

ORIGINAL ARTICLE

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

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Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1 A (*DYRK1A*) maps to the Down syndrome critical region; copy number increase of this gene is thought to have a major role in the neurocognitive deficits associated with Trisomy 21. Truncation of *DYRK1A* in patients with 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 7162 ASD/DD patients (2446 previously reported) and 2169 unaffected siblings and performed a detailed phenotypic assessment on nine patients. Comparison of our data and published cases with 8696 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 with previous case reports, including larger CNV and translocation events ( $n=7$ ), identified a syndromal disorder among the 15 patients. It was 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.

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## INTRODUCTION

Autism spectrum disorder (ASD) is a genetically and clinically heterogeneous neurodevelopmental disorder representing various subtypes of social communication dysfunction and unusually restricted interests or repetitive behavior.<sup>1</sup> 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.<sup>2,3</sup> Recently, a potentially novel form of syndromic ASD caused by *de novo* mutations in *ADNP* (MIM 611386) was reported.<sup>4</sup> 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.<sup>5,6</sup> 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.<sup>7,8</sup> Recurrent disruptive mutations in six genes were shown to contribute to 1% of sporadic ASD, including dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1 A (*DYRK1A*; MIM 600855) in three

individuals (3/2446) with ASD and microcephaly, suggesting that it might be one of the most common genes associated with *de novo* truncating mutations after *CHD8*.<sup>7</sup>

*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 the two genes thought to be primarily responsible for neurocognitive deficits associated with Down syndrome.<sup>9,10</sup> 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.<sup>11–13</sup> 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.<sup>13–15</sup> 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.

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## MATERIALS AND METHODS

### Patient and control samples

The resequencing cohort consisted of 3387 patients with ID/developmental delay (DD) and 1329 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 2193 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 out of 20 samples (including the one reported in this paper) parental DNA was available for library preparation, capturing and whole-exome sequencing, allowing trio-based variant filtering. This study was approved by the institutional review boards at the 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 2446 probands from the SSC initially sequenced in O'Roak *et al.*<sup>7</sup> An additional 6503 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.<sup>7</sup> 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 the 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, 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.*<sup>7</sup> 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 Burrows-Wheeler Aligner 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 the variants were validated using standard polymerase chain reaction (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 and 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.1751toc.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, nonsilent 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).

