Systematic Functional Characterization of Human 21st Chromosome Orthologs in Caenorhabditis elegans

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ABSTRACT Individuals with Down syndrome have neurological and muscle impairments due to an additional copy of the human 21st chromosome (HSA21). Only a few of ~200 HSA21 genes encoding proteins have been linked to specific Down syndrome phenotypes, while the remainder are understudied. To identify poorly characterized HSA21 genes required for nervous system function, we studied behavioral phenotypes caused by loss-of-function mutations in conserved HSA21 orthologs in the nematode Caenorhabditis elegans. We identified 10 HSA21 orthologs that are required for neuromuscular behaviors: cle-1 (COL18A1), cysl-2 (CBS), dnsn-1 (DONSON), eva-1 (EVA1C), mtq-2 (N6ATM1), ncam-1 (NCAM2), pad-2 (POFUT2), pdxk-1 (PDXK), mt-1 (RUNX1), and unc-26 (SYNJ1). We also found that three of these genes are required for normal release of the neurotransmitter acetylcholine. This includes a known synaptic gene unc-26 (SYNJ1), as well as uncharacterized genes pdxk-1 (PDXK) and mtq-2 (N6ATM1). As the first systematic functional analysis of HSA21 orthologs, this study may serve as a platform to understand genes that underlie phenotypes associated with Down syndrome.

Common symptoms of DS, hypotonia (muscle weakness), causes deficits in both gross and fine motor skills, causing people with DS to have trouble speaking, writing, and moving efficiently (Pitetti et al. 1992). For over 50 yr, researchers have known that an extra copy of the 21st chromosome (Human somatic autosome 21, HSA21) underlies DS (Jacobs et al. 1959). However, the precise mechanisms by which trisomy 21 causes DS-associated phenotypes are largely unknown. An early hypothesis explaining the link between DS and trisomy 21 argued that the burden of the extra genetic material strains cellular processes in the DS patient, resulting in all DS symptoms (Patterson 2009; Shapiro 1975). Yet research on rare individuals with partial trisomy 21 has shown that the amount of extraneous genetic material does not readily account for the collection of symptoms associated with DS nor their degree of manifestation (Korenberg et al. 1994; Lyle et al. 2009). A second hypothesis posited that a region containing ~30 protein-coding genes on HSA21, termed the Down Syndrome Critical Region (DSCR), is responsible for all DS-associated phenotypes. This was based on the genomic area of overlap shared between two individuals with partial trisomy 21 who still displayed many DS phenotypes (Rahmani et al. 1989). A mouse model (Ts1Rhr) containing trisomy of only DSCR orthologous genes was developed to investigate the role of the DSCR (Olson et al. 2004). Many, but not all, of the phenotypes expected for
DS were observed in this mouse model, suggesting that the DSCR is important but not solely responsible for the whole set of DS-associated phenotypes (Belichenko et al. 2009; Olson et al. 2004). Mouse models of DS trisomic for syntenic regions of HSA21 other than the DSCR have also been instrumental in the analysis of the genetic origins of DS-related phenotypes, including craniofacial defects (Richtsmeier et al. 2000, 2002), cerebellar cell loss (Baxter et al. 2000), as well as synaptic and hippocampal circuit dysfunction (Belichenko et al. 2004; Demas et al. 1998; Escorihuela et al. 1995; Holtzman et al. 1996; Reeves et al. 1995).

Studies focusing on individual orthologous genes on HSA21 in animal models have also provided insight into the genetic basis of DS phenotypes. Single gene studies are attractive because the underlying genetic contributions of a phenotype are more readily dissected; single genes can also offer clearer insight into pharmacotherapeutic targeting of specific gene products (Dierssen 2012). Determining the biological function of a single gene can be approached with either a loss-of-function or gain-of-function (e.g., overexpression) approach. Both have merit. For genes for which there is no or incomplete redundancy, loss-of-function experiments provide valuable insight into gene function. Several HSA21 orthologous genes were initially characterized in this way in invertebrate models. Early work conducted in Drosophila melanogaster, for instance, revealed that the gene mnb (minibrain) played a critical role in the embryonic neurogenesis (Tejedor et al. 1995). Subsequent research linked fly mnb to its mammalian ortholog, DYRK1A; and, as in fly, loss of Dyrk1a in mice was also shown to result in abnormal neurogenesis (Patil et al. 1995; Shindoh et al. 1996; Song et al. 1996, 1997). Intriguingly, overexpression of Dyrk1a alone in a mouse transgenic model results in neurodevelopmental delay, motor abnormalities, and spatial memory defects, which suggests a potential role in DS-associated cognitive impairment (Ahn et al. 2006; Alfatj et al. 2001). Similarly, the neuronal role of the basic helix-loop-helix protein, SIM2 (single-minded family bHLH transcription factor 2) on HSA21 was also initially identified in fly. Mutations in the fly ortholog, sim, impair development of cells in the midline of the central nervous system (Crews et al. 1988; Thomas et al. 1988). Subsequent experiments identified the mouse homolog and found that a targeted deletion of the gene led to craniofacial malformations (Chen et al. 1995; Shambrot et al. 2002). Overexpression of Sim2 in mouse also recapitulated behavioral aspects of established mouse models of DS, including reduced exploratory behaviors and hypersensitivity to pain (Chrst et al. 2000). Thus, single gene studies and invertebrate models complement research with traditional DS mouse models that overexpress combinations of many HSA21 genes.

Despite this progress, the in vivo function of a majority of genes on HSA21 remains unknown. It is impractical to perform a systematic analysis of gene function through gene knockdown in mouse models. Therefore, we set out to gain knowledge about roles of HSA21 genes by studying the function of HSA21 orthologs in the nematode Caenorhabditis elegans.

C. elegans is a well-established genetic model and exhibits sequence similarity with at least 42% of genes associated with human disease (Culetto and Sattelle 2000). Additionally, the C. elegans nervous system is compact, with only 302 neurons (White et al. 1986). Because any one neuron may be connected to any other by only six or fewer synapses, defects in even a single neuron are often detectable with behavioral phenotypes such as locomotion (Chen et al. 2006). Proper function of the muscular feeding organ, called a pharynx, also requires precise communication between neurons and muscle. Defects in pharyngeal pumping have helped identify a conserved vesicular glutamate transporter (e.g., cat-4) and nicotinic cholinergic receptor subunits (Avery and You 2012). Finally, C. elegans synapses are also similar to those of mammals. Genetic screens using the acetylcholinesterase inhibitor aldicarb in worm have identified genes critical for synaptic function in mammals (Richmond 2005).

