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Archival Report

Domain-Specific Cognitive Impairments in Humans and Flies With Reduced *CYFIP1* Dosage

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ABSTRACT

BACKGROUND: Deletions encompassing a four-gene region on chromosome 15 (BP1-BP2 at 15q11.2), seen at a population frequency of 1 in 500, are associated with increased risk for schizophrenia, epilepsy, and other common neurodevelopmental disorders. However, little is known in terms of how these common deletions impact cognition.

METHODS: We used a Web-based tool to characterize cognitive function in a novel cohort of adult carriers and their noncarrier family members. Results from 31 carrier and 38 noncarrier parents from 40 families were compared with control data from 6530 individuals who self-registered on the Lumosity platform and opted in to participate in research. We then examined aspects of sensory and cognitive function in flies harboring a mutation in *Cyfip*, the homologue of one of the genes within the deletion. For the fly studies, 10 or more groups of 50 individuals per genotype were included.

RESULTS: Our human studies revealed profound deficits in grammatical reasoning, arithmetic reasoning, and working memory in BP1-BP2 deletion carriers. No such deficits were observed in noncarrier spouses. Our fly studies revealed deficits in associative and nonassociative learning despite intact sensory perception.

CONCLUSIONS: Our results provide new insights into outcomes associated with BP1-BP2 deletions and call for a discussion on how to appropriately communicate these findings to unaffected carriers. Findings also highlight the utility of an online tool in characterizing cognitive function in a geographically distributed population.

Keywords: 15q11.2, Cognitive impairment, CYFIP1, *Drosophila*, Neurodevelopmental disorders, Online phenotyping

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Deletions spanning a four-gene region on chromosome 15 (BP1-BP2 at 15q11.2), present at a population frequency of approximately 1 in 500, are associated with increased risk for multiple neurodevelopmental disorders (NDDs) (1). A large case-control study showed deletion carriers to be at approximately threefold increased risk for schizophrenia (2–4). Later work revealed an association between BP1-BP2 deletions and idiopathic generalized epilepsy, identifying a fivefold increased risk for carriers (5,6). Separate work has pointed to the deletion as a susceptibility locus for developmental disability (7,8). Consistent with this result, the rates of speech and motor delays were significantly increased in carriers versus noncarriers (9). Lastly, case series summarizing presentation in carriers have suggested that the variant may be associated with additional outcomes, including dysmorphic features, autism, attention-deficit/hyperactivity disorder, and intellectual disability (10–15).

Relatively little, however, has been done outside of clinically ascertained populations, potentially obscuring the relationship between BP1-BP2 deletion status and disease risk. Some investigators have looked to outcomes in carrier parents (10–14,16), and as summarized by Cafferkey *et al.* (9), only 22 of 32 evaluated were reported to be healthy. Not captured there, however, were phenotype data needed for the interpretation of this observation. Also unaddressed is whether deletions are associated with subclinical features. Results from a separate study identified carriers through population-based genotyping and performed neuropsychological testing that determined that deletion carriers struggled more with challenges of daily living than control subjects (17). Variations in regional gray matter volumes were also observed, and separate work has extended this finding to white matter microstructure (17,18). Stefansson *et al.* (17) also found that deletion carriers reported difficulties with reading and math at higher than expected rates. However, results from these questionnaires related to reading and math are subjective, and because they are based on historical impressions, they cannot be used in assessing the efficacy of possible interventions. Formal neurocognitive evaluations were also performed but did not identify differences between deletion carriers and noncarriers.

Separate from the identification of deficits resulting from deletions at BP1-BP2 is the development of appropriate models for interrogation of mechanisms, something that cannot be done through study of deletion carriers. Whereas four genes are present within the human BP1-BP2 region (*TUBGCP5, CYFIP1, NIPA2,* and *NIPA1*), mounting evidence

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points to the importance of cytoplasmic FMR1 interacting protein 1 (CYFIP1). Through physical interaction with ras-related c3 botulinum toxin substrate 1 (RAC1), CYFIP1 activates the WAVE regulatory complex and promotes cytoskeletal remodeling, a key aspect of dendritic spine formation (19–21). CYFIP1 also binds to the fragile X mental retardation protein, forming a translational inhibitory complex (22–25). Altered *CYFIP1* dosage perturbs neuronal morphology (21,26,27), synaptic excitation (28,29), and brain patterning and function (30). Importantly, alterations in white matter architecture and functional connectivity, similar to those observed in humans with BP1-BP2 deletions, were observed in mice and rats heterozygous for a mutation in *Cyfip1* (31,32). Regarding disease, individuals with schizophrenia showed a reduction in CYFIP1 in prefrontal cortex relative to control subjects (33). Separately, *CYFIP1* transcript levels are reportedly upregulated relative to control subjects in blood from individuals with autism and epilepsy (34,35). Associations between regulatory variants at the *CYFIP1* locus and both schizophrenia and autism have also been observed (36,37). Lastly, we have shown in human neural progenitor cells that *CYFIP1* knockdown resulted in dysregulation of schizophrenia and epilepsy gene networks (38).

