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A Recurrent De Novo Variant in *NACCI* Causes a Syndrome Characterized by Infantile Epilepsy, Cataracts, and Profound Developmental Delay

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Whole-exome sequencing (WES) has increasingly enabled new pathogenic gene variant identification for undiagnosed neurodevelopmental disorders and provided insights into both gene function and disease biology. Here, we describe seven children with a neurodevelopmental disorder characterized by microcephaly, profound developmental delays and/or intellectual disability, cataracts, severe epilepsy including infantile spasms, irritability, failure to thrive, and stereotypic hand movements. Brain imaging in these individuals reveals delay in myelination and cerebral atrophy. We observe an identical recurrent de novo heterozygous c.892C>T (p.Arg298Trp) variant in the nucleus accumbens associated 1 (*NACCI*) gene in seven affected individuals. One of the seven individuals is mosaic for this variant. *NACCI* encodes a transcriptional repressor implicated in gene expression and has not previously been associated with germline disorders. The probability of finding the same missense *NACCI* variant by chance in 7 out of 17,228 individuals who underwent WES for diagnoses of neurodevelopmental phenotypes is extremely small and achieves genome-wide significance ($p = 1.25 \times 10^{-14}$). Selective constraint against missense variants in *NACCI* makes this excess of an identical missense variant in all seven individuals more remarkable. Our findings are consistent with a germline recurrent mutational hotspot associated with an allele-specific neurodevelopmental phenotype in *NACCI*.

Advances in massively parallel, next generation sequencing such as whole-exome sequencing (WES) have led to improved molecular diagnostic rates for rare and undiagnosed Mendelian disorders, identification of new pathogenic gene variants, better treatment options, and accurate prediction of recurrence risk.¹ WES in large cohorts of neurologic phenotypes including autism spectrum disorders,^{2,3} intellectual disability,^{4–6} and epilepsy^{7,8} have

demonstrated a major contribution of rare, de novo variants to disease phenotypes.^{9,10} However, since many of these de novo variants are often seen in a singleton setting, it can be challenging to conclusively infer causality. Moreover, the extent to which the phenotype includes findings in addition to the developmental delay may not be initially appreciated.¹¹ This can be resolved by ascertaining a number of affected individuals with similar phenotypes who

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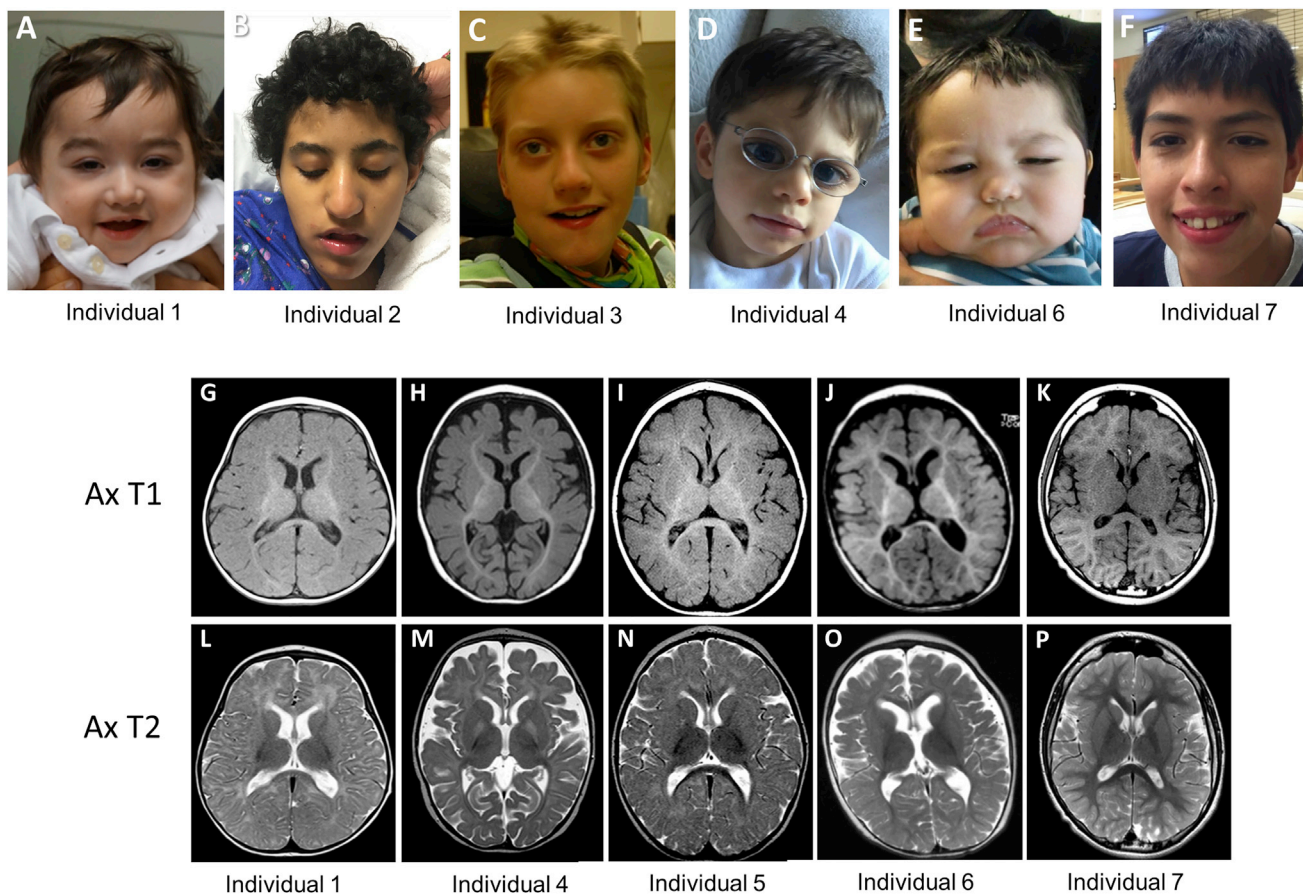