Because the in vivo functions of HSA21 genes have not been systematically studied, we suspected that some HSA21 genes with important neuronal or muscle roles were overlooked. The goal for our study was to determine HSA21 orthologs involved in neuronal and/or muscle function. To this end, we analyzed behavioral phenotypes induced by loss-of-function mutations and found that disruption of several characterized HSA21 orthologs in worm yielded significant neuromuscular phenotypes. To determine if any HSA21 orthologs were involved in neurotransmission, we performed a second screen on loss-of-function mutants measuring their sensitivity to the acetylcholinesterase inhibitor aldicarb. We identified three genes involved in neurotransmitter release: unc-26 (SYNTJ1), which has previously been characterized, and mtq-2 (N3017/T08A10) and pdk-1 (PDKX), both of which have received minimal attention. The results from our behavioral and pharmacological screens may inform predictions for which genes contribute to the neuronal and muscle phenotypes associated with DS.

**MATERIALS AND METHODS**

**Strains**

C. elegans were grown on NGM (nematode growth media) agar plates seeded with OP50 bacteria at 20°C as described (Brenner 1974). N2 Bristol served as wild type and was also used for outcrossing (Brenner 1974). For a list of outcrossed strains, refer to Supplemental Material, Table S1 (sheet Worm strains). The following strains were used in this study: BB3 adr-2 (e424 III); CB211 lev-1(e211) IV; C21853 ztg-10 (tm6127) II; DR97 unc-26(e345) IV; EK228 mbk-1(ok1389) X; FX1756 pad-2(tm1756) III; FX2021 rcm-1(tm2011) III; FX2626 Y105E8A1 (tm2626) I; FX2657 ndr-1(tm2657) I; FX3322 dun-1(tm3322) II; FX3565 mtq-2(tm3565) II; FX6706 B0024.13(tm6706) V; FX776 sod-1(tm776) II; GA503 sod-5(tm1146) II; MT1072 egl-4(e4777) IV; NS3026 ikb-1(n1227) I; PR678 sax-4(pd678) III; PS2627 dgy-1(ky428) I; RB1849 D037.1(ok1746) I; RB2097 set-29(ok2772) I; RB2436 cyl-4(ok3359) V; RB2535 cyl-2 (ok3516) II; RB870 gcn-1(ok711) X; RB899 cyl-1(ok762) X; RB979 dip-2(ok883) I; VC200 ntr-1(ok351) I; VC201 itsn-1(ok268) IV; VC3037 mtq-2 (ok3740) II; VC40866 pdk-1(ks855208) I; VC868 evr-1(ok1133) I; VC943 cle-1(ok421) I; V11860 rcm-1(hd49) X.

**Transgenic strains**

We used standard techniques to generate constructs used to make the transgenic strains shown in Table 1.

**RNA interference**

We performed RNA interference (RNAi) by feeding as described (Timmons et al. 2001). All RNAi clones were obtained from Source-BioScience (Nottingham, UK). First, RNAi-expressing AMP-resistant bacteria were cultured overnight at 37°C with shaking in LB broth containing ampicillin (50 mg/ml) to prevent contamination of liquid cultures. The following day, ~100 μl of bacterial liquid culture was seeded on NGM plates containing 1-mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to induce expression of exogenous RNA by the T7 promoter. Once a bacterial lawn had sufficiently grown, a mixed age population of BZ1272 nce-1(hud20) lin-15(hud120) double-mutant worms was placed in a 2:1 mixture of bleach and 1-M NaOH to kill bacteria and postembryonic worms. This strain was selected due to its heightened sensitivity to RNAi in the nervous system (Schmitz et al. 2007). Eggs were allowed to hatch and grow over the next week at 20°C, and observed for the following week. This period covers two generations. The first generation of RNAi-treated
worms experienced postembryonic effects of the RNAi treatment, while the second generation also experienced maternal and preembryonic effects. We scored viability phenotypes including embryonic lethality, larval lethality, and larval arrest and sterility in either the P0 or F1 generation. Control-treated worms were fed L440 background-strain bacteria that harbored an empty RNAi vector.

**Behavioral assays**

All behavioral assays were performed blind to the genotype or RNAi treatment and conducted at room temperature (~20°C).

**Radial dispersion assay:** To measure radial distance traveled, we placed five to eight d-one adult worms in the center of a 10-cm diameter plate thinly seeded with OP50 bacteria. The distance traveled from the center of the plate was measured at 10 min (Topalidou et al. 2017). A minimum of 30 worms were tested per genotype on at least two separate days.

**Exploration assay:** A single L4-stage worm was placed on a 3.5-cm diameter plate thinly seeded with OP50 bacteria and allowed to crawl freely. After 16 hr, we removed the worm and used the worm track to count how many of 69 squares the explored across. At least 15 worms per genotype were tested on at least two separate days.

**Aldicarb assay:** Sensitivity to the acetylcholinesterase inhibitor aldicarb was quantified as described (Mahoney et al. 2006). At least 25 d-one adult-stage worms were evaluated per trial. For the aldicarb assay using RNAi-treated worms, worms were examined at a single time point (100 min) on 1-mM aldicarb and scored for paralysis. Trials were performed blind to RNAi treatment and in triplicate for two generations (if viable as F1). For the aldicarb assay performed on mutant worms, the number of paralyzed worms on 1-mM aldicarb was noted every half hour for 3 hr. A worm was considered paralyzed when it showed neither spontaneous movement nor movement in response to being prodded three times on the head and tail with a platinum wire. Assays were repeated a minimum of three times.

**Levamisole assay:** Sensitivity to the acetylcholine receptor agonist levamisole was measured as described (Lewis et al. 1980). At least 25 d-one adult-stage worms were placed on plates treated with 800-μM levamisole. We scored worms for paralysis every 10 min for 1 hr. Assays were performed in triplicate.

**Pharyngeal pumping assay:** We quantified the pumping rate of d-one adult-stage worms by eye for 30 sec under a stereomicroscope at 100× magnification using a handheld counter. A single pump was defined as the backward movement of the grinder (Albertson and Thomson 1976; Raizen et al. 1995). At least 30 worms per genotype were analyzed.

**Statistical analysis**

For radial dispersion, exploration, and pharyngeal pumping assays, we compared values to the wild-type control with a one-way ANOVA with a Bonferroni correction to evaluate select comparisons or Dunnett’s test when all comparisons were to wild type. Aldicarb and levamisole responses of mutants were compared to wild-type control with a two-way ANOVA and a Bonferroni correction for select comparisons or Dunnett’s test when all comparisons were to wild type. We used GraphPad Prism7 software. To minimize false positives from our screen, we set significance to $P < 0.001$. For all subsequent assays, significance was considered $P < 0.05$.

**Data availability**

Strains are available upon request. Table S1, sheet Worm strains contains detailed descriptions and sources of all strains used in this study.
For instance, the human gene paralogous human genes in our analysis: both four paralogs: C. elegans genes results in many human genes being orthologous to worm. These 47 HSA21 human genes are represented by 51 genes in InParanoid predicted orthologous in worm (three wedges highlighted by red arc in Figure 1B). These 47 HSA21 predicted orthologs in worm (light gray and blue) with an available RNAi clone were tested in the RNAI viability screen (Table S1). (B) Representation of InParanoid-predicted orthologs with their human to worm relationship (shades of blue). We tested all predicted InParanoid orthologs except those within a many-to-many relationship with RNAi for viability and mutants for behavioral and aldicarb assays (red).