Less is known about tubulin gamma complex associated protein 5, although its regulation of mitotic spindle formation is done in cooperation with glycogen synthase kinase 3 beta, a protein implicated in schizophrenia (39–44). Separately, a recent case report describing a BP1-BP2 deletion carrier with a missense variant in *TUBGCP5* suggested that this second variant may contribute to the primary microcephaly and mild developmental delay observed (45). Nonimprinted in Prader-Willi/Angelman syndrome 1 and 2 (NIPA1 and NIPA2), encoding proteins involved in magnesium transport, have also each been linked to neurological disease (46). Dominant negative mutations in *NIPA1* cause hereditary spastic paraplegia and expanded polyalanine repeats within the protein and are associated with increased risk for amyotrophic lateral sclerosis, a disorder resulting from death of motor neurons in the brain and spinal cord (47–49). Disruptive mutations in *NIPA2* have been identified in childhood absence epilepsy, and an association between a possible regulatory variant and schizophrenia has been reported (36,50,51). Toward mechanistic insights, we characterized flies heterozygous for a null mutation in the *CYFIP1* homologue (*Cyfip*$^{85.1/+}$).

METHODS AND MATERIALS

Human Studies

Subject Ascertainment and Enrollment. Families in which a child had been identified as a BP1-BP2 deletion carrier following clinical evaluation were invited to participate in our study. Some families were referred to us from clinicians, whereas others contacted us directly through the "15q11.2 Advocacy, Research, and Support" Facebook site (https://www.facebook.com/groups/1419874888238714/). Initial screening was based on a review of clinical array-based comparative genomic hybridization results provided to us by subjects or their physicians. Subjects harboring structural variants spanning the adjacent BP2-BP3 region were excluded, but beyond this, neither secondary variants nor diagnoses were considered in terms of inclusion or exclusion. The Albert Einstein College of Medicine Institutional Review Board approved our study, consent forms, and procedures for obtaining informed consent. All subjects, or their parent in the case of minors, provided written informed consent before participation. No genotype or phenotype data generated in the course of our study were made available to participants. Control data correspond to results from 6530 anonymized individuals who self-registered on the Lumosity platform (Lumos Labs, San Francisco, CA) and opted in to participate in research. These individuals were selected on the basis of demographic information from individuals within our familial cohort as well as the absence of any self-reported clinical diagnosis. For each subject we enrolled, Lumosity provided us with deidentified results from 100 or more individuals matched for age, gender, and education level. Similar to subjects we recruited, these individuals were tested remotely and without supervision.

Genotyping. A saliva or blood sample was obtained from each subject. The primary determinant here was the subject's geographical proximity to a phlebotomy laboratory. Regardless, all phenotyping was done online. Saliva samples were collected in Oragene OG-500 kits (DNA Genotek, Ottawa, ON, Canada), and blood samples were collected in purple-top ethylenediamine tetraacetate–containing tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). DNA were extracted on a QuickGene 610L instrument (Autogen, Holliston, MA) using QuickGene SP kits (Kurabo Industries Ltd., Osaka, Japan) for saliva and Puregene Blood Kits (Qiagen, Hilden, Germany) for blood. BP1-BP2 copy number was determined for all subjects using a TaqMan assay Hs01476346_cn (Thermo Fisher Scientific, Waltham, MA) as described elsewhere (38). DNA samples from Lumosity control subjects were not available for determination of BP1-BP2 deletion status.

Phenotyping. Parents were asked whether a clinician had ever given them a diagnosis of any of 10 NDDs (Table 1). We included subjects reporting pervasive developmental disorder–not otherwise specified within the autism group. Individuals reporting dyslexia, language delay, or another learning disability were said to have a learning disability. Each participant was also provided with a unique login and asked to complete the Web-based NeuroCognitive Performance Test (NCPT) (52) at home during a single 20- to 30-minute session.

Statistical Analyses. Two-tailed Fisher's exact test was used to evaluate the relationship between deletion status and gender. Frequencies of NDDs were compared between carriers and noncarriers using one-tailed Fisher's exact tests. For these exploratory analyses, p values $< .05$ were deemed nominally significant. For NCPT outcomes, raw scores for each subject enrolled was compared with the mean of $\geq$100 anonymous control subjects matched for gender, age, and education.