Figure 1. Clinical and Neuroradiologic Characteristics of the Seven Probands

(A) Individual 1 at 20 months of age.

(B) Individual 2 at 12 years of age.

(C) Individual 3 at 9 years of age.

(D) Individual 4 at 3.5 years of age.

(E) Individual 6 at 13 months of age.

(F) Individual 7 at 12 years of age.

(G–P) Axial T1 and T2 brain MRI images demonstrating mildly delayed myelination in individual 1 at 9 months old (G, L); severely delayed myelination and volume loss in individual 4 at 3 years old (H, M); severely delayed myelination and minimal volume loss in individual 5 at 10 years old (I, N); volume loss with normal myelination in individual 6 at 12 months old (J, O); and normal myelination and brain volume in individual 7 at 10 years old (K, P).

harbor the same recurrent *de novo*, potentially pathogenic variant, as seen in this series of seven individuals all with the same missense variant in the nucleus accumbens associated 1 gene (*NACCI* [MIM: 610672]). *NACCI* is a member of the BTB/POZ domain-containing gene family and encodes for NAC1, a protein that functions as a transcriptional regulator.¹² Although *NACCI* has not previously been associated with human Mendelian disease, its function makes it a plausible disease-associated gene, since it has been increasingly shown that a number of neurodevelopmental disorders are caused by misregulated gene expression.¹³

The seven individuals we report here (Figure 1) have a clinical constellation of severe to profound developmental delay and/or intellectual disability (HP: 0012736), epilepsy (HP: 0001250), feeding difficulties and/or feeding intolerance (HP: 0011968), irritability (HP: 0000737), and in the majority, postnatal microcephaly (HP: 0005484), bilateral

cataracts (HP: 0000519), sleep disorder (HP: 0002360), and stereotypic motor behaviors (HP: 0000733). Detailed medical summaries and WES results are available in the [Supplemental Data](#). Consent for publication was obtained from parents of all subjects, and procedures were followed in accordance with guidelines specified by Institutional Review Boards and Ethics Committees of each institution. Experienced pediatricians, geneticists, and neurologists clinically assessed the individuals.

The key clinical phenotypes are summarized in [Table 1](#). Most individuals had profound developmental impairment such that ambulation and speech were absent or greatly impaired. Infantile epilepsy had been noted in all seven individuals, with infantile spasms in four. Overall seizure control had been variable in the seven individuals but generally required multiple anti-epileptics and sub-specialized neurologic care. Bilateral cataracts requiring surgical extraction were present in five out of seven

Table 1. Phenotypic Features in Individuals with *NAC1* c.892C>T (p.Arg298Trp) Variant