**RESULTS**

**Estimate of HSA21 protein-coding genes**

To determine a conservative number of protein-coding genes on the human 21st chromosome, we queried both Ensembl and Human Gene Nomenclature Committee databases and selected only those proteins reviewed by SwissProt. This public database provides manual curation and review for each protein, which ensures high-quality, nonredundant entries (UniProt Consortium 2014). This strategy yielded a total number of 213 protein-coding genes on the 21st chromosome (see Orthologs and Other Ortholog sheets in Table S1).

**Determining HSA21 orthologs in C. elegans**

To identify putative worm orthologs of protein-coding genes on the human 21st chromosome (HSA21), we relied on OrthoList, a publicly available database. OrthoList compiles results from a meta-analysis of four orthology prediction programs—Ensembl Compara, OrthoMCL, InParanoid, and Homologene (Shaye and Greenwald 2011). OrthoList predicts at least one worm ortholog for 85 of the 213 HSA21 protein-coding genes (blue and light-gray wedges in Figure 1A). HSA21 encodes 48 predicted keratin proteins, which have no orthologs in worm (Shaye and Greenwald 2011). Excluding these keratin-encoding genes, C. elegans has at least one predicted orthologous gene to 52% of the remaining HSA21 genes (Figure 1A). To focus our screen on genes that most likely share conserved functions, we chose to study orthologous genes existing in any relationship except many-to-many as defined by the InParanoid algorithm (Sonhammer and Östlund 2015). This included one-to-one, one-to-many, and many-to-one relationships. By this measure, InParanoid identifies 47 unique HSA21 genes with predicted orthologs in worm (three wedges highlighted by red arc in Figure 1B). These 47 HSA21 human genes are represented by 51 genes in worm.

The imperfect evolutionary matchup of HSA21 human genes with C. elegans genes results in many human genes being orthologous to multiple worm genes (Figure 1B and see Orthology sheet in Table S1). For instance, the human gene CBS is predicted to be orthologous to four paralogs: cysl-1, cysl-2, cysl-3, and cysl-4. There was only one case of paralogous human genes in our analysis: both RRPI and RRPIB human genes are predicted to be orthologous to the single gene C47E12.7. Of the 47 HSA21 human genes, 19 are orthologous one-to-one with worm genes, 25 are orthologous many-to-one with 25 worm genes, and three are orthologous one-to-many with eight worm genes. An additional 35 HSA21 human genes had one or more orthologs predicted by the OrthoList algorithm in C. elegans (depicted by light-gray wedge in Figure 1A and listed in Other Orthologs sheet in Table S1). Because most of these HSA21 human genes are predicted to be orthologous to multiple worm genes, the orthology of the 35 human genes expands to represent 111 worm genes in total. For example, the human gene ABCG1 is predicted to be orthologous to four paralogs: wht-2, wht-3, wht-6, and wht-8. In four cases, distinct but paralogous human genes are orthologous to the same worm gene. For instance, both OLIG1 and OLIG2 human genes are both predicted to be orthologous to hhl-16 in worm. Due to the divergent nature of these orthologies, these 111 orthologs in worm are less likely to represent functional orthologs of HSA21 human genes. Therefore, we did not pursue them in this study.

Lastly, we found that an additional 128 protein-coding genes on HSA21 have no predicted OrthoList ortholog in C. elegans (depicted by black wedge in Figure 1A and listed in No Orthologs sheet in Table S1).

Taken together, our analysis above shows that discounting the keratin genes, about half of the 213 HSA21 human genes are predicted to be orthologous to genes in C. elegans. We focused on the set of 47 HSA21 human genes represented by 51 InParanoid orthologs in worm in this study.

**HSA21 orthologs required for viability in C. elegans**

Before embarking on our behavioral screen using mutants, we first sought to determine which of the 51 HSA21 predicted orthologs are required for viability using RNAi (see Orthologs sheet in Table S1). Previous large-scale and small-scale RNAi screens studied 38 of the 51 genes (Geron et al. 2007; Cui et al. 2008; Fraser et al. 2000; Gottschalk et al. 2005; Kamath et al. 2003; Maeda et al. 2001; Simmer et al. 2003; Sönntig et al. 2005). These studies reported that RNAi treatments caused viability phenotypes for 15 of these orthologs that severely effect development, growth, and/or reproduction. These include embryonic and larval lethal, larval arrest, severe growth defects, and sterility phenotypes. For an additional 24 orthologs, these studies reported that RNAi treatment caused an apparent wild-type phenotype. Out of the 51 HSA21 worm orthologs, 14 were not studied in previous RNAi screens, in part, because no RNAi reagent was available at the time.

To confirm and extend these results we performed RNAi on all 51 HSA21 orthologs for which an RNAi clone was currently available using 45 unique RNAi treatments (see Orthologs sheet in Table S1). We found concordance for 82% or 37 out of the 45 genes tested. Only 4% or
two of our RNAi treatments produced results discrepant from previous studies. RNAi treatment of *irk-1* (KCNJ6) or *chaf-2* (CHAF1B) were previously reported to cause severe viability phenotypes whereas we found no effect for both treatments (Gottschalk et al. 2005; Kamath et al. 2003; Nakano et al. 2011; Sönntichsen et al. 2005). We found that 14 out of 16 of the genes that caused inviability in our study or in previous studies when knocked down, including *irk-1* and *chaf-2*, lacked a publicly available viable loss-of-function mutant. This finding is consistent with the idea that these genes are probably essential. Two additional genes, *dip-2* and *dasm-1*, had corresponding viable predicted null mutants, but we and others found that they caused inviability when knocked down by RNAi (Kamath et al. 2003; Simmer et al. 2003); perhaps this reflects a viability phenotype with low penetrance. We acknowledge that additional genes may later prove to represent essential genes too, especially if the gene lacks a corresponding null mutant, e.g., *pdkk-1*. Thus, overall our RNAi screen confirms most viability phenotype results from previous studies and suggests that at least 33% or 15 out of 45 of tested HSA21 predicted orthologs are inviable when reduced in function in *C. elegans*.

**HSA21 orthologs required for neuromuscular behaviors**

Having identified which HSA21 orthologs are probably essential, we turned next to investigate the in vivo function of nonessential HSA21 orthologs. We tested every publicly available, viable mutant, which represented 27 genes in total (see Orthologs sheet in Table S1). We performed a phenotypic screen, selecting a battery of three behavioral assays to probe neuromuscular function. Overall, we identified 10 mutants that were significantly defective in performance in these assays. To better link genotype to phenotype, we generated transgenic rescue lines for any mutant deficient in any of the three behaviors and/or tested additional mutant alleles if available.