After excluding values greater than 3 SD from each group mean, one-tailed paired Student's t tests were used to identify deficits in deletion subjects and noncarriers. Based on self-reported difficulties with reading and mathematics among carriers (17), performance on grammatical reasoning and arithmetic reasoning tasks was evaluated under the a priori

Table 1. Learning Disabilities Are Present at a Higher Rate in Adult BP1-BP2 Deletion Carriers Than in Noncarrier Adults From the Same Homes

Diagnosis	Deletion (n = 31)	Noncarrier (n = 38)	p Value
ADHD	6 (19.4%)	7 (18.4%)	5.8×10^{-1}
Autism[a]	1 (3.2%)	0 (0.0%)	4.5×10^{-1}
Bipolar Disorder	2 (6.5%)	0 (0.0%)	2.0×10^{-1}
Depression	6 (19.4%)	7 (18.4%)	5.8×10^{-1}
Intellectual Disability	1 (3.2%)	0 (0.0%)	4.5×10^{-1}
Learning Disabilities[b]	9 (29.0%)	3 (7.9%)	2.3×10^{-2}[c]
OCD	2 (6.5%)	1 (2.6%)	4.2×10^{-1}
Schizophrenia	0 (0.0%)	0 (0.0%)	1.0
Seizures or Epilepsy	2 (6.5%)	0 (0.0%)	2.0×10^{-1}
Sensory Integration Disorder	1 (3.2%)	1 (2.6%)	7.0×10^{-1}
Any of Above[d]	14 (45.2%)	16 (42.1%)	5.0×10^{-1}

ADHD, attention-deficit/hyperactivity disorder; OCD, obsessive-compulsive disorder.

[a]Includes individuals reported to have a pervasive developmental disorder–not otherwise specified diagnosis.

[b]Individuals reporting a diagnosis of dyslexia, language delay, or a nonspecified learning disability were included here.

[c]$p < .05$ (uncorrected).

[d]Multiple individuals reported more than one diagnosis, and so percentages do not sum to 100%.

hypothesis that deletion carriers would show deficits (Table 1). We used Nyholt's method to obtain a p value threshold for these analyses that would ensure appropriate correction for multiple comparisons but also take into account the correlation between outcomes (effective test number = 2.73; $p_{hypothesis\ corrected} < 1.8 \times 10^{-2}$) (52,53). This same procedure was used to identify significant effects across the full set of measures available to us (effective test number = 9.75; $p_{all\ corrected} < 5.1 \times 10^{-3}$). Data visualization and statistics were done using R 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

Drosophila Studies

***Drosophila* Stocks, Rearing Conditions, and Behavioral Analyses.** Flies were cultured in vials containing a standard *Drosophila* medium at 25°C with 60% to 80% humidity in a 12-hour light/dark cycle. Flies heterozygous for a mutation that eliminates two thirds of the *dCyfip*-coding region ($Cyfip^{85.1/+}$) have been described previously (20). The fly line used as control was wild-type Canton-S w^{1118} (iso1CJ). Flies were tested at 3 to 5 days of age, and in all cases animals of each genotype were tested on the same day. All experiments were performed at 25°C and 75% to 80% relative humidity with the experimenter blinded to genotype. Student's t tests were performed in GraphPad Prism, version 8.1.0 (GraphPad Software, San Diego, CA) to identify between-group differences.

Sensory Perception. Olfactory and shock avoidance assays were performed as described previously to assess sensory function (54). Briefly, 10 or more groups of 50 odor-naïve flies of each genotype were placed in a T-maze. After 90 seconds, the number of flies in each arm was counted, and an avoidance index was calculated. The avoidance index corresponds to the number of flies found in the air-containing compartment minus the number of flies in the odor-containing compartment divided by the total number of flies. Shock avoidance was tested in a similar fashion with 90-V stimuli 1.25 seconds in duration. In these experiments, air was passed through both arms at constant flow throughout the testing period.

Associative Learning. We employed a previously described negative reinforcement paradigm to assay associative learning (54–56). During a 2-minute training phase, 10 or more groups of 50 to 60 flies of each genotype were placed into the single upper arm of a T-maze and exposed to various stimuli. Animals were first exposed to six shocks (90V; 1-second in duration every 5 seconds for 30 seconds) and a novel shock-associated odor (CS+). In this same compartment, animals were then exposed to odor-free air for 30 seconds, followed by a second unfamiliar odor for 30 seconds (without shock, CS−), and finally odor-free air again for a final 30 seconds. Flies were transferred immediately after this training phase to the lower part of the T-maze for testing. A performance index was calculated as described before (54–56). Briefly, half of the performance index was obtained by calculating the fraction of flies that avoided the shock-associated odor over 90 seconds minus the number of flies avoiding the control odor divided by the total number of flies. In parallel, another population of the same genotype of flies was trained with the CS+ and CS− odors reversed. A final performance index was the average of the two values.

Habituation: Electric Shock. Habituation, a measure of experience-dependent attenuation of reactivity to a stimulus, was assayed as described previously (57,58). In a training phase, 50 to 60 flies were placed in the upper arm of a standard T-maze with an electrifiable grid, then exposed to 45-V shocks over 75 to 90 seconds (15 shocks of 1.2-second duration, 4-second interstimulus interval). Air was not drawn through the tube during training to avoid association of the shocks with air. After a 30-second rest period, animals were transferred over 120 seconds to the lower part of the maze and allowed to move freely for 90 seconds during which time stimuli were delivered as above to the electrified arm of the maze. At the end of the choice period, the flies in each arm were trapped and counted, and an avoidance index was calculated as above.

