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

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Abstract

Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (*DYRK1A*) maps to the Down syndrome critical region; copy number increase of this gene are thought to play a major role in the neurocognitive deficits associated with Trisomy 21. Truncation of *DYRK1A* in patients with

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CONFLICT OF INTEREST

E.E.E. is on the scientific advisory board (SAB) of DNAnexus, Inc.

WEB RESOURCES [September 2014 accessed]

The URLs for data presented herein are as follows:

Database of Genomic Variants, <http://projects.tcag.ca/variation/>

Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle WA: <http://evs.gs.washington.edu/EVS/>

The Genotype-Tissue Expression project portal <http://www.gtexportal.org/home/>

Human protein reference database: <http://www.hprd.org>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

UCSC genome browser: <http://genome.ucsc.edu/>

Universal Protein Resource: <http://www.uniprot.org>

Supplementary information is available at Molecular Psychiatry's website

developmental delay (DD) and autism spectrum disorder (ASD) suggests a different pathology associated with loss-of-function mutations. To understand the phenotypic spectrum associated with *DYRK1A* mutations, we resequenced the gene in 7,162 ASD/DD patients (2,446 previously reported) and 2,169 unaffected siblings and performed a detailed phenotypic assessment on nine patients. Comparison of our data and published cases with 8,696 controls identified a significant enrichment of *DYRK1A* truncating mutations ($p = 0.00851$) and an excess of *de novo* mutations ($p = 2.53 \times 10^{-10}$) among ASD/intellectual disability (ID) patients. Phenotypic comparison of all novel ($n = 5$) and recontacted ($n = 3$) cases to previous case reports, including larger CNV and translocation events ($n = 7$), identifies a syndromal disorder among the 15 patients. It is characterized by ID, ASD, microcephaly, intrauterine growth retardation, febrile seizures in infancy, impaired speech, stereotypic behavior, hypertonia, and a specific facial gestalt. We conclude that mutations in *DYRK1A* define a syndromic form of ASD and ID with neurodevelopmental defects consistent with murine and *Drosophila* knockout models.

INTRODUCTION

Autism spectrum disorder (ASD) is a genetically and clinically heterogeneous neurodevelopmental disorder representing various different subtypes of social communication dysfunction and unusually restricted interests or repetitive behavior.¹ Rarely, monogenic ASD subtypes can be clinically recognized, such as in individuals with macrocephaly and a *PTEN* or *CHD8* mutation, developmental regression in Rett syndrome, and typical dysmorphisms in Fragile X syndrome and tuberous sclerosis.^{2, 3} Recently, a potentially novel form of syndromic ASD caused by *de novo* mutations in *ADNP* (MIM 611386) was reported.⁴ Exome sequencing studies of ASD have identified *de novo* mutations in candidate genes, with a limited number of recurrent mutations owing to the extensive locus heterogeneity underlying this disease.^{5, 6} We recently applied a targeted resequencing approach of candidate genes to a large cohort of affected individuals to identify potentially monogenic subforms of ASD for further investigation.^{7, 8} Recurrent disruptive mutations in six genes were shown to contribute to 1% of sporadic ASD, including *DYRK1A* (MIM 600855) in three individuals (3/2,446) with ASD and microcephaly suggesting that it might be one of the most common genes associated with *de novo* truncating mutations after *CHD8*.⁷

DYRK1A, a member of the dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase (DYRK) family, is a highly conserved gene located in the Down syndrome critical region on chromosome 21. When present in three copies, *DYRK1A* is one of two genes thought to be primarily responsible for neurocognitive deficits associated with Down syndrome.^{9, 10} Previously, disruption due to translocation or deletion of *DYRK1A* was reported in four individuals with intellectual disability (ID), primary microcephaly and overlapping facial dysmorphisms (Supplementary Figure 1) pointing towards a possible emerging syndrome.^{11–13} Four individuals with *DYRK1A* variants have recently been concisely reported. The lack of microcephaly in one of these questioned the variability of the associated phenotype.^{13–15} To determine the core phenotype of the *DYRK1A* disruption, we targeted *DYRK1A* (NM_001396.3) for resequencing in a mixed cohort of individuals referred for ID, epilepsy and/or ASD.