We first examined short-term locomotion with a radial dispersion assay, which provides a general measure of locomotor function over 10 min. In this assay, the distance displaced over time reflects a combination of locomotor traits including frequency of body bends, reversal rate, and efficient coordination (Topalidou et al. 2017). Aside from the well-characterized uncoordinated mutant *unc-26*, an ortholog of synaptojanin (Harris et al. 2000), we also identified two other mutants with rescuable deficits in radial dispersion: *cysl-2* (CBS) and *rnt-1* (RUNX1) (Figure 2).

Second, we examined the longer-term behavior of mutants with an exploration assay. This assay measures not only the general locomotor ability of worms, but also their tendency to explore a cultivation plate seeded with bacterial food over the course of 16 hr. While foraging, worms alternate between two states: roaming, an active state characterized by a burst of locomotion associated with a search for food; and dwelling, a more passive state associated with feeding or resting after satiation. Time spent in roaming and dwelling states depends on integrating internal neuromodulatory cues with external sensory cues. The absence of such sensory transduction leads to extended dwelling as observed in the *tax-4* mutant, which encodes a cyclic nucleotide-gated channel subunit (Fujisawa et al. 2002; Greene et al. 2016). In contrast, mutants with constitutive sensory input, such as the *egl-4* mutant, show extended roaming (Fujisawa et al. 2002). Extended dwelling or roaming compared to wild-type behavior can be quantified by counting the number of squares that a worm traverses over 16 hr (Figure 3).

As expected, we found that the uncoordinated mutant *unc-26* (SYNJ) moved poorly in the exploration assay. We also identified the following four mutants with rescuable defects in extended dwelling: *eva-1* (EVA1C), *dasm-1* (DONSON), *mtq-2* (N6AMT1), and *pdkk-1* (PDXK1) (Figure 3A). These mutants appeared to move superficially similar to wild type, suggesting sensory-motor integration deficits. We rescued the exploration behavior of each mutant by transformation with wild-type copies of corresponding transgenes (Figure 3B).

To obtain a measure of neuromuscular activity that was distinct from locomotion, we performed a feeding assay. *C. elegans* pumps bacterial food into its gut through a feeding organ called a pharynx that uses muscles and neurons distinct from those needed for locomotion. The rhythmic contractions and relaxations of the pharynx require precise coordination between pharyngeal neurons and muscle. We identified the following four genes required for normal pharyngeal pumping: *cle-1* (*COL18A1*), *mtq-2* (*N6AMT1*), *ncam-1* (*NCAM2*), and *pad-2* (*POFUT2*) (Figure 4A). Independent null alleles of *mtq-2* displayed similar deficits in pharyngeal pumping (Figure 4B). Transformation with wild-type copies of each gene rescued the slow pumping phenotype of each mutant (Figure 4B).
In summary, by screening three behaviors, we identified 10 genes total with rescuable defects in one or more behaviors designed to probe neuromuscular function. Several among these genes—dron-1 (DONSON), pdkk-1 (PDXK), and mtq-2 (N6AMT1)—are poorly characterized, yet may mediate nervous system function, development, or both.

**HSA21 orthologs required for proper synaptic function**

To determine the potential role of HSA21 predicted orthologs in neurotransmission, we tested whether the same set of loss-of-function mutants displayed altered sensitivity to paralysis by the acetylcholinesterase inhibitor aldicarb. Many genes critical for key steps in synaptic function have been identified by testing mutants for altered sensitivity to aldicarb (Richmond 2005). Aldicarb causes paralysis of worms by chronically activating body-wall muscle after prolonged presence of acetylcholine at the neuromuscular junction. Mutants defective in any aspect of synaptic transmission show altered sensitivity to paralysis by aldicarb. For instance, mutants defective in acetylcholine release show resistance to paralysis by aldicarb due to decreased levels of acetylcholine in the cleft (Mahoney et al. 2006). Conversely, mutations that increase acetylcholine release lead to increased sensitivity to paralysis (Gracheva et al. 2006; McEwen et al. 2006).

We identified three mutants that displayed significant resistance to paralysis by aldicarb: unc-26 (SYNJ1), mtq-2 (N6AMT1), and pdkk-1 (PDXK). Compared to wild type, these three mutant strains took longer to paralyze when treated with aldicarb (Figure 5A and Figure 6). By contrast, 24 other mutants became paralyzed at a similar rate as wild-type worms. Although we measured the 180-min time course of paralysis for all 27 mutants, we plot only the percent moving on aldicarb at the 180-min time point for ease of comparison (Figure 5B). Transformation of mtq-2 and pdkk-1 mutants with wild-type copies of these genes rescued aldicarb sensitivity for each mutant (Figure 6). As a complementary strategy to the mutant screen, we also tested aldicarb sensitivity for HSA21 predicted orthologs using RNAi. With this independent approach, we confirmed the aldicarb resistance of pdkk-1 (PDXK-1) and observed suggestive results for mtq-2 (MTQ-2) (Figure S1).

**Figure 3** Screen for exploration defects. Histograms show the mean percentage of the assay plate traversed (percent of squares entered ± SEM) by individual worms over a 16-hr exploration assay. (A) Five mutants (green) covered significantly less area than wild type (black). *** P < 0.001, n > 15. (B) Reductions in exploration were rescued in the eva-1, C24H12.5, mtq-2(tm3565), and pdkk-1 mutants (green) by extrachromosomal expression of each of the wild-type genes (blue). Wild-type performance shown for comparison (black). * P < 0.05, ** P < 0.01, *** P < 0.001, n > 15.

**Figure 4** Screen for pumping defects. Histograms showing mean pharyngeal pumps per minute (±SD) in the presence of standard bacterial food. (A) Seven mutants (green) showed significant defects in pharyngeal pumping relative to wild type (black). *** P < 0.001, n > 30. (B) Mutants with pharyngeal pumping defects mtq-2(tm3565), pack-2, ncam-1, and cle-1 (green) were rescued by extrachromosomal expression of the wild-type genes (blue). We did not observe rescue of B0024.15. * P < 0.05, *** P < 0.001, n > 30.
We found that RNAi treatment targeting ncam-1 provided resistance to aldicarb; however, we also found that a null allele of ncam-1 failed to show any resistance to paralysis by aldicarb (Figure 4B and Figure 5), suggesting that our RNAi result for ncam-1 was a false positive. This result is consistent with a previous RNAi study that found negative results for ncam-1 on aldicarb (Sieburth et al. 2005).

Several decades of studies have concluded that resistance to aldicarb is primarily caused by presynaptic deficiencies in acetylcholine release or postsynaptic deficiencies in receptor response (Mahoney et al. 2006). To distinguish between these possibilities, we measured the sensitivity of mutants to paralysis by levamisole, a potent agonist for the nicotinic cholinergic receptor (Lewis et al. 1980). Levamisole causes paralysis of wild-type worms by chronically activating body-wall muscle irrespective of synaptic release defects. Mutants resistant to paralysis by levamisole are defective in cholinergic receptor signaling or muscle function.