Habituation: Light-Off Jump Reflex. There were 128 male flies per genotype subjected to 100 light-off stimuli (15-ms each; 1-second interstimulus interval), and a response was recorded if a jump occurred during or within 500 ms of the stimulus event. The proportion of responders within each group, or average jump response, was determined for each trial.

RESULTS

Marked Cognitive Deficits Observed in Adults Harboring BP1-BP2 Deletions

Given high rates of NDDs in clinically ascertained children harboring BP1-BP2 deletions (9–13), we sought to

characterize the frequency of various conditions in carrier and noncarrier parents. Eligibility was based on having a child referred to geneticists and determined to have a BP1-BP2 deletion. DNA analysis in our laboratory identified 31 carrier and 38 noncarrier parents across 69 individuals from 40 families. Consistent with published work, deletions were largely inherited (9,15) with only two de novo events observed across the 33 families in which inheritance could be resolved. The frequency of deletions was not significantly different between mothers and fathers. As detailed in Table 1, analyses showed that NDDs were reported at similar frequencies in carriers and noncarriers (14/31 vs. 17/38, $p = .58$), although learning disabilities were more than three times as common in carriers (9/31 vs. 3/38, $p = 2.3 \times 10^{-2}$).

To permit standardized and objective quantification of cognitive performance in our global cohort, we had subjects complete the Web-based NCPT (52). Composed of classic neuropsychological instruments adapted for computerized administration, the NCPT permits rapid assessment of grammatical reasoning, arithmetic reasoning, psychomotor speed, attention, working memory, nonverbal problem solving, response inhibition, and visual search (Supplemental Table S1). Good concordance between results from the NCPT and analogous paper-and-pencil tests is observed (52). Moreover, individuals reporting mild cognitive impairment showed significantly poorer performance than matched control subjects (52). To surmount issues relating to ascertainment bias, we studied parental carriers recruited on the basis of a child referred for genetic testing. Noncarrier parents were also evaluated to guard against misattributing effects of environment to genotype (59,60). The test battery was completed by 22 carriers (7 men and 15 women; mean age 39.7 ± 9.6 years) and 22 noncarriers (9 men and 13 women; mean age 42.6 ± 9.0 years).

Based on work suggesting that carriers reported difficulties with reading and mathematics (17), performance on the Grammatical Reasoning and the Arithmetic Reasoning tasks within the NCPT was evaluated first, under the a priori hypothesis that deletion carriers would do worse than population control subjects ($p_{hypothesis\ corrected} < 1.8 \times 10^{-2}$). Deficits were observed for both tasks (Table 2 and Figure 1). Deletion

Table 2. Domain Specific Cognitive Impairment in Adult BP1-BP2 Deletion Carriers but Not Noncarrier Adults From the Same Homes

	Deletions			Non-Carriers		
Test	Mean Difference (SD)[a]	Proportion Worse Than Control Subjects[b]	*p* Value	Mean Difference (SD)[a]	Proportion Worse Than Control Subjects[b]	*p* Value
A Priori Tests[c]						
Grammatical Reasoning, Time per Correct Trial, ms	599.23 (1001.79)	16/21	6.3×10^{-3}	−106.44 (1196.10)	8/21	6.6×10^{-1}
Grammatical Reasoning, No. Correct − No. Incorrect	−1.35 (3.15)	15/22	2.9×10^{-2}	−0.53 (4.67)	12/22	3.0×10^{-1}
Arithmetic Reasoning, No. Correct − No. Incorrect	−1.93 (3.76)	15/22	1.3×10^{-2}	−1.18 (5.77)	14/22	1.7×10^{-1}
Independent Tests[d,e]						
Digit Symbol Coding, No. Correct − No. Incorrect	−1.86 (5.99)	12/22	8.0×10^{-2}	−0.32 (8.07)	10/22	4.3×10^{-1}
Digit Symbol Coding, Time per Trial, ms	80.68 (331.84)	12/22	1.3×10^{-1}	−62.52 (402.35)	8/21	7.6×10^{-1}
Divided Visual Attention, Minimum Time, ms	−40.05 (121.75)	8/22	9.3×10^{-1}	−34.35 (145.33)	8/22	8.6×10^{-1}
Reverse Memory Span, No. Correct	−0.65 (0.89)	15/22	1.2×10^{-3}	0.02 (0.76)	10/22	5.6×10^{-1}
Forward Memory Span, No. Correct	−0.05 (1.05)	10/22	4.2×10^{-1}	−0.46 (0.93)	13/21	1.7×10^{-2}
Progressive Matrices, No. Correct	0.43 (3.44)	8/22	7.2×10^{-1}	−0.67 (3.36)	10/22	1.8×10^{-1}
Go/No-Go, Time per Trial, ms	−17.02 (67.28)	8/22	8.8×10^{-1}	12.39 (55.50)	10/22	1.5×10^{-1}
Trail Making A, Time to Complete, ms	−1628.59 (3785.56)	4/22	9.7×10^{-1}	−3402.18 (4605.57)	5/21	1.0
Trail Making B, Time to Complete, ms	−1704.08 (11,065.08)	8/21	7.6×10^{-1}	74.52 (15,860.89)	10/21	4.9×10^{-1}

[a]Performance for each subject was compared with a population of control subjects matched for age, education, and gender, and differences were calculated. For score-based measures, negative values correspond to relatively worse performance. For time-based measures, positive values correspond to relatively poorer performance.