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Total
Age	20 months	12 years	18 years	3 years	9 years	13 months	12 years	
Gender	M	F	M	M	F	M	M	
<i>NAC1</i> c.892C>T (Arg298Trp) variant	constitutional, de novo	constitutional, de novo	constitutional, de novo	constitutional, de novo	constitutional, de novo	constitutional, de novo	mosaic, de novo	7/7
Postnatal microcephaly	yes	yes	yes	yes	yes	no	no	5/7
Intellectual/developmental disability	profound	profound	profound	profound	profound	profound	severe	7/7
Bilateral cataracts	lamellar, diagnosed at 7 months old	diagnosed at 10 and 11 years old	diagnosed at 6 weeks old	anterior subcapsular, congenital	nuclear, diagnosed at 11 and 12 months old	no	no	5/7
GI problems/feeding difficulties	feeding intolerance, failure to thrive, gastrostomy at 9 months	dysphagia	severe feeding problems, PEG at 6 years old	feeding intolerance, failure to thrive, gastrostomy at 9 months	feeding intolerance, cyclic vomiting, gastrostomy at 2.5 years old	dysphagia, failure to thrive	feeding difficulties, GERD	7/7
Hypotonia	present	present	present	present	present	present	no	6/7
Seizures	infantile spasms	focal seizures	generalized tonic	infantile spasms	infantile spasms, mixed generalized and multifocal epilepsy	infantile spasms, tonic seizures	single febrile seizure, nocturnal seizures	7/7
Irritability	very fussy, breath-holding spells	yes	tactile aversion, unable to tolerate being held, periods of inconsolability	bouts of irritability, breath-holding spells	cyclical bouts of severe irritability, breath-holding spells	yes	yes	7/7
Sleep disorder	yes	–	yes	yes	episodes of dysautonomia (tachycardia and insomnia)	–	obstructive sleep apnea	5/7
Stereotypic movements	repetitive movement bringing hands to midline and pulling shirt down	autistic features, Rett-like hand automatisms; hands always in her mouth	Rett-like stereotypic hand movements	repetitive hand movements	random choreiform movements of the hands	no	hand flapping, autistic features	6/7
Brain MRI findings	delayed myelination, mildly decreased brain volume, slight enlargement of ventricles	brain atrophy, temporal arachnoid cyst, MRS showed decreased NAA over basal ganglia	reduced white matter, delayed and incomplete myelination with temporal lobes most affected, mildly decreased brain volume, MRS showed decreased NAA levels	delayed myelination and volume loss	minimal volume loss, delayed myelination	volume loss	scattered areas of subcortical white matter T2/FLAIR hyperintensity	7/7

