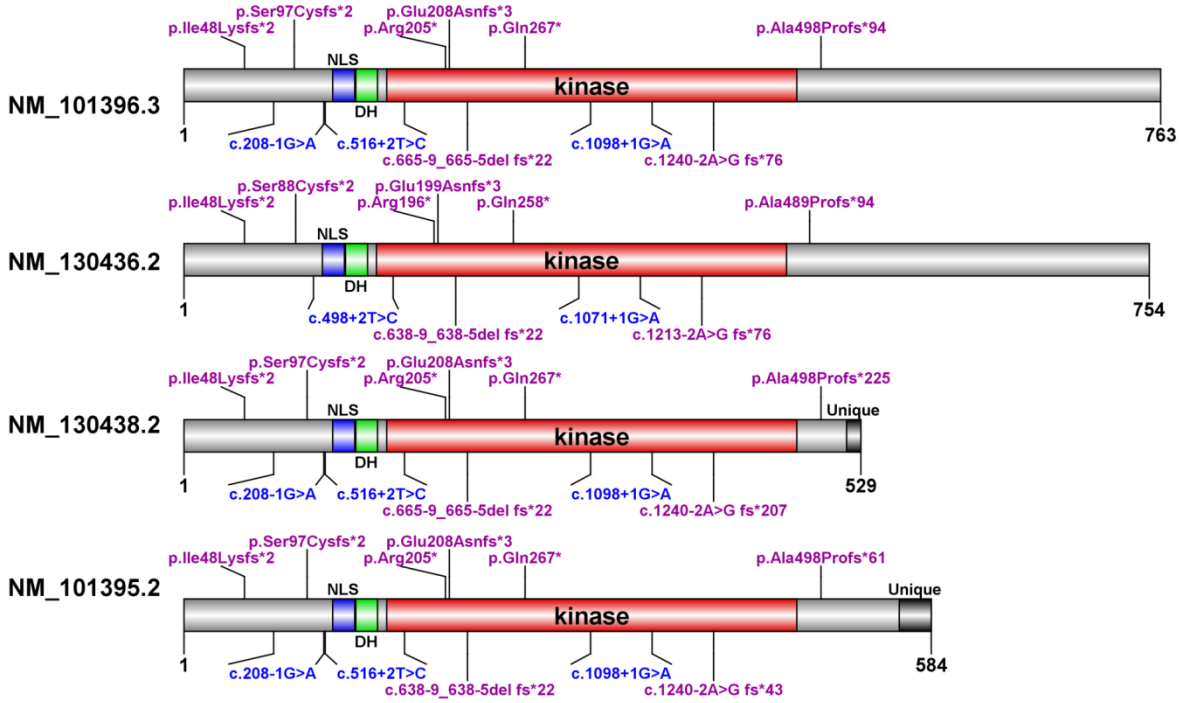
Sample	Variant	Splice Score Type	SSF [0-100]	MaxEnt [0-12]	NNSPLICE [0-1]	GeneSplicer [0-15]	HSF [0-100]	Average Splice Score Difference
SSC13890	NM_001396.3:c.1098+1G>A	Donor	84.79 ⇒—	8.39 ⇒—	0.98 ⇒—	0.56 ⇒—	86.07 ⇒—	-100.00%
UMCN 2	NM_001396.3:c.1240-2A>G	Acceptor	94.09 ⇒—	12.67 ⇒—	0.99 ⇒—	9.23 ⇒—	94.38 ⇒—	-100.00%
Troina	NM_001396.3:c.516+2T>C	Donor	87.61 ⇒87.25	8.55 ⇒—	0.88 ⇒—	1.20 ⇒—	87.68 ⇒—	-80.08%
Leuven	NM_001396.3c.665-9_665-5del	Acceptor	91.42 ⇒77.27	10.32 ⇒4.82	0.94 ⇒—	6.74 ⇒—	91.99 ⇒ 84.46	-55.39%
Murdoch	NM_001396.3:c.208-1G>A	Acceptor	82.92 ⇒—	12.16 ⇒—	—	8.96 ⇒—	81.27 ⇒—	-100.00%
		Acceptor at c.209	— ⇒76.79	— ⇒7.32	—	— ⇒4.69	— ⇒77.62	