[b]Outliers (>3 SD from the mean) were removed before analyses, resulting in differences from end point to end point in the number of subjects considered.

[c]Based on previously published work (17), performance on the grammatical reasoning and arithmetic reasoning tests was evaluated under the a priori hypothesis that deletion carriers would do worse than population control subjects ($p_{threshold} < 1.8 \times 10^{-2}$).

[d]For the remaining end points, *p* values were corrected for the full set of measures evaluated ($p_{threshold} < 5.1 \times 10^{-3}$). In each case, one-tailed tests were carried out to identify deficits present in either group.

[e]Surprisingly, noncarrier subjects performed significantly better than matched population control subjects on the Trail Making A test ($p = 2.9 \times 10^{-3}$). Performance on this task was also nominally better in deletion carriers ($p = 5.7 \times 10^{-2}$).

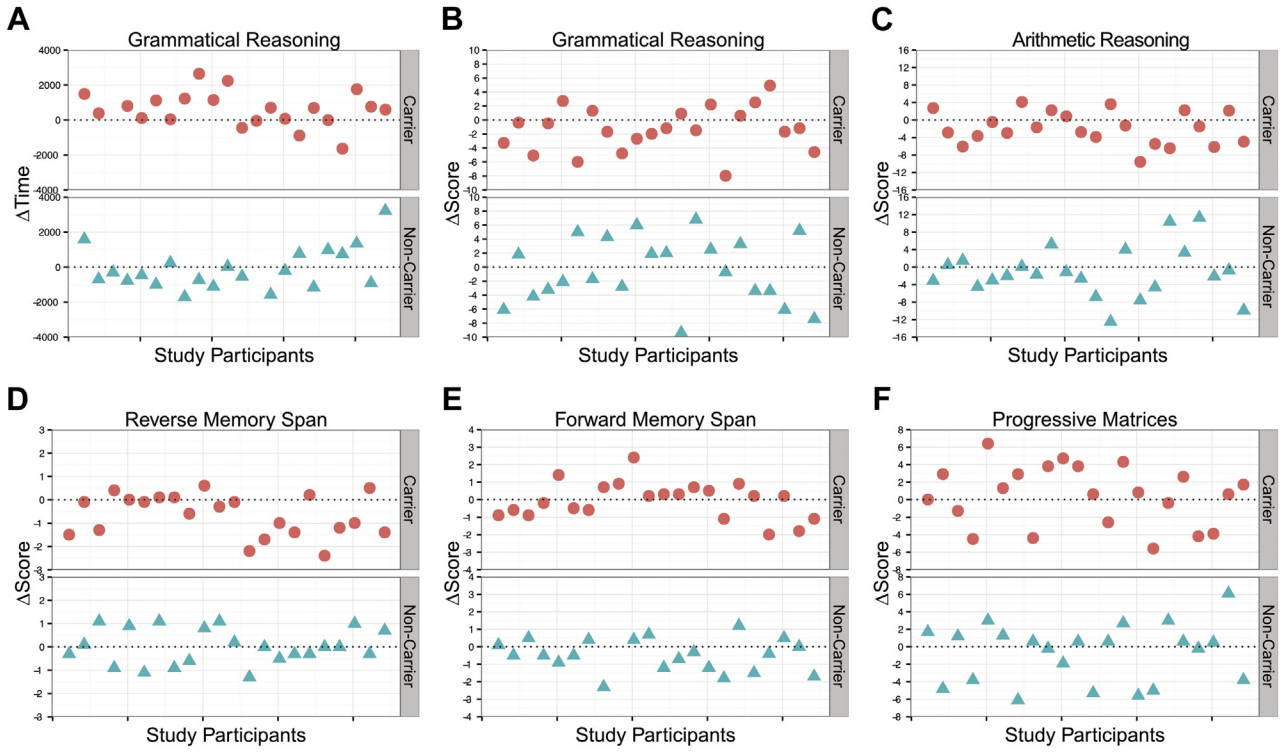

Figure 1. BP1-BP2 deletion–specific impairments in grammatical reasoning, arithmetic reasoning, and working memory. Task performance for individuals harboring deletions (red circles) and noncarrier adult family members (green triangles) was compared with matched population control subjects, and difference scores were obtained (y-axis). **(A)** Based on previously published work (17), performance on the grammatical reasoning and arithmetic reasoning tests was evaluated under the a priori hypothesis that deletion carriers would do worse than population control subjects ($p_{hypothesis\ corrected} < 1.8 \times 10^{-2}$). Relative to population control subjects, deletion carriers took significantly longer to answer individual questions within a grammatical reasoning task ($p = 6.3 \times 10^{-3}$; Cohen's $d = +0.72$, 95% confidence interval [CI] +0.06 to +1.4). No such effect was observed in noncarriers ($p = .66$). **(B)** A near significant decrease in grammatical reasoning score (number correct minus number incorrect) was observed in deletion carriers ($p = 2.9 \times 10^{-2}$; Cohen's $d = -0.55$, 95% CI −1.2 to +0.08), but not noncarriers ($p = .30$). **(C)** Deletion carriers scored significantly lower than population control subjects on an arithmetic reasoning task ($p = 1.3 \times 10^{-2}$; Cohen's $d = -0.66$, 95% CI −1.3 to −0.02). No such effect was observed in noncarriers ($p = .17$). **(D)** Performance on additional tasks was also examined, but a more stringent p value threshold was employed to correct for all 12 end points evaluated ($p_{all\ corrected} < 5.1 \times 10^{-3}$). Deletion carriers scored significantly worse than population control subjects on a reverse memory span task ($p = 1.2 \times 10^{-3}$; Cohen's $d = -1.0$, 95% CI −1.7 to −0.36). No such effect was observed in noncarriers ($p = .18$). **(E)** Neither deletion subjects nor noncarriers showed deficits on the less taxing forward memory span task ($p = .42$ and $p = .02$, respectively). **(F)** Performance IQ as assessed by the Raven's Progressive Matrices task was similar to control subjects in both deletion carriers ($p = .72$) and noncarriers ($p = .18$).