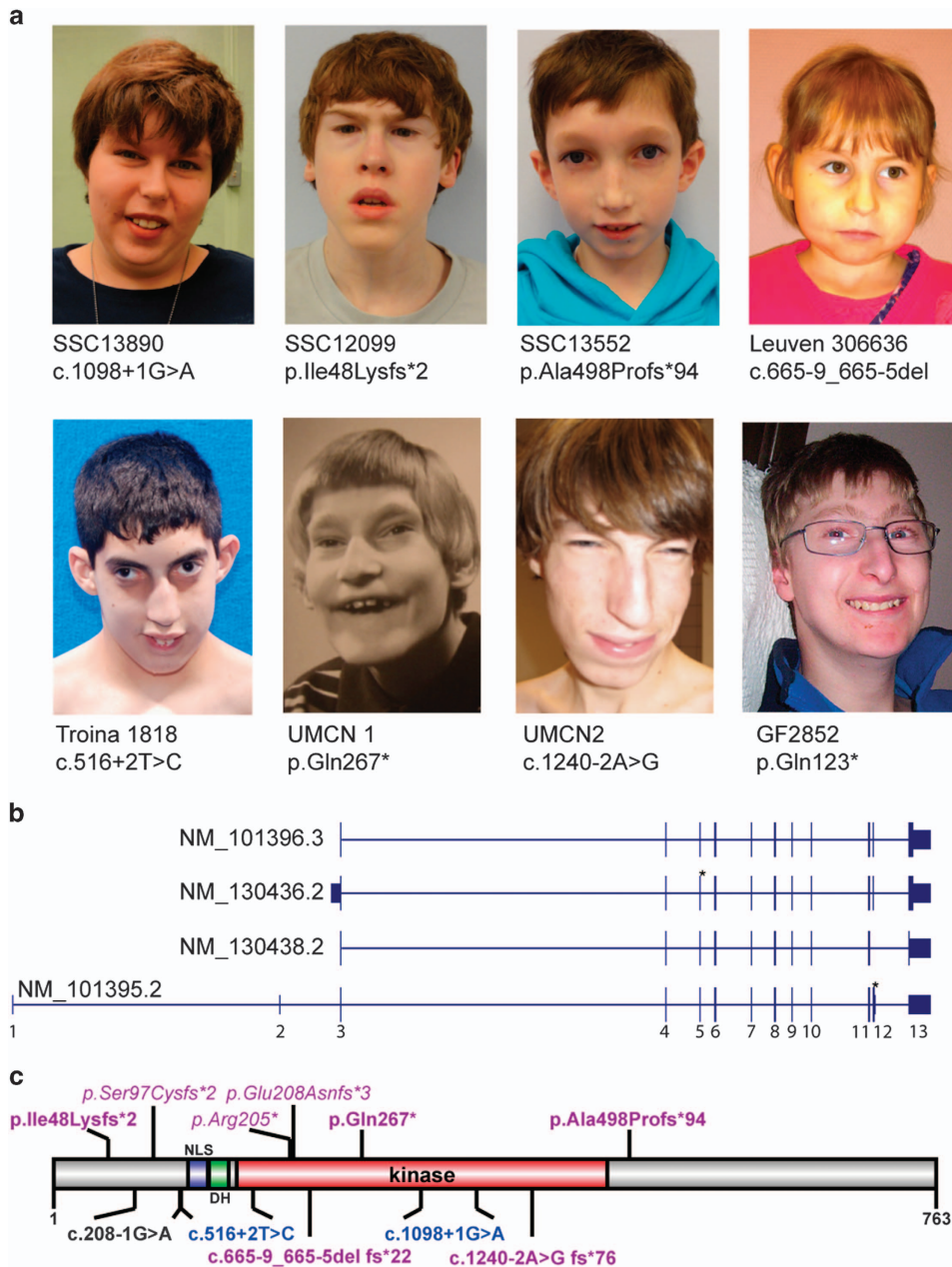
## RESULTS

To determine the frequency and core phenotype of the *DYRK1A* disruption, we targeted *DYRK1A* using MIP sequencing, which was applied to 4716 new cases (3387 cases of ID/DD and 1329 cases of ASD).<sup>7</sup> In addition, we incorporated 2446 cases of ASD previously assayed for *DYRK1A* mutations.<sup>7</sup> The inheritance status of potentially disruptive mutations was assessed using standard Sanger sequencing approaches on DNA extracted from the peripheral blood of probands and parents where available.

In total, we observed eight putative truncating variants (three from a previous screen<sup>7</sup> and five from the 4716 new cases) in a screen of 7162 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 in 6503 individuals. We also resequenced the gene in 2193 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,<sup>7</sup> 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 inframe 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 inframe 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.<sup>16</sup>

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



**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 inframe exon loss (blue, or black if not likely pathogenic) or likely protein effect. Previously published variants from Redin *et al.*<sup>14</sup> and Courcet *et al.*<sup>13</sup> are indicated in italics. *DYRK1A*, dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A.

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 inframe 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

**Table 1.** Clinical features

Individuals' ID	Total	%	Moller et al.	van Bon et al.	Courcet et al.	Courcet et al.	Redin et al.	Redin et al.	O'Roak et al.	UMCN1	UMCN2	Troina 1818	GF2852	Leuven 306636	Murdoch CRI				
									SSC13552	SSC12099	SSC13890								
Age (years)	15/15	100	2	13	37	4	14	16	5	10	12	17	59	23	16	32	10	18	
Genotype	15/15	100	T(9;21)(p12;q22)	T(2;21)(q22;q22)	Intragenic deletion 9-11	Intragenic deletion	c.290_291delCT	c.613C>T	c.621_624delinsGAA	c.1491del	c.143_144del	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			Trans location M	Trans location F	Del exon 9-11 F	Del exon 1 F	Frameshift +	Nonsense +	Frameshift +	Frameshift +	Frameshift +	Splice +	Nonsense +	Splice +	Splice +	Nonsense +	Splice +	Splice +	
Mutation type			M	F	F	F	F	M	M	M	M	F	F	M	M	M	M	M	M
Gender																			
Microcephaly ID	15/15	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ASD	7/8	88	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Stereotypic behavior	10/11	91	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Anxious behavior	5/9	56	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Hyperactive behavior	3/11	27	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Impaired speech	15/15	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IUGR	9/11	82	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Short stature	6/13	46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Febrie seizures infancy	10/13	77	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epilepsy	5/15	33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Normal brain MRI/CT	6/11	55	CCH	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Hypertonia	8/9	89	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Abnormal gait	9/12	75	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Neonatal feeding problems	15/15	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Childhood feeding problems	7/12	58	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Typical facial gestalt	10/12	83	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Slender build	9/13	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Scoliosis/kyphosis/pectus excavatum	6/12	50	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Foot anomalies	10/10	100	U	SFT	HW, SFT	U	U	U	U	CT	CS	FD	FD	CS, SFT	IW, CS, SFT, HV	HAF, LH	HAF, LH	HAF, LH	
Other skeletal anomalies	6/9	67	U	U	U	U	U	U	U	U	A	SN	C, K	-	TOCE	SH	-	-	
Cardiac defect	2/11	18	-	VSD, AOV	-	U	U	U	U	U	-	-	AS	-	-	-	-	-	
Sleeping disturbances	4/8	50	U	U	BAP	U	U	U	U	U	U	U	U	U	U	U	U	U	
Additional features			IH, HM				HM, AST, OP			CSF	HL, RD	DPD, AST	DPD						