Supplementary Figure 1



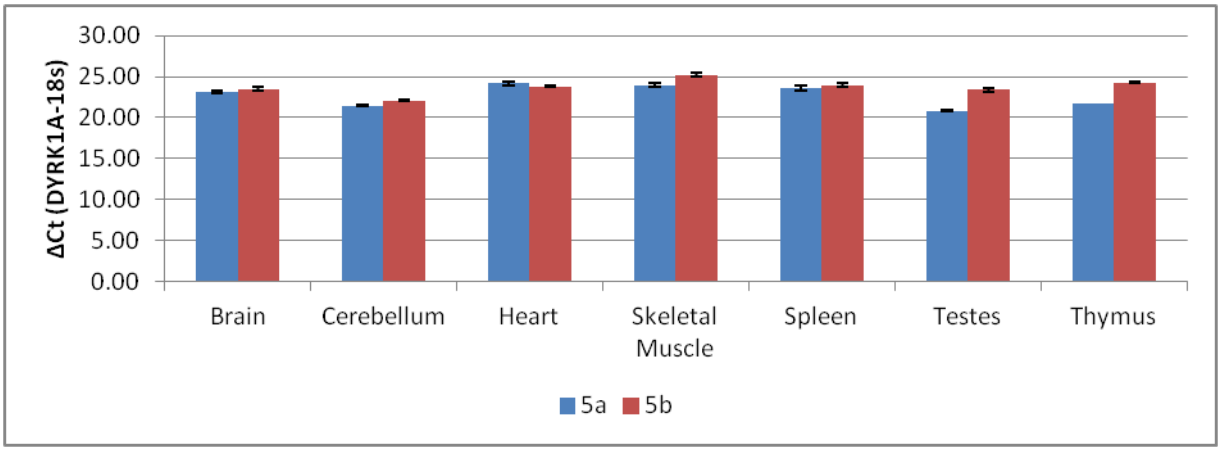
Previously published deletions are shown in red (n=2) and translocations in blue (n=2).

Supplementary Figure 2



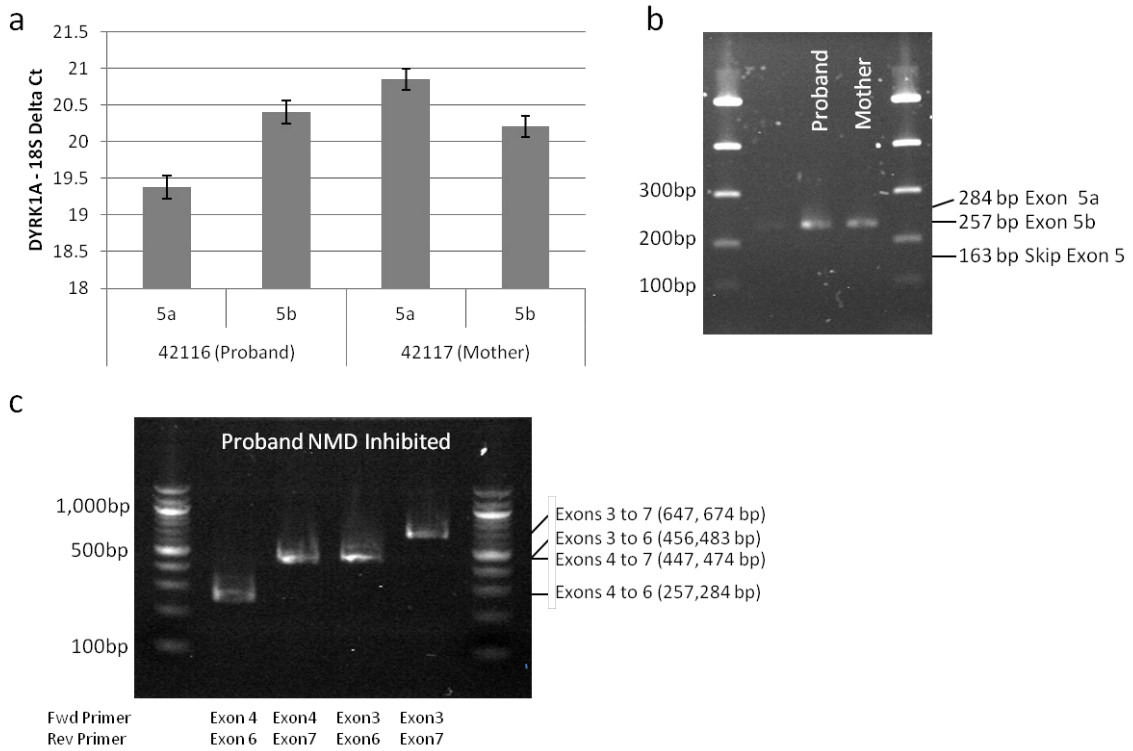
Alternative splicing events yield at least four isoforms of *DYRK1A*. Three utilize a longer version of exon 5 (exon 5a) and one uses a shorter variant (exon 5b).