carriers took significantly longer than control subjects to correctly complete trials within the Grammatical Reasoning task, which has subjects evaluate whether sentences accurately describe the positioning of shapes presented alongside them ($p = 6.3 \times 10^{-3}$; Cohen's $d = +0.72$, 95% confidence interval [CI] +0.06 to +1.4) (Figure 1A, top). No such effect was observed in noncarriers ($p = .66$) (Figure 1A, bottom). A near significant decrease in Grammatical Reasoning score was also observed in carriers ($p = 2.9 \times 10^{-2}$; Cohen's $d = -0.55$, 95% CI −1.2 to +0.08) (Figure 1B, top), but not in noncarriers ($p = 0.30$) (Figure 1B, bottom). Carriers likewise scored significantly lower than population control subjects on the Arithmetic Reasoning task, in which subjects are presented with an arithmetic problem written out in words and asked to answer using numbers ($p = 1.3 \times 10^{-2}$; Cohen's $d = -0.66$, 95% CI −1.3 to −0.02) (Figure 1C, top). No such effect was observed in noncarriers ($p = .17$) (Figure 1C, bottom).

Performance on additional tasks was also examined (Table 2), but a more stringent p value threshold was employed to correct for multiple comparisons ($p_{all\ corrected} < 5.1 \times 10^{-3}$). These analyses determined that deletion carriers scored significantly worse than population control subjects on a reverse memory span task that has subjects click on rectangles in the opposite order to which they changed color ($p = 1.2 \times 10^{-3}$; Cohen's $d = -1.0$, 95% CI −1.7 to −0.36) (Figure 1D, top). No such working memory effect was observed in noncarriers ($p = .18$) (Figure 1D, bottom). Neither deletion carriers nor noncarriers showed deficits on a cognitively less taxing forward memory span task ($p = .42$ and $p = .02$, respectively) (Figure 1E). Performance IQ as assessed by the Raven's Progressive Matrices task was similar to matched control subjects in carriers and noncarriers ($p = .72$ and $p = .18$, respectively) (Figure 1F). Similarly, no deficits were observed in either group for additional end points examined (Table 2 and Supplemental Figure S1), although noncarriers were faster than matched population control subjects on the Trail Making A test used to assess visual search ability ($p_{two\ tailed} = 2.9 \times 10^{-3}$; Cohen's $d = -1.0$, 95% CI −1.7 to −0.36). For this test,

subjects were tasked with tracing a path between numbered circles as rapidly as possible. Performance on this task was nominally better in deletion carriers relative to control subjects, although this difference was not significant ($p_{two\ tailed} = 5.7 \times 10^{-2}$; Cohen's $d = -0.50$, 95% CI −0.13 to +1.1).