MATERIALS AND METHODS

Patient and Control Samples

The resequencing cohort consisted of 3,387 patients with ID/developmental delay (DD) and 1,329 patients with DD or a clinical or psychiatric definition of autism. For the purpose of this study, ID was defined as individuals with an IQ < 70. In addition, we resequenced 2,193 unaffected siblings from the Simons Simplex Collection (SSC) as controls. The independent exome sequencing cohort consisted of 20 sporadic patients displaying syndromic forms of microcephaly. For 17/20 samples (including the one reported in this paper) parental DNA was available for library preparation, capturing and whole exome sequencing (WES) allowing trio-based variant filtering. This study was approved by the institutional review boards at collaborating institutions (Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen NL36191.091.11; University of Washington HSD #42744; for the exome sequencing cohort Commissie Medische Ethiek (MEC) UZ KU Leuven/Onderzoek S-52853). Written informed consent was obtained for all individuals. We also included three patients identified in a resequencing screen of 2,446 probands from the SSC initially sequenced in O’Roak et al.⁷ An additional 6,503 controls were sourced from the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project.

Clinical Assessment

We performed a detailed clinical assessment of all eight patients with sporadic *DYRK1A* mutations, including the five new patients described here as well as three individuals with ASD for whom there was previously only limited clinical information available.⁷ Additionally we assessed a ninth patient whose variant was determined to not likely lead to *DYRK1A* loss of function. Patients underwent a thorough assessment, including physical exam, dysmorphological assessment, a review of medical history, and comprehensive neurocognitive and diagnostic battery. The battery included clinician observation and parent reporting across domains of cognition, memory, language, motor, executive functioning, social, repetitive and atypical behaviors, and adaptive ability. In addition, psychiatric presentation was evaluated and gold standard ASD assessments (Autism Diagnostic Observation Schedule (ADOS), Autism Diagnostic Interview - Revised, clinical judgment) were completed for patients where possible.

Molecular Inversion Probe (MIP) Resequencing

We designed 38 overlapping MIPs designed to cover the protein-coding sequence of the RefSeq *DYRK1A* isoforms (NM_101396.3, NM_130436.2, NM_101395.2, and NM_130438.2) and performed sequencing and analysis as described in O’Roak et al. Briefly, samples were barcoded and 192 samples were sequenced using a paired-end 101 bp protocol per HiSeq 2000 (Illumina, San Diego, CA, USA) lane. Sequences were then trimmed to remove the MIP sequencing primer sites and to remove any overlap between the paired reads. Sequences were aligned using BWA v0.5.6 and variants were identified using SAMtools v0.1.7. Annotation of predicted functions was performed using SeattleSeq, while Alamut (Interactive Biosoftware, Rouen, France) was used to predict splice effects. All variants were validated using standard PCR and Sanger sequencing. The majority of MIPs demonstrated high coverage (>20X) in >95% of QC-passing samples, covering 93.3% of the

largest isoforms' coding sequence. Four MIP designs failed to generate coverage (<8% of samples covered) representing a loss of 6.7% of the largest isoform, with 99% of the missing sequence at a low complexity segment of exon 13: NM_101396.3:c.950_951 in exon 8, NM_101396.3:c.1768_1921 in exon 13. The additional low-coverage region is specific to one short isoform in exon 12b NM_101395.2:c.1751to c.1755.

Whole-Exome Sequencing

Sequencing libraries were prepared with the TruSeq DNA Sample Prep Kit (Illumina, San Diego, CA, USA) and enriched with the SeqCap EZ Human Exome Library v3.0 kit (Roche NimbleGen, Madison, WI, USA). The samples were then sequenced on an HiSeq 2000 or 2500 machine (Illumina). Paired-end sequence reads were aligned to the human genome reference sequence (hg19) with the Burrows-Wheeler Aligner (v0.6.2). SAMtools (v0.1.18) was used for SAM to BAM files conversion, sorting and indexing alignments. Quality metrics were calculated using Picard tools (v1.78) and PCR-generated duplicates marked for downstream analysis. The Genome Analysis Toolkit (GATK v2.4.9) software package was used to perform local realignment, base call recalibration and single-nucleotide polymorphism (SNP) calling. For each called SNP, the GATK Unified Genotyper tool was used to estimate the most likely genotypes. Variant annotation was performed with ANNOVAR (v11-02-2013), including data sets from dbSNP137, the NHLBI 6500 Exome and 1000 Genomes projects for variant frequencies, amino acid change functional predictions from SIFT, Polyphen2, LRT, MutationTaster and PhyloP, GERP++ conservation scores. Trio-based filtering of the annotated variants was manually conducted according to a *de novo* inheritance pattern for genotype prediction filtering; "knowngene"-based annotations were used for further filtering: only exonic, splicing, non-silent and rare variants (MAF < 1% or absent in all the aforementioned databases) were included. Candidate *de novo* variants were confirmed by standard Sanger sequencing using the BigDye Terminator v3.1 chemistry (Life Technologies). Sequencing traces were then visualized with Alamut (Interactive Biosoftware).