Supplementary Figure 3



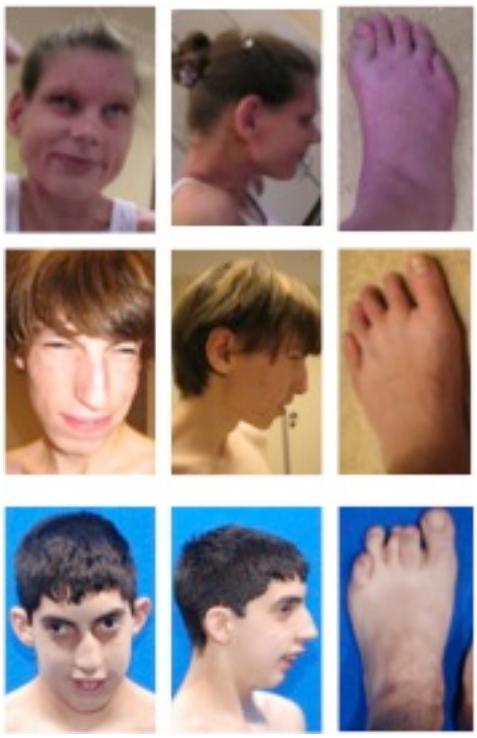
Real-time PCR detects expression of isoforms containing exons 5a and 5b across multiple tissues. PCR was performed using standard procedures with the following TaqMan primers: Hs01061661_m1 to detect exon 5a containing isoforms (NM_001396.3, NM_101395.2, NM_130438.2), and Hs01066006_m1 to detect exon 5b containing isoforms (NM_130436.2) (Life Technologies, Grand Island, NY). The cDNA panel was generated from 2ug of RNA with an ABI High-Capacity cDNA Reverse Transcription Kit (50ul reactions) using the following total RNA samples: Brain (Clontech 636530), Cerebellum (Clontech 636535), Heart (Clontech 636532), Skeletal Muscle (Stratagene MVP 540029-41), Spleen (Stratagene MVP 540035-41), Thymus (Stratagene MVP 540141-41), and Testes (Stratagene MVP 540049-14).

Supplementary Figure 4



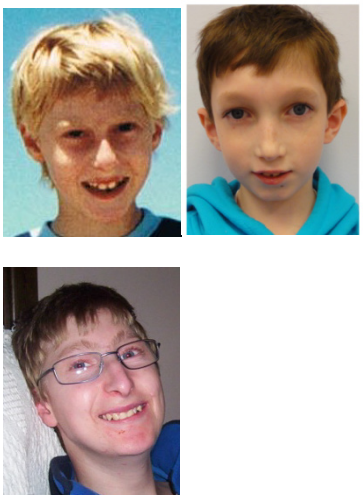
(a) Real-time PCR of the family with the inherited c.208-1G>A DYRK1A variant detects intact copies of isoforms containing both exon 5a and 5b. **(b)** PCR using primers in exon 4 (CGCCAGCCAAACATAAGTG) and exon 6 (TCATTGTAAACCTTCCGTTCC) detected no products skipping exon 5. Cloning of blunt PCR products into PCR®4 Blunt TOPO® (Life Technologies, Grand Island, NY) and Sanger sequencing detected canonical exon 5a and 5b splicing events (4 clones with 5a, 40 with 5b). **(c)** Treatment of patient cell lines with cyclohexamide to inhibit nonsense mediated decay (NMD), followed by cDNA PCR using additional primers in exon 3 (TAGTTTTGCCGCTGGACTCT), exon 4 (see above), exon 6 (see above), and exon 7 (TCTGTGCTTGATTCAGAAAAGC) did not detect any aberrant splicing events involving skipping additional exons adjacent to exon 5, or a difference in the ratio of 5a/5b containing bands (not shown, ratios of 5a/5b bands were 0.095 untreated and 0.1 treated). An additional cloning experiment using gel enrichment for the larger band again detected 10 clones with functional splicing of exon 5a. One clone with a non-canonical splice event using an alternate intronic 3' splice-site, incorporating 19 additional nucleotides was detected, which would results in a frameshift (p.Val70Serfs*6); however, this was derived from the wild-type allele evidenced by the presence of a G at the included canonical splice-site.