Abbreviations: A, arachnoidecty; AOV, aortic valve insufficiency; AS, aortic stenosis; ASD, autism spectrum disorder; AST, astigmatism; BA, brain atrophy; BAP, breast aplasia; C, contractures; CCH, corpus callosum hypoplasia; CS, partial cutaneous syndactyly 2-4 toes; CSF, mild 2-3 syndactyly of fingers; CT, camptodactyly of toes; E, exon; EV, enlarged ventricles; DPD, delayed primary dentition; F, female; FD, cavali-varus foot deformity; HAF, high-arch feet; HL, hearing loss; HM, hypermetropia +6dpt; HV, hallux valgus; IH, inguinal hernia; ID, intellectual disability; IT, irregular toe implant; IW, interdigital webbing; K, kyphosis; LH, long hallux; M, male; MD, myelination delay; MRI, magnetic resonance imaging; N, normal; nr, number; OP, opisthotonos episodes; PD, polydactyly; RD, retinal detachment; SDP, short distal phalanges; SEC, small ear canal; SFT, short fifth toe; SH, small hands; SN, supernumary teeth; TOCE, tibial osteochondrosis and exostoses; U, unknown; VSD, ventricle-septum defect. <sup>3</sup>Only mother available for testing; no mutation. <sup>4</sup>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 cutoff for ASD on the Autism Diagnostic Observation Schedule - 2nd edition (ADOS-2). <sup>5</sup>Patient was microcephalic during infancy, but within range of normocephaly during puberty (-1.6 s.d.).

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 7162 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).<sup>11–14</sup>

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 noncanonical human splice sites with a GT-AA sequence.<sup>17</sup> This is consistent with the patient's rather atypical phenotype when compared with 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.<sup>15</sup> 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.<sup>18</sup> 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 s.d. at 4 years of age), which remarkably resolved to –1.6 s.d. 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 ( $\leq 5$  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 with receptive language as the majority did not speak or only used one- to two-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 than 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 the 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.<sup>19,20</sup> 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.<sup>21</sup> 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.<sup>19,20</sup> 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.<sup>22</sup> 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.<sup>23</sup> *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.<sup>24–26</sup>

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 with 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.<sup>27</sup> 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.<sup>19</sup>

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 *INI1* orthologue, which is a member of the switch/sucrose nonfermentable complex involved in the morphogenesis of dendritic arbors in *Drosophila* sensory neurons.<sup>28,29</sup> Similarly, in mice, *DYRK1A* binds a switch/sucrose nonfermentable complex, suggesting a potential role in chromatin remodeling.<sup>30</sup> In the light of the importance of germline mutations in chromatin remodeling complexes for neurodevelopmental disease,<sup>3,31–35</sup> detailed investigation into these complexes and networks may reveal additional pathogenic mutations and potential novel syndromes associated with ID and autism.<sup>3,36</sup> 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.

### CONFLICT OF INTEREST

EEE is on the scientific advisory board (SAB) of DNAnexus. The remaining authors declare no conflict of interest.

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### WEB RESOURCES

The URLs for data presented herein are as follows (accessed September 2014): 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>.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)