RESULTS

To determine the frequency and core phenotype of the *DYRK1A* disruption, we targeted *DYRK1A* using MIP sequencing, which was applied to 4,716 new cases (3,387 cases of ID/DD and 1,329 cases of ASD).⁷ In addition, we incorporated 2,446 cases of ASD previously assayed for *DYRK1A* mutations.⁷ Inheritance status of potentially disruptive mutations were assessed using standard Sanger sequencing approaches on DNA extracted from peripheral blood of probands and parents where available.

In total, we observed eight putative truncating variants (3 from a previous screen⁷ and 5 from the 4,716 new cases) in a screen of 7,162 probands and one additional patient based on whole-exome sequencing in a separate cohort of 20 individuals presenting with microcephaly. No truncating variants in *DYRK1A* were observed in the NHLBI Exome Sequencing Project (ESP) in 6,503 individuals. We also resequenced the gene in 2,193 unaffected siblings from the SSC and found no occurrences of truncating mutations, indicating that recurrent truncating mutations were significant for ID/ASD ($p = 0.0017$,

Fisher's exact test). In all but one case where parental DNA was available (8/9), the *DYRK1A* mutations were shown to be *de novo*. Applying a *de novo* rate of 1.2 nonsynonymous coding events per individual and a probabilistic model derived from human-chimpanzee fixed differences and sequence context,⁷ we calculated the probability of detecting seven *de novo* truncating events in *DYRK1A* within our cohort as $p = 2.53 \times 10^{-10}$ (binomial test).

The *de novo* heterozygous disruptive mutations included two nonsense (c.798_799delinsTT and c.367C>T), two frameshift (c.143_144del and c.1491del), and four splice-site (c.1098+1G>A, c.1240-2A>G, c.516+2T>C and c.665-9_665-5delTTCTC) variants, which are predicted to disrupt the local splice-site (Figure 1, Supplementary Table 1). The nonsense, frameshift and one splice-site variants all predict premature stop codons leading to a potential loss of function. The c.143_144del frameshift mutation leads to p.Ile48Lysfs*2 (the earliest truncation event identified in this study) and occurs before all annotated functional elements in the *DYRK1A* protein. The c.1491del leads to p.Ala498Profs*94, which occurs after the known functional elements. The c.1240-2A>G splice-site mutation likely results in the loss of exon 11 leading to p.Glu414Valfs*76 in the protein kinase domain disrupting the helix-turn-helix functional element. The c.516+2T>C splice-site mutation is predicted to lead to an in-frame deletion of exon 6, containing the nuclear localization sequence, DYRK homology box, a confirmed phosphorylation site, and part of the kinase domain. The c.1098+1G>A splice-site mutation is predicted to result in an in-frame deletion of exon 9, which is internal to the protein kinase domain, eliminating several annotated helices and disrupting the critical activation segment that is likely to affect the function of the protein.¹⁶

The sole inherited c.208-1G>A splice-site variant was detected in an individual who had been diagnosed with Asperger's disorder during childhood. 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 2). Both the long and short isoforms are expressed in various tissues, including the brain (Supplementary Figure 3). In contrast to the *de novo* splice variants, this *DYRK1A* variant does not affect all four isoforms. It is predicted to lead to loss of the in-frame exon 5a, which occurs before any annotated functional or structural elements, and was also predicted to create a novel splice acceptor site one base into exon 5a, potentially generating a premature stop (p.Val70*) in the three RefSeq isoforms that utilize exon 5a but not in the isoform that utilizes exon 5b (Supplementary Table 1). qPCR and cloning of the splice junction from cDNA showed that the proband expresses intact copies of both isoforms (Supplementary Figure 4). It is, therefore, unlikely that this variant results in a loss of *DYRK1A* function. The clinical details of these and previously reported individuals, including translocation and deletion patients (n = 15, age range 2–69 years), are presented in Table 1, Figure 1, Supplementary Note case descriptions, Supplementary Video and Supplementary Figures 5 and 6.