We found that mtq-2, unc-26, and pdxk-1 mutants all displayed wild-type-like or hypersensitive responses to levamisole (Figure 6). These results suggest that resistance to aldicarb among these three mutants is due to defective presynaptic release of neurotransmitter.

Expression pattern of HSA21 orthologs

We identified nine genes linked to rescuable neuromuscular defects in our study (Table 2). Five genes were previously shown to be expressed in the nervous system: cle-1 (COL18A1), eva-1 (EVA1C), ncam-1 (NCAM2), pad-2 (POFUT2), rnt-1 (RUNX1), and unc-26 (SYNJ) (Ackley et al. 2001; Fujisawa et al. 2007; Hunt-Newbury et al. 2007; McKay et al. 2003; Menzel et al. 2004; Schwarz et al. 2009). An additional four genes had no reported expression patterns: cyls-2 (CBS), dnsn-1 (DONSON), mtq-2 (N6AMT1), and pdxk-1 (PDXK). To identify the tissues in which these genes may function, we generated mCherry transcriptional reporter strains. We found that mtq-2 expressed exclusively throughout the nervous system. Expression overlapped with a subset of cholinergic neurons in the head and throughout the ventral nerve cord as defined by the Punc-17::GFP reporter (Figure 7A4). Expression of mtq-2 mCherry reporter was not observed in GABAergic neurons defined by the Punc-47::GFP reporter (Figure 7A5). Interestingly, our mCherry reporter for pdxk-1 was observed in GABAergic, but not cholinergic neurons (Figure 7B). Our transcriptional reporter for dnsn-1 expressed robustly within the developing embryo, but not within the adult nervous system (Figure 7C). This suggests a potential developmental role for this gene. Lastly, we found that our mCherry cyls-2 reporter expressed broadly throughout head and body-wall muscle (Figure 7D).

DISCUSSION

Our research predicts new functional annotation of genes on the human 21st chromosome by characterizing in vivo roles of putative orthologs in C. elegans. Within the field of DS, research has primarily focused on the functions of contiguous groups of genes using mice, such as those included in the so-called DSCR (Jiang et al. 2015). Meanwhile, the vast majority of HSA21 genes remain functionally uncharacterized. One proven approach to reveal the function of large numbers of genes, even at the level of the whole genome, is to study their roles in more tractable invertebrate models such as C. elegans. Although several large-scale reverse genetics screens in C. elegans have begun to uncover novel in vivo roles for thousands of genes, they have focused less explicitly on neuronal phenotypes and excluded more genes than may be generally acknowledged (Fraser et al. 2000; Kamath et al. 2003; Sieburth et al. 2003; Vashlishan et al. 2008). For instance, two seminal whole-genome RNAi screens tested only 33 of the 51 HSA21 orthologs focused on in this study (Fraser et al. 2000; Kamath et al. 2003). These studies primarily focused on viability phenotypes such as gross development and fertility. We expanded this analysis to test an additional eight orthologs...
not tested in these seminal or subsequent RNAi studies (Ceron et al. 2007; Gottschalk et al. 2005; Nakano et al. 2011; Pujol et al. 2001; Rual et al. 2004; Sönntichsen et al. 2005). With the addition of our current study, out of the 51 HSA21 orthologs, only five genes remain unstudied using RNAi or mutant in any study due to lack of reagents: D1086.9 (CLIC6), H39E23.3 (CLIC6), Y54E10A.11 (LTN1), Y74C10AL.2 (TMEM50B), and unc-14 (UBE2G2). Overall, however, our results were consistent with earlier studies. We found only one discrepancy, where a previous study found that RNAi targeting irk-2 caused sterility while we found it had no effect (Kamath et al. 2003).

Other seminal RNAi screens in C. elegans searched for genes critical for synaptic transmission. Even though these screens were prodigious, testing 2068 genes in total, they only tested five of the HSA21 orthologs: mbk-1, itsn-1, ncam-1, K02C4.3, and wdr-4 (Sieburt et al. 2005; Vashlishan et al. 2008). These studies reported negative results for all five genes in agreement with our RNAi and mutant findings here. Notably, the synaptic RNAi screens did not test the three genes that we found to be critical for normal synaptic transmission: mtq-2, pdsk-1, and unc-26. Thus, despite the whole genome and large-scale nature of previous screens, many genes with important in vivo functions likely remain to be discovered using C. elegans. Because our study largely replicated results from previous studies, however, we are optimistic that a systematic approach with C. elegans, as used here, may reveal the function of important sets of genes involved in human polygenic or chromosomal disorders.

We also performed several behavioral screens to identify HSA21 predicted orthologs that function in nervous system and muscle in ways that previous reverse-genetic screens may have missed. This included a radial dispersion assay that reflects short-term locomotor ability, an exploration assay that reflects longer-term sensory and motor integration, and a feeding assay that probes an independent neuromuscular circuit. We identified 10 mutants with deficiencies in these behaviors that we were able to rescue and/or confirm with independent alleles (Table 2): cle-1 (COL18A1), cysl-2 (CBS), dnsn-1 (DONSON), eva-1 (EVA1C), mtq-2 (N6AMT1), ncam-1 (NCAM2), pad-2 (POFUT2), pdsk-1 (PDXK), rnt-1 (RUNX1), and unc-26 (SYN1). These 10 orthologs represent alluring candidates as genes important in nervous system development and/or function and should be prioritized for further study in worm and mammalian model systems.

In general, our targeted screen of HSA21 orthologs revealed three primary findings for the 10 HSA21 orthologs. First, we found genes with established links to nervous system function (e.g., cle-1, eva-1, ncam-1, and unc-26). For instance, cle-1 (COL18A1), eva-1 (EVA1C), and ncam-1 (NCAM2) and have all been linked to deficits in axon guidance in worm. The type XVIII collagen gene, cle-1, mediates both neuronal and axonal migrations (Ackley et al. 2001). eva-1 encodes a Slit receptor required for axon guidance, and ncam-1 encodes a neural cell adhesion molecule that functions redundantly with other cell adhesion molecules to direct axonal outgrowth (Fujiwara et al. 2007; Schwarz et al. 2009). Additionally, the unc-26 (SYN1) mutant showed resistance to aldicarb but sensitivity to levamisole indicative of presynaptic defects. This is consistent with its known role in synaptic vesicle recycling (Harris et al. 2000).