Cyfip Haploinsufficiency in Flies Revealed Associative Learning and Habituation Deficits

Given published work linking one of four genes within the BP1-BP2 deletion to synaptic plasticity and long-term potentiation (20,21,23,24,29), we reasoned that the cognitive deficits we observed in carriers might be attributable to haploinsufficiency of *CYFIP1*. To test this hypothesis, we characterized associative and nonassociative learning paradigms in *Cyfip*$^{85.1/+}$ flies, heterozygous for a null mutation in the *Drosophila* homologue of the human *CYFIP1* gene. No between-group differences were evident for avoidance of aversive odors (octanol and benzaldehyde) or an electric shock ($p > .05$) (Figure 2A), demonstrating intact sensory capabilities in *Cyfip*$^{85.1/+}$ flies. Behavior in an associative learning paradigm, however, identified a deficit in *Cyfip*$^{85.1/+}$

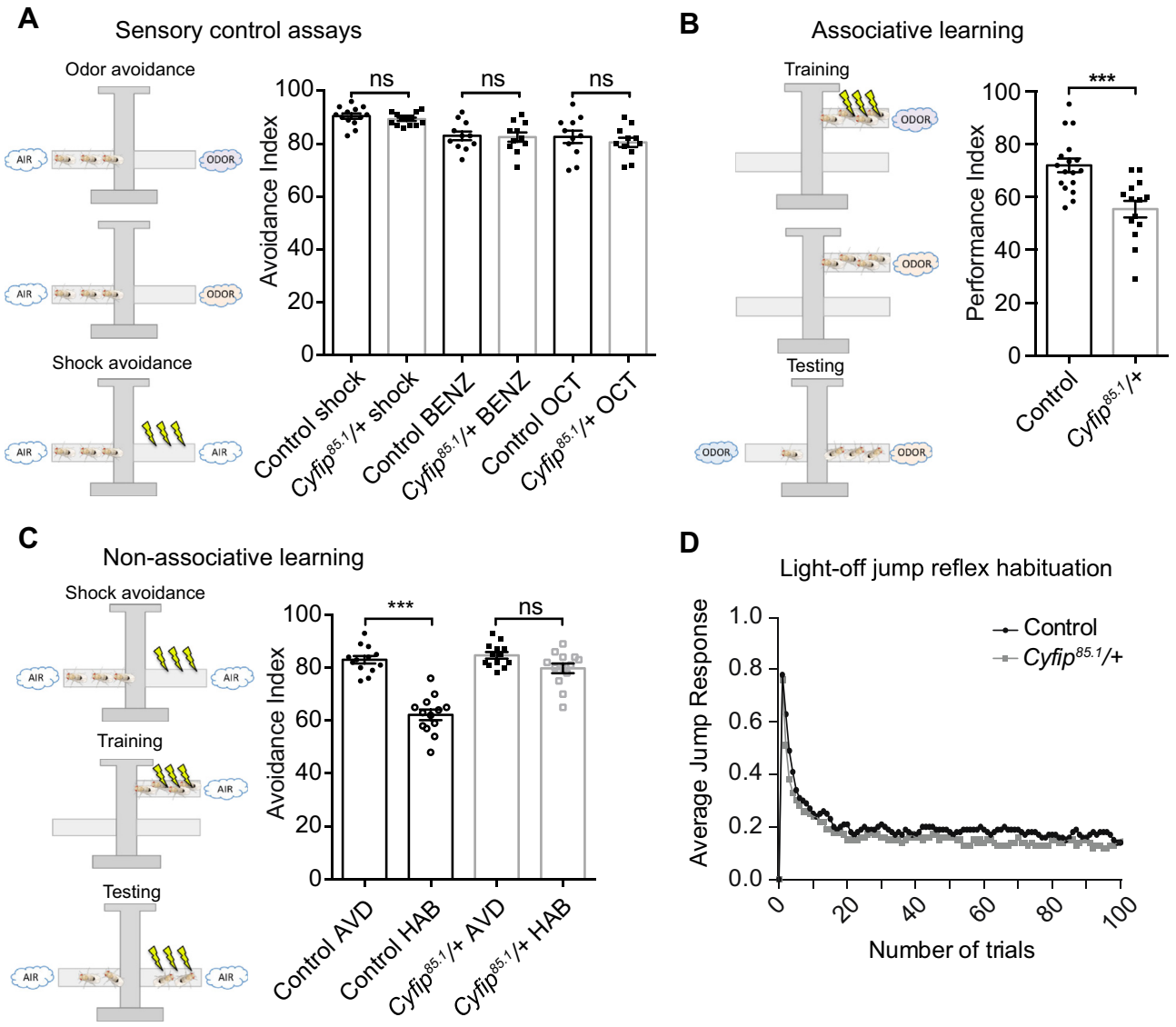

Figure 2. Associative learning and habituation deficits in flies with reduced *Cyfip* dosage. **(A)** Control and *Cyfip*$^{85.1/+}$ flies avoided an environment containing 3-octanol (OCT) or benzaldehyde (BENZ). Both genotypes likewise avoided entering an environment in which they received an electric shock, indicating intact sensory perception. No significant between-group differences were observed ($p > .05$; ≥11 groups of 50 flies per genotype). **(B)** *Cyfip*$^{85.1/+}$ mutant flies showed significantly impaired associative learning in a negative reinforcement paradigm compared with control animals (***$p < 1.0 \times 10^{-3}$; 17 groups of 50 flies per genotype). **(C)** *Cyfip*$^{85.1/+}$ flies showed diminished habituation to shock, a deficit in nonassociative learning, compared with control animals (***$p < 1.0 \times 10^{-3}$; 13 groups of 50 flies per genotype). **(D)** No difference between control and *Cyfip*$^{85.1/+}$ flies was observed in a second test of habituation, the light-off jump assay ($p > .05$). AVD, avoidance; HAB, habituation; ns, not significant.