DISCUSSION

To determine the core phenotype of the *DYRK1A* disruption, we targeted *DYRK1A* for resequencing in a mixed cohort of individuals referred for ID, epilepsy and/or ASD. In total,

we observed eight *de novo* truncating variants in a screen of 7,162 probands and one additional patient based on whole-exome sequencing in a separate cohort of 20 individuals presenting with microcephaly. In all but one case where parents were available, the *DYRK1A* mutations were shown to be *de novo*. We estimated the probability of detecting seven *de novo* truncating events in *DYRK1A* within our cohort as $p = 2.53 \times 10^{-10}$. Moreover, all other previously reported cases, which included parental testing, including two balanced translocations, two single gene deletions and three mutations, were also shown to be *de novo* (Supplementary Figure 1).^{11–14}

Unlike the *de novo* splice variants, the inherited *DYRK1A* variant affects only three of the four isoforms. We showed that the proband expresses intact copies of both major isoforms (Supplementary Figure 4) despite the fact that five different algorithms predicted 100% loss of the splice-site (Supplementary Table 1). It is interesting that the variant sequence observed in this patient and its mother is consistent with nine non-canonical human splice-sites with a GT-AA sequence.¹⁷ This is consistent with the patient's rather atypical phenotype when compared to the other patients. This individual did not meet the criteria for ASD at 18 years of age and showed a normal intelligence without microcephaly. Moreover, his carrier mother is a normal healthy individual. Although it is possible that this variant may still contribute to splicing during development and that the mother may be buffered for its effect, it is more likely that this variant has no consequence despite its prediction. Similar to this case, we excluded another recently published variant from this review due to uncertain pathogenicity.¹⁵ Not only was parental testing not described for this case but the variant (c.1699C>T/p.Gln557*) is located late in the protein past all annotated functional domains and is unlikely to induce nonsense-mediated decay.¹⁸ These examples serve as a cautionary note in the interpretation of putative loss-of-function mutations and diagnostic interpretation.

Microcephaly was noted in all 15 individuals after birth or in the first months thereafter. One individual showed microcephaly in infancy (-4.5 SD at four years of age), which remarkably resolved to -1.6 SD at 17 years of age. Information on the presence or absence of ASD was not provided in six out of seven of the previously reported cases and it may be questioned whether ASD was formally assessed, especially considering the young age of assessment for three of these individuals (< 5 years). In the remainder of the cohort, ASD was reported in the majority of cases (88%). Stereotypic behavior was present in 91% of individuals and anxious behavior in 56%. All individuals experienced apparent speech problems; notably, expressive language was more severely affected compared to receptive language as the majority did not speak or only used 1- to 2-word sentences (Supplementary Video).

In almost all individuals a specific facial gestalt could be recognized, especially at an older age. During infancy and childhood, the face is characterized by deep-set eyes, mild upslanting palpebral fissures, a short nose with a broad tip, and retrognathia with a broad chin. In adulthood, the nasal bridge becomes high and the alae nasi short, giving the nose a more prominent appearance. In case the facial gestalt is not clearly visible in childhood, it may still develop by adulthood (Supplementary Figure 6). Febrile seizures were present in the majority of individuals (77%), whereas at a later age, epilepsy was diagnosed in only five individuals (33%). Nine individuals showed clear motor disturbances characterized by

an abnormal gait and eight individuals showed hypertonic musculature. In general, the level of ID was variable. Moderate to severe ID was noted in 12 individuals (80%). Mild ID was noted in three individuals (20%)—one with a translocation and two with a frameshift mutation. This may reflect a variable genotype–phenotype correlation based upon the type of *DYRK1A* truncation as, for instance, the p.Ala498Profs*94 mutation does not disrupt the main functional domain and is predicted to be less impactful to the alternative shorter isoforms. The previously reported translocation does not affect these *DYRK1A* isoforms either.