Second, we uncovered novel neuronal functions for the otherwise well-characterized HSA21 ortholog rnt-1 (RUNX1). In mammals, the runt-related transcription factor RUNX1 plays a vital role in development, including regulation of the developing nervous system (Wang and Stifani 2017). In worm, the runt-related transcription factor rnt-1 has also been studied for its role in general development, including cell proliferation and male tail development (Hajduškova et al. 2009;
### Table 2 Summary of HSA21 gene orthologs required for normal behaviors in C. elegans

| Gene      | Description                      | Behaviors                               | Expression                                                                 | HSA21 Gene | Mouse Gene | Mouse Viability |
|-----------|----------------------------------|-----------------------------------------|----------------------------------------------------------------------------|-------------|-------------|-----------------|
| cle-1     | Collagen                         | Dispersal, Exploration, Pumping, Aldicarb | ✓                                                                           | COL18A1     | Col18a1     | Viable          |
| cysl-2    | Cysteine synthase                | Dispersal, Exploration                  | ✓                                                                           | CBS         | Cbs         | Postnatal lethal |
| dnsn-1    | Novel                            | Dispersal                               | ✓                                                                           | DONSON      | Donson      | Viable          |
| eva-1     | Slit receptor                    | Dispersal                               | ✓                                                                           | EVA1C       | Eva1c       | Viable          |
| mtq-2     | Glutamine methyltransferase      | Dispersal, Pumping                      | ✓                                                                           | N6AMT1      | N6amt1      | Embryonic lethal |
| ncam-1    | Neural cell adhesion molecule    | Dispersal                               | ✓                                                                           | NCAM2       | Ncam2       | Viable          |
| pad-2     | O-fucosyltransferase             | Dispersal                               | ✓                                                                           | POFUT2      | Pofut2      | Preweaning lethal |
| pdxk-1    | Pyridoxal kinase                 | Dispersal, Pumping                      | ✓                                                                           | PDXK        | Pdxk        | Preweaning lethal |
| rnt-1     | Runx transcription factor        | Dispersal                               | ✓                                                                           | RUNX1       | Runx1       | Embryonic lethal |
| unc-26    | Synaptojanin                    | Dispersal, Pumping                      | ✓                                                                           | SYNJ1       | Synj1       | Perinatal lethal |

- ▼ indicates the direction of changes that significantly differ from wild type (P < 0.001, one-way ANOVA). RIC (Resistant to Inhibitors of Cholinesterase) indicates mutants with significant resistance to aldicarb vs. wild type (P < 0.001, two-way ANOVA). ✓ indicates tissue(s) in which the gene is expressed. Data were derived from WormBase. Underlining signifies expression results found in this study.

- aHSA21 gene and mouse gene orthologs derived from InParanoid.
- bMouse viability data derived from the Mouse Genome Informatics database. Specific citations are indicated with a footnote.

Nimmo et al. 2005. Interestingly, loss of rnt-1 may also cause defects in phasmid neuron morphology; however, this was not linked to a detectable behavioral phenotype (Hajduskova et al. 2009). The deficiency that the rnt-1 mutant displayed in our radial dispersion assay might be due to developmental defects affecting the nervous system.

Third, whereas the majority of DS research has focused on well-studied genes, we also uncovered novel neuronal functions of three poorly characterized HSA21 orthologs that have weak or nonexistent links to the nervous system: dnsn-1 (DONSON), mtq-2 (N6AMT1), and pdxk-1 (PDXK). These three genes are so poorly characterized that they only had placeholder names when we started our study. To encourage predicted interacting partners warrant more detailed study to determine how it functions in synaptic transmission. C. elegans may provide an additional informative system to study mtq-2 as deletion of N6AMT1 causes embryonic lethality in knockout mice (Liu et al. 2010).

PDXK phosphorylates vitamin B6, converting it to PLP (pyridoxal-5’-phosphate), a key cofactor in the metabolism of hundreds of enzymatic reactions, including synthesis of neurotransmitters (Cao et al. 2006; Shetty and Gaitonde 1980). Loss of PDXK in mice causes preweaning lethality, so its behavioral role has not yet been studied in mammals (Mouse Genome Informatics and the International Mouse Phenotyping Consortium). Interestingly, PDXK is predicted to be haploinsufficient in mammals and is a candidate dose-sensitive gene contributing to phenotypes in DS (Antonarakis 2016). We found that pdxk-1 mutant worms showed general deficiencies in behaviors as well as specific defects in synaptic transmission as determined by aldicarb screening. Our finding that the pdxk-1 mutant was readily paralyzed by levamisole suggests a presynaptic role for pdxk-1 in the nervous system.

There is a rich body of literature in the field of DS devoted to exploring the underlying genetic contributions of the disorder. Single gene studies have helped to elucidate the role of individual genes such as Dyrk1A, both as a transgenic single gene triplication and within a trisomic context, and have led to the development of pharmacotherapeutic treatments (Ahn et al. 2006; Altafa et al. 2001; Shindoh et al. 1996; Song et al. 1996). Mouse models have also been instrumental in...
Figure 7 Expression pattern of select genes.
Fluorescent micrographs of all HSA21 ortholog hits for which expression patterns were unknown. (A1–A3) A transcriptional reporter for mtq-2 expressed mCherry throughout the adult nervous system including in head (A3). Bright field image shown in (A1) for reference. Scale bar, 100 μm. Dual color images show coexpression of mtq-2 reporter in cholinergic (arrowheads, A4) but not in GABAergic neurons (A5) in the ventral nerve cord. Scale bar, 50 μm. (B1–B3) A transcriptional reporter for pdkk-1 expressed mCherry throughout the adult nervous system including in head (B3). Bright field image shown in B1 for reference. Scale bar, 100 μm. Dual color images show coexpression of pdkk-1 reporter in GABAergic neurons (B5) but not cholinergic neurons (B4) in the ventral nerve cord. Scale bar, 50 μm. (C) A transcriptional reporter for dnsn-1 expressed mCherry widely in developing embryo. Scale bar, 50 μm. (D) A transcriptional reporter for cysl-2 expressed mCherry throughout body-wall and vulval muscle. Scale bar, 100 μm.
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LITERATURE CITED

Bujnicki, J. M., and M. Radlinska, 1999 Is the HemK family of putative S-adenosylmethionine-dependent methyltransferases a “missing” zeta subfamily of adenine methyltransferases? A hypothesis. IUBMB Life 48: 247–249.

Canfield, M. A., M. A. Honein, N. Yuskiw, J. Xing, C. T. Mai et al., 2006 National estimates and race/ethnic-specific variation of selected birth defects in the United States, 1999–2001. Birth Defects Res. A Clin. Mol. Teratol. 76: 747–756.

Cao, P., Y. Gong, L. Tang, Y.-C. Leung, and T. Jiang, 2006 Crystal structure of human pyridoxal kinase. J. Struct. Biol. 154: 327–332.

Ceron, J., J.-F. Rual, A. Chandra, D. Dupuy, M. Vidal et al., 2007 Large-scale RNAi screens identify novel genes that interact with the C. elegans retinoblastoma pathway as well as splicing-related components with synMuv B activity. BMC Dev. Biol. 7: 30.

Chen, B. L., D. H. Hall, and D. B. Chklovskii, 2006 Wiring optimization can relate neuronal structure and function. Proc. Natl. Acad. Sci. USA 103: 4723–4728.

Chen, H., R. Chrast, C. Rossier, A. Gos, S. E. Antonarakis et al., 1995 Single-minded and Down syndrome? Nat. Genet. 10: 9–10.