Supplementary Figure 5



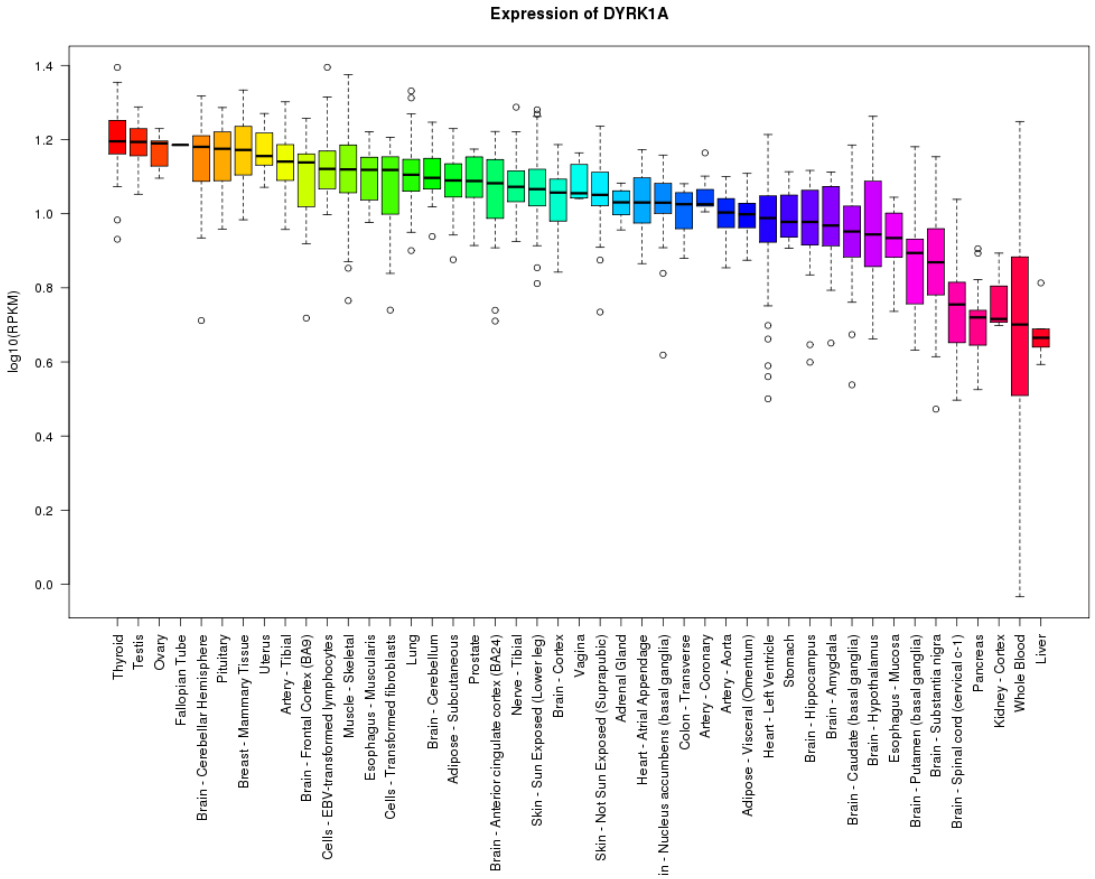
Photographs of a previously reported individual (van Bon et al.) and two novel individuals (UMCN2 and Troina1818), illustrating the typical frontal facial gestalt with deep-set eyes, mild upslanting palpebral fissures, a prominent nose with short alae nasi and the lateral facial profile illustrating the high nasal bridge and broad chin with retrognathia. In addition, most individuals show a combination of mild cutaneous syndactyly of toes 2-4, hallux valgus and a short fifth toe.

Supplementary Figure 6



The facial phenotype may be less clear during mid-childhood but may develop at later age. The individuals in the upper row are both photographed at 10 years of age (GF2852 and SSC13552). Neither showed the typical facial gestalt. However, GF2852 had developed a typical facial gestalt at 27 years of age (lower left corner). Whether SSC13552 will develop a typical facial phenotype at a later age is yet to be seen.

Supplementary Figure 7



GTEX expression data *DYRK1A* http://www.gtexportal.org/home/