From our detailed clinical evaluation, we conclude that truncating mutations in *DYRK1A* lead to a well-recognizable syndromic form of ASD and ID, characterized by a specific facial gestalt, microcephaly, lack of speech, seizures, neonatal feeding problems, hypertonia, and gait disturbances. Considering the core phenotype delineated within this study, *DYRK1A* testing should be considered when autism and/or ID is accompanied by microcephaly, especially when two or more of the additional characteristic key features are present, such as the typical facial gestalt, seizures, hypertonia, feeding problems, or an abnormal gait. Cardiac and ophthalmologic evaluation should be performed in all affected individuals as anomalies have been reported in 18% and 27% of cases, respectively (Table 1).

The clinical presentation of individuals with *DYRK1A* mutations demonstrates some neurodevelopmental features in common with *Drosophila* and mouse models where mutants show defects in cell proliferation, neurogenesis, neuronal differentiation, cell death and synaptic plasticity. Mutants of the *Drosophila* ortholog, Minibrain (*Mnb*) gene show greatly reduced adult brains (40–50%) with no gross changes in neuronal architecture reported.^{19, 20} Similarly, newborn *Dyrk1a* +/- mice show a decreased viability, pre- and postnatal growth retardation, DD and behavioral abnormalities, including increased anxiety and altered reactivity to stress.²¹ Loss of function of *Dyrk1a* in mice results in a 30% reduction in brain size, with a disproportionate reduction in the size of the mid and hindbrain regions.^{19, 20} Brain architecture remains largely unchanged, which is in concordance with brain imaging results in humans where, except for corpus callosum hypoplasia and cortical atrophy, no major structural anomalies were noted (Table 1). In humans, expression of *DYRK1A* is high in multiple tissues, including different areas in the developing fetal and adult brain (Supplementary Figures 3 and 7). In mice, *Dyrk1a* is highly expressed in the developing and adult nervous system, most abundantly in the cerebellar cortex and functionally related structures, the spinal cord and most of the motor nuclei of the midbrain and brain stem.²² These data are in line with the altered locomotor activity, including gait disturbances, observed in both adult *Dyrk1a* +/- mice and affected individuals. In addition, the presence of *DYRK1A* in the presynaptic terminal of the neuromuscular junction suggests a physiological role of *DYRK1A* in the function of the nervous system.²³ *DYRK1A* has also been linked to retinal development in *Drosophila* and mouse models, which may relate to the observed retinal detachment in one of the individuals.^{24–26}

Half of the individuals displayed sleep disturbances characterized by difficulties in falling asleep and nighttime awakenings, although this observation does not reach statistical significance when compared to its prevalence among ASD patients. Of note, *DYRK1A* has been proposed as a clock-related protein kinase, influencing the regulation of the protein

level of CRY2 and shortening the period length of the circadian clock in *Dyrk1a* knockdown mice.²⁷ ASD, stereotypic behavior and anxious behavior were frequently noted. Similarly, *Dyrk1a*^{+/-} mice demonstrate an enhanced freezing response, suggesting either increased anxiety or changes in emotional behavior.¹⁹