Chrast, R. H., S. S. Scott, R. Madani, L. Huber, D. P. Wolfer et al., 2000 Mice trisomic for a bacterial artificial chromosome with the single-minded 2 gene (Sim2) show phenotypes similar to some of those present in the partial trisomy 16 mouse models of Down syndrome. Hum. Mol. Genet. 9: 1853–1864.

Coyle, J. T., M. L. Oster-Granite, and R. H. Reeves, 1988 Down syndrome, Alzheimer’s disease and the trisomy 16 mouse. Trends Neurosci. 11: 390–394.

Cremona, O., G. Di Paolo, M. R. Wenk, A. Luthi, W. T. Kim et al., 1999 Essential Role of Phosphoinositide Metabolism in Synaptic Vesicle Recycling. Cell 99: 179–88.

Crews, S. T., J. B. Thomas, and C. S. Goodman, 1988 The Drosophila single-minded gene encodes a nuclear protein with sequence similarity to the per gene product. Cell 52: 143–151.

Cui, M. M., A. Allen, A. Larsen, M. Macnorniris, M. Han et al., 2008 Genes involved in pre-mRNA 3’-end formation and transcription termination revealed by a lin-15 operon Muv suppressor screen. Proc. Natl. Acad. Sci. USA 105: 16665–16670.

Culettò, E., and D. B. Sattelle, 2000 A role for Caenorhabditis elegans in understanding the function and interactions of human disease genes. Hum. Mol. Genet. 9: 869–877.

Das, I., J.-M. Park, J. H. Shin, S. K. Jeon, H. Lorenzi et al., 2013 Hedgehog agonist therapy corrects structural and cognitive deficits in a down syndrome mouse model. Sci. Transl. Med. 5: 201ra120.

Demas, G. E., R. J. Nelson, B. K. Krueger, and P. J. Yarowsky, 1998 Impaired spatial working and reference memory in segmental trisomy (Ts65Dn) mice. Behav. Brain Res. 90: 199–201.

Dierssen, M., 2012 Down syndrome: the brain in trisomic mode. Nat. Rev. Neurosci. 13: 844–858.

Escuriola, R. M., A. Fernández-Teruel, I. F. Vallina, C. Baamonde, M. A. Lumbrares et al., 1995 A behavioral assessment of Ts65Dn mice: a putative Down syndrome model. Neurosci. Lett. 199: 143–146.

Fletcher, A. G., R. S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann et al., 2000 Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. Nature 408: 325–330.

Fujisawa, K., J. L. Wrana, and I. G. Culotti, 2007 The slit receptor EVA-1 coactivates a SAX-3/Robo mediated guidance signal in C. elegans. Science 317: 1934–1938.

Fujisawa, M., P. Sengupta, and S. L. McIntire, 2002 Regulation of body size and behavioral state of C. elegans by sensory perception and the EGL-4 cGMP-dependent protein kinase. Neuron 36: 1102.

Gottschalk, A., R. B. Almedom, T. Schedletzky, S. D. Anderson, J. R. Yates et al., 2005 Identification and characterization of novel nicotinic receptor-associated proteins in Caenorhabditis elegans. EMBO J. 24: 2566–2578.

Gracheva, E. O., A. O. Burdina, A. M. Holgado, M. Berthelot-Grosjean, B. D. Ackley et al., 2006 Tomosyn inhibits synaptic vesicle priming in Caenorhabditis elegans. PLoS Biol. 4: e261.

Bax, L. L., M. T. Morgan, H. T. Honein, T. Pihlajaniemi, C. J. Kuo et al., 2000 The NCI/endostatin domain of Caenorhabditis elegans type XVIII collagen affects cell migration and axon guidance. J. Cell Biol. 152: 1219–1232.

Ahn, K.-J., H. K. Jeong, H.-S. Choi, S.-R. Ryoo, Y. J. Kim et al., 2006 Dyrk1A BAC transgenic mice show altered synaptic plasticity with learning and memory defects. Neurobiol. Dis. 22: 463–472.

Albertson, D. G., and J. N. Thomson, 1976 The pharynx of Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B Biol. Sci. 275: 299–325.

Altfaix, J., M. Dierssen, C. Baamonde, E. Marti, J. Visa et al., 2001 Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down’s syndrome. Hum. Mol. Genet. 10: 1915–1923.

Antonarakis, S. E., 2012 Phosphoinositide metabolism in synaptic diseases. Nat. Rev. Genet. 13: 147–163.

Avery, L., and Y.-J. You, 2012 C. elegans feeding (May 21, 2012). WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.150.1. http://www.wormbook.org.

Baxter, L. L., T. H. Moran, J. T. Richtsmeier, J. Troncoso, and R. H. Reeves, 2000 Discovery and genetic localization of Down syndrome cerebellar phenotypes using the Ts65Dn mouse. Hum. Mol. Genet. 9: 195–202.

Belichenko, N. P., P. V. Belichenko, A. M. Kleschevnikov, A. Salehi, R. H. Reeves et al., 2009 The “down syndrome critical region” is sufficient in the mouse model to confer behavioral, neuropathological, and synaptic phenotypes characteristic of down syndrome. J. Neurosci. 29: 5938–5948.

Belichenko, P. V., E. Masliah, A. M. Kleschevnikov, A. J. Villar, C. J. Epstein et al., 2004 Synaptic structural abnormalities in the Ts65Dn mouse model of down syndrome. J. Comp. Neurol. 480: 281–298.

Belichenko, P. V., A. M. Kleschevnikov, A. Becker, G. E. Wagner, L. V. Lysenko et al., 2015 Down syndrome cognitive phenotypes modeled in mice trisomic for all HSA 21 homologues. PLoS One 10: e0134861.

Blake, J. A., J. T. Eppig, J. A. Kadin, E. J. Richardson, C. L. Smith et al., 2017 Mouse Genome Database (MGD)-2017: Community Knowledge Resource for the Laboratory Mouse Nucleic Acids Research 45: D723–29.

Brenner, S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.
Greene, J. S., M. Brown, M. Dobosiewicz, I. G. Ishida, E. Z. Macosko et al. 2016 Balancing selection shapes density-dependent foraging behaviour. Nature 539: 254–258.

Hajduskova, M., M. Jindra, M. A. Herman, and M. Asahina, 2009 The nuclear receptor NHR-25 cooperates with the Wnt/beta-catenin asymmetry pathway to control differentiation of the T seam cell in C. elegans. J. Cell Sci. 122: 3051–3060.

Harris, T. W., E. Hartweg, H. R. Horvitz, and E. M. Jorgensen, 2000 Mutations in synaptopodin disrupt synaptic vesicle recycling. J. Cell Biol. 150: 589–600.

Heurgué-Hamard, V., M. Graille, N. Scrima, N. Ulryck, S. Champ, 2008 Protein expression profiling reveals differentially expressed genes in Caenorhabditis elegans. PLoS Biol. 6: e237.