animals. More specifically, avoidance of a shock-paired odor was significantly reduced in $Cyfip^{85.1/+}$ flies relative to control animals ($p < 1.0 \times 10^{-3}$) (Figure 2B). Furthermore, whereas control flies showed significantly reduced habituation to electric shock, a form of nonassociative learning ($p < 1.0 \times 10^{-3}$) (Figure 2C), mutant flies were resistant to habituation. Behavior in the light-off jump reflex assay was similar between the two genotypes ($p > .05$) (Figure 2D), however, suggesting that at least some forms of short-term habituation remained intact.

DISCUSSION

BP1-BP2 deletions are associated with increased risk for NDDs and are present in 1 in 500 individuals. We show in this study that carriers, recruited for this study because of genotype as opposed to clinical diagnosis, show marked deficits in grammatical and mathematical reasoning. These results are consistent with an earlier questionnaire-based study that determined that BP1-BP2 deletion carriers reported significantly greater difficulties than noncarriers in reading (adult reading history questionnaire) and math (adult mathematical history questionnaire) (17). Whereas Stefansson *et al.* (17) inferred competency from self-report, we assessed subject performance directly. This is an important distinction in that subjectivity is reduced and inherent biases are diminished. For example, adult reading history questionnaire scores are highly sensitive to subject age (61). It is unclear whether we would have identified deficits in our much smaller cohort had we relied on self-report. It is also still unclear what specific aspects of cognition are compromised in deletion carriers. Stefansson *et al.* (17) found no effect of genotype on verbal IQ as measured by the Wechsler Abbreviated Scale of Intelligence or verbal fluency as determined by the Controlled Oral Word Association Test. Similarly, we saw no effect on the Digit Symbol Coding task despite a strong correlation between outcomes here and those for the Arithmetic Reasoning task (Pearson's $r = .48$). Underscoring the complexity here, this correlation is greater than that between performance on the Grammatical Reasoning and Arithmetic Reasoning tasks in which deficits were identified (Pearson's $r = .4$). Additional clarity may emerge from brain imaging studies, in that rare and common variants at the BP1-BP2 locus have been associated with variation in gray matter volume and white matter integrity (17,18,62).

We also report for the first time impairments in working memory that could underlie the observed deficits in reading-related and math-related performance. No memory-related findings were observed by Stefansson *et al.* (17). In their study, memory was assessed using the Logical Memory subtest from the Wechsler Memory Scale III, which has subjects retell stories told to them either immediately or after a delay. Working memory was also evaluated by these authors by having subjects perform the Spatial Working Memory subtest within the Cambridge Neuropsychological Test Automated Battery; subjects were presented with colored squares and asked to identify hidden tokens through a process of elimination. The effects we observed are large, with Cohen's *d* values as high as 1.0, corresponding to a 76% chance that a randomly selected BP1-BP2 deletion carrier will perform worse than a noncarrier (63). As such, results argue against the deletion being either benign or of unknown significance and suggest that findings be shared with families. This said, because outcomes for individuals cannot be predicted with good precision, accurate communication of results will be challenging.

Results from flies, demonstrating deficits in associative learning and habituation in $Cyfip^{85.1/+}$ animals, show that in this species reduced dosage of this single gene is sufficient to cause the cognitive deficits we observed in human deletion carriers. Consistent with this idea is that individuals with fragile X syndrome, a disorder arising from dysregulation of the CYFIP1 interacting protein fragile X mental retardation protein, do especially poorly on tasks involving arithmetic processing and memory span (64–66). Also consistent is that regulatory variants in *CYFIP1* linked to *FOXP2* are associated with variation in surface area of the language-related supramarginal gyrus (62). In this context, additional study of $Cyfip^{85.1/+}$ animals may identify new strategies for addressing the cognitive impairments observed in disorders such as schizophrenia for which individuals with BP1-BP2 deletions are at increased risk. A limitation, however, is that it is not yet clear the degree to which *Cyfip*-associated cognitive deficits in flies will inform our understanding of the impact of BP1-BP2 deletions in humans. As reviewed immediately above, this issue is particularly relevant for deficits associated with BP1-BP2 deletion given distinct findings from subtly different neuropsychological tests.

Finally, results from this study demonstrate the utility of a Web-based approach in the characterization of a geographically distributed population. Given ever-falling costs in generating and analyzing complex genetic data, acquiring phenotype data at scale represents the primary bottleneck in uncovering new genotype-phenotype relationships. The application of high-throughput remote phenotyping represents a potential solution and may make possible studies that could not have been undertaken otherwise.

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YJW, AKK, CB, and BSA designed the study. YJW, AKK, PH, JK, RAN, and BSA generated and/or analyzed data. JK oversaw family recruitment. TW provided expertise regarding statistical analyses. YJW and BSA wrote the article.

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