Although the molecular function of *DYRK1A* is not completely understood, it is noteworthy that Mnb, the *Drosophila* orthologue of *DYRK1A*, has been shown to interact with *Snr1*, the *INI* orthologue, which is a member of the SWI/SNF complex involved in the morphogenesis of dendritic arbors in *Drosophila* sensory neurons.^{28, 29} Similarly, in mice, *DYRK1A* binds a SWI/SNF complex suggesting a potential role in chromatin remodelling.³⁰ In the light of the importance of germline mutations in chromatin remodelling complexes for neurodevelopmental disease,^{3, 31–35} detailed investigation into these complexes and networks may reveal additional pathogenic mutations and potential novel syndromes associated with ID and autism.^{3, 36} Our results, however, caution that diagnostic interpretation of mutations should not rely solely on the predicted impact based on gene annotation even for high-penetrant genes such as *DYRK1A*. Rather, it is critical to consider familial transmission, isoform differences, and functional assessment of putative truncating mutations in conjunction with the clinical assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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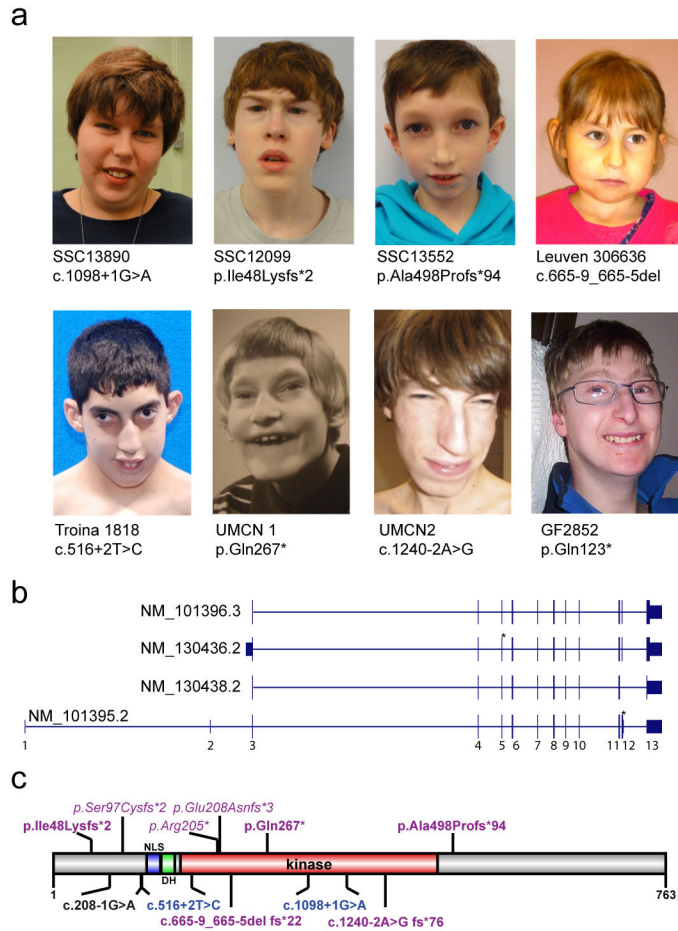


Figure 1.
a) Characteristic facial features can be noted in SSC12099, Troina1818, UMCN1, GF2852, UMCN2, and Leuven 306636. During infancy and childhood, the face is characterized by deep-set eyes, mild upslanting palpebral fissures, a short nose with a broad tip, and a retrognathic but prominent chin. In adulthood, the nasal bridge becomes high and the alae nasi short, giving the nose a more prominent appearance. If the facial gestalt is not clearly visible in childhood, it may still develop in adulthood (Supplementary Figure 1B). **b)** RefSeq *DYRK1A* splice isoforms examined in this study. Resequencing covered all coding exons (3 to 13). An asterisk indicates alternatively spliced exon variants. **c)** Diagram of the largest protein isoform (RefSeq: NM_101396.3, UniProt: Q13627). Truncating variants are listed in purple. Splice variants are on the bottom, referenced by their cDNA effect for in frame exon loss (blue, or black if not likely pathogenic) or likely protein effect. Previously published variants from Redin et al. and Courcet et al. are indicated in italics.^{13, 14}