Jacobs, P. A., A. G. Baikie, W. M. Court Brown, and J. A. Strong, 1959 The somatic chromosomes in mongolism. Lancet 1: 710.

Jiang, X., C. Liu, T. Yu, L. Zhang, K. Meng et al., 2015 Genetic dissection of the Down syndrome critical region. Hum. Mol. Genet. 24: 6540–6551.

Koremberg, J. R., X.N. Chen, R.S. Schipper, Z. Sun, R. Gonsky, 2011 Replication-coupled chromatin assembly generates a neuronal bilateral asymmetry in C. elegans. Cell 147: 1525–1536.

Kimmo, R., A. Antehi, and A. Woollard, 2005 mob-2 encodes RNT-1, a C. elegans Runx homologue essential for controlling cell proliferation in a stem cell-like developmental lineage. Development 132: 5043–5054.

Okuda, T., J. van Deursen, S. W. Hiebert, G. Grosveld, and J. R. Downing, 1996 AML1, the Target of Multiple Chromosomal Translocations in Human Leukemia, Is Essential for Normal Fetal Liver Hematopoiesis. Cell 26: 321–330.

Olson, E. L., J. T. Richtsmeier, J. Leszl, and R. H. Reeves, 2004 A chromosome 21 critical region does not cause specific down syndrome phenotypes. Science 306: 687–690.

Olson, E. L., R. J. Roper, C. L. Sengstaken, E. A. Peterson, V. Aquino et al., 2007 Trisomy for the Down syndrome “critical region” is necessary but not sufficient for brain phenotypes of trisomic mice. Hum. Mol. Genet. 16: 774–782.

Patil, N., D. R. Cox, D. Bhat, M. Faham, R. M. Myers et al., 1995 A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. Nat. Genet. 11: 126–129.

Patterson, D., 2009 Molecular genetic analysis of Down syndrome. Hum. Genet. 126: 195–214.

Piette, K. H., M. Climstein, M. J. Mays, and P. J. Barrett, 1992 Isokinetic arm and leg strength of adults with Down syndrome: a comparative study. Arch. Phys. Med. Rehabil. 73: 847–850.

Polevoda, B., L. Span, and F. Sherman, 2006 The yeast translation release factors Mrf1p and Sup45p (eRF1) are methylated, respectively, by the methyltransferases Mtq1p and Mtq2p. J. Biol. Chem. 281: 2562–2571.

Pujol, N., E. M. Link, L. X. Liu, C. L. Kurz, G. Alloing et al., 2001 A reverse genetic analysis of components of the Toll signaling pathway in Caenorhabditis elegans.Curr. Biol. 11: 809–821.

Rahmani, Z., J. L. Blouin, N. Creau-Goldberg, P. C. Watkins, J. F. Mattei et al., 1989 Critical role of the D21S55 region on chromosome 21 in the pathogenesis of Down syndrome. Proc. Natl. Acad. Sci. USA 86: 5958–5962.

Raizen, D. M., R. Y. Lee, and L. Avery, 1995 Interacting genes required for pharyngeal excitation by motor neuron MC in Caenorhabditis elegans. Genetics 141: 1365–1382.

BRAF mutation frequency in tumors from the TCGA Data Portal.

BRAF mutation frequency in tumors from the TCGA Data Portal.

BRAF mutation frequency in tumors from the TCGA Data Portal.

BRAF mutation frequency in tumors from the TCGA Data Portal.
Shamblott, M. J., E. M. Bugg, A. M. Lawler, and J. D. Gearhart, 2002 Craniofacial abnormalities resulting from targeted disruption of the murine Sim2 gene. Dev. Dyn. 224: 373–380.

Shapiro, B. L., 1975 Amplified developmental instability in Down's syndrome. Ann. Hum. Genet. 38: 429–437.

Shaye, D. D., and I. Greenwald, 2011 OrthoList: a compendium of C. elegans genes with human orthologs. PLoS One 6: e20085 (erratum: PLoS One 9).

Shetty, K. T., and B. B. Gaitonde, 1980 Effect of contraceptive steroids on gamma-aminobutyric acid metabolism and pyridoxal kinase activity in rat brain. Exp. Neurol. 70: 146–154.

Shindoh, N., J. Kudoh, H. Maeda, A. Yamaki, S. Minoshima et al., 1996 Cloning of a human homolog of the Drosophila minibrain/rat Dyrk gene from “the Down syndrome critical region” of Chromosome 21. Biochem. Biophys. Res. Commun. 225: 92–99.

Sieburth, D., Q. Ch'ng, M. Dybbs, M. Tavaozie, S. Kennedy et al., 2005 Systematic analysis of genes required for synapse structure and function. Nature 436: 510–517.

Simmer, F., C. Moorman, A. M. van der Linden, E. Kuijk, P. van den Berghe et al., 2003 Genome-wide RNAi of C. elegans using the hypersensitive rrf-3 strain reveals novel gene functions. PLoS Biol. 1: E12.

Song, W. J., L. R. Sternberg, C. Kasten-Sportès, M. I. Keuren, S. H. Chung et al., 1996 Isolation of human and murine homologues of the Drosophila minibrain gene: human homologue maps to 21q22.2 in the Down syndrome "critical region." Genomics 38: 331–339.

Song, W. J., S. H. Chung, and D. M. Kurnit, 1997 The murine Dyrk protein maps to chromosome 16, localizes to the nucleus, and can form multimers. Biochem. Biophys. Res. Commun. 231: 640–644.

Sonnhammer, E. L. L., and G. Östlund, 2015 InParanoid 8: orthology analysis between 273 proteomes, mostly eukaryotic. Nucleic Acids Res. 43: D234–D239.

Sönnichsen, B., L. B. Koski, A. Walsh, P. Marschall, B. Neumann et al., 2005 Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans. Nature 434: 462–469.

Shusterman, X., T. L. Le, M. M. Ahmed, and K. J. Gardiner, 2012 Pathways to cognitive deficits in Down syndrome. Prog. Brain Res. 197: 73–100.

Topalidou, I., P.-A. Chen, K. Cooper, S. Watanabe, E. M. Jorgensen et al., 2017 The NCA-1 and NCA-2 ion channels function downstream of Gq and Rho to regulate locomotion in Caenorhabditis elegans. Genetics 206: 265–282 (erratum: Genetics 206: 2225).

Vis, J. C., M. G. J. Duffels, M. M. Winter, M. E. Weijerman, J. M. Cobben et al., 2009 Down syndrome: a cardiovascular perspective. J. Intellect. Disabil. Res. 53: 419–425.

Wang, J. W., and S. Stifani, 2017 Roles of Runx genes in nervous system development. Adv. Exp. Med. Biol. 962: 103–116.

White, J. G., E. Southgate, J. N. Thomson, S. Brenner, 1986 The structure of the nervous system of Caenorhabditis elegans. Phil. Trans. Royal Soc. London. Series B, Biol Sci. 1165: 1–340.

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