TABLE 1

CLINICAL FEATURES

Individuals ID	Total	%	Moller et al	Moller et al	van Bon et al	Couret et al	Couret et al	Redin et al	Redin et al	SSC1352 O'Roak et al.	SSC12099 O'Roak et al.	SSC1890 O'Roak et al.	UMCN1	UMCN2	Troina 1818	GF2852	Laxen 30656	Murdock CRI	
Age (years)			2	13	37	4	14	16	5	10	12	17	59	23	16	32	10	18	
Genotype			T(9;21)(p12;q22)	T(2;21)(q22;q22)	intra-genic deletion	intra-genic deletion	c.290_291 delCT	c.613C>T	c.621_624 delinsGAA	c.1491del	c.143_144 del	c.1098 +1G>A	c.799C>T	c.1240>2A>G	c.516 +2T>C	c.367C>T	c.665>9_665-5delTTCTC	c.208-1G>A	
De novo occurrence					+	+	+	+	+	+	+	+	+	+	+	+	+	***	
Mutation type			Trans location	Trans location	del exon 9-11	del exon 1	Frameshift	Nonsense	Frameshift	Frameshift	Frameshift	Splice	Nonsense	Splice	Splice	Nonsense	Splice	Splice	
Gender			M	F	F	F	M	M	M	M	M	F	F	M	M	M	F	M	
Microcephaly	15/15	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
ID	15/15	100	Mild	Severe	Severe	Global	Moderate	Moderate	Moderate	Mild	Mild	Severe	Severe	Moderate	Severe	Mild-moderate	Mild-moderate	-	-
ASD	7/8	88	U	U	+	U	U	U	U	+	+	+	U	+	-	+	+	+	***
Stereotypic behavior	10/11	91	+	U	+	U	U	U	U	+	+	+	-	+	U	+	+	+	-
Anxious behavior	5/9	56	U	U	+	U	U	U	U	-	+	-	-	-	+	+	+	+	+
Hyperactive behavior	3/11	27	U	U	-	+	+	+	U	-	-	-	-	+	U	-	-	-	-
Impaired speech	15/15	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
IUGR	9/11	82	+	+	+	+	U	U	U	U	-	U	+	+	+	+	+	+	-
Short stature	6/13	46	+	-	-	+	U	U	U	-	-	-	+	+	-	-	+	+	+
Febile seizures infancy	10/13	77	+	+	+	+	U	U	U	+	+	+	+	+	+	+	+	+	-
Epilepsy	5/15	33	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
Normal brain MRI/CT	6/11	55	CCH	U	BA	N	U	EV	U	N	U	N	U	N	MD, CCH, BA	N	N	U	U
Hypertonia	8/9	89	U	U	U	-	U	U	U	U	+	+	+	+	+	+	+	+	-
Abnormal gait	9/12	75	+	U	+	+	U	U	U	-	+	+	+	-	+	+	+	+	-
Neonatal feeding problems	15/15	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Childhood feeding problems	7/12	58	U	+	+	+	U	U	U	-	-	-	+	+	U	-	-	-	-
Typical facial gestalt	10/12	83	+	+	+	+	U	U	U	-	+	+	+	+	+	+	+	+	-
Slender Build	9/13	69	-	+	+	+	U	U	U	+	+	-	-	+	-	+	+	+	-
Scoliosis/kyphosis/pectus excavatum	6/12	50	-	+	-	U	U	U	U	-	+	-	+	+	+	-	-	-	-
Foot anomalies	10/10	100	U	SFT	HV, SFT	U	U	U	U	CT	CS	FD	FD	CS, SFT	IW, CS, SFT, HV	HAF, LH	HAF, LH	-	
Other skeletal anomalies	6/9	67	U	U	U	U	U	U	U	-	A	SN	C, K	-	TOCE	SH	-	-	
Cardiac defect	2/11	18	-	VSD, AOV	-	U	U	U	U	-	-	-	AS	-	-	-	-	-	-
Sleeping disturbances	4/8	50	U	U	U	U	U	U	U	+	+	-	-	-	U	+	-	+	+
Additional features			IH, HM	BAP	BAP		HM, AST, OP			CSF		SEC	HL, RD		DPD, AST		DPD		-

Abbreviations: A = arachnodactyly; AOV = aortic valve insufficiency; AS = aortic stenosis; AST = astigmatism; BA = brain atrophy; BAP = breast aplasia; C=contractures, CCH = high arch foot; CS = corpus callosum hypoplasia; CS = partial cutaneous syndactyly 2-4 toes, CSF = mild 2-3 syndactyly of fingers, CT = camptodactyly toes, E = exon; EV= enlarged ventricles; DPD = delayed primary dentition; F = female; EV= hearing loss; HM = hypermetropia +6dpt; HAF= hallux valgus; IH = inguinal hernia;

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IT=irregular toe implant; IW= interdigital webbing; K=kyphosis, LH=long hallux; M = male; MD = myelination delay; N = normal; nr = number of opisthodontos episodes; PD = polydactyly; RD = retinal detachment; SDP = short distal phalanges; SFT = short fifth toe; SEC=small ear canal; SH=small hands, SN = supernumary teeth; TOCE = tibial osteochondrosis and exostoses; u = unknown; VSD = ventricle septum defect;

* Patient was microcephalic during infancy, but within range of normocephaly during puberty (-1.6 SD).

** Only mother available for testing; no mutation.

*** Patient with inherited variant, listed separately as it is unlikely that this variant results in a loss of *DYRK1A*. At 18 years of age, this individual did not meet the cut-off for ASD on the Autism Diagnostic Observation Schedule – 2nd edition (ADOS-2).