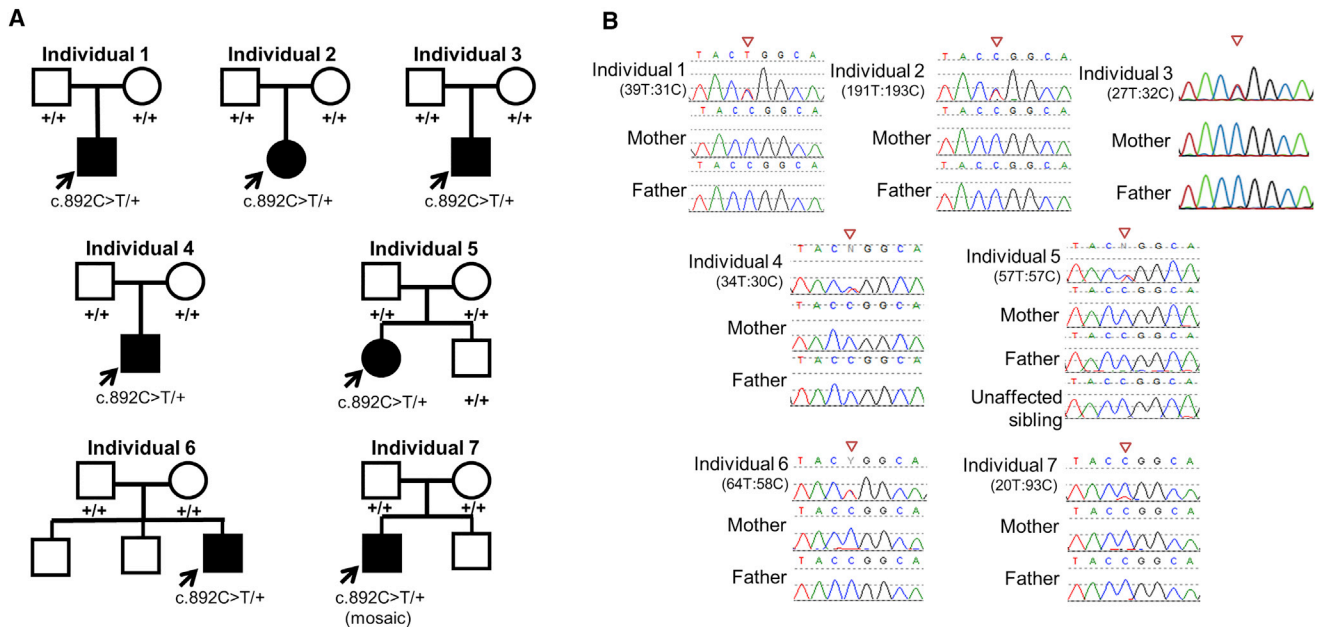


Figure 2. Recurrent De Novo c.892C>T (p.Arg298Trp) Missense Change in *NACCI* Observed in All Seven Proband

(A) Family pedigrees for individuals 1–7 with de novo events in each affected proband. Note that unaffected sibling of individual 5 was also sequenced and found not to have the de novo variant.

(B) Sanger sequencing traces for individuals 1–7 showing the heterozygous de novo C-T transition at the c.892 CGG codon encoding arginine, resulting in a TGG codon encoding tryptophan. Individual 7 was found to be mosaic for this variant in peripheral blood. Numbers below each individual represent allele count of alternative to reference alleles from exome sequencing.

individuals, with age of onset ranging from birth to 10 years, with variability in the type of cataracts. All individuals had a history of feeding difficulties or intolerance, with four out of seven requiring feeding by gastrostomy tube. Postnatal microcephaly was noted in five out of seven individuals, being evident prior to the onset of seizures in some individuals. All individuals were described as being irritable or having bouts of severe irritability. Hypotonia, sleep disorders, breath-holding spells, and repetitive or choreiform movements, hand flapping, or Rett-like hand automatisms were present in the majority of individuals. Interestingly, individuals presented here were consistently evaluated for severe infantile epilepsy disorders such as *CDKL5* (MIM: 300203)- and *ARX* (MIM: 300382)-related diseases, in addition to *MECP2* (MIM: 300005) and other Rett-like disorders due to the repetitive hand movements. Brain MR imaging showed delayed myelination in four individuals, decreased brain volume in six individuals, and focal cortical dysplasia in one individual (Figure 1). The appearance of a broad nasal tip was the only consistent facial dysmorphic feature noted in these individuals.

Individuals 1, 2, 6, and 7 had trio exome sequencing, and individuals 3–5 had proband exome sequencing followed by Sanger confirmation (Figure 2). Detailed WES platform^{14–16} and coverage for the seven individuals are listed in Table S1. The WES data on individual 1 were re-analyzed after he was enrolled into the Undiagnosed Diseases Network (UDN). GeneMatcher,¹⁷ a web-based tool for researchers and clinicians with interest in common genes, connected researchers and clinicians involved

with individuals 1 and 2. Individual 3 was identified from the Baylor-Hopkins Center for Mendelian Genomics Database (BHCMG) database,¹⁴ and individuals 4 and 5 were identified from clinical exome data from the Baylor Genetics laboratory.¹⁵ Finally, individuals 6 and 7 were connected through a UDN webpage designed to connect individuals with overlapping clinical features and candidate genes.

WES in all seven individuals identified a recurrent, heterozygous, de novo variant c.892C>T (p.Arg298Trp) in *NACCI* (GenBank: NM_052876.3) (Figures 2A and 2B). Sanger sequencing of this variant in all families confirmed de novo inheritance. Low alternative allele fraction observed in the exome data (93 reference allele and 20 alternate allele) and Sanger sequencing for individual 7 suggested *NACCI* mosaicism. The significance of the allele balance bias observed at c.892C>T was evaluated by binomial test of the observed alternate allele counts and overall allele coverage of all well-covered (>10×) heterozygous positions in the affected individual's exome. After adjusting p values using the Benjamini-Hochberg method with an FDR of 0.05, the *NACCI* variant was confirmed mosaic ($p < 0.0001$). Parentage of six of the seven trio families was confirmed by trio exome sequencing (individuals 1, 2, 6, 7) or Sanger sequencing of rare variants (individuals 4 and 5) (Table S2).

The c.892C>T transition occurs at a CpG dinucleotide within an arginine codon, a hypermutable CpG pattern reported in association with de novo events at numerous loci in the setting of advanced paternal age.^{18–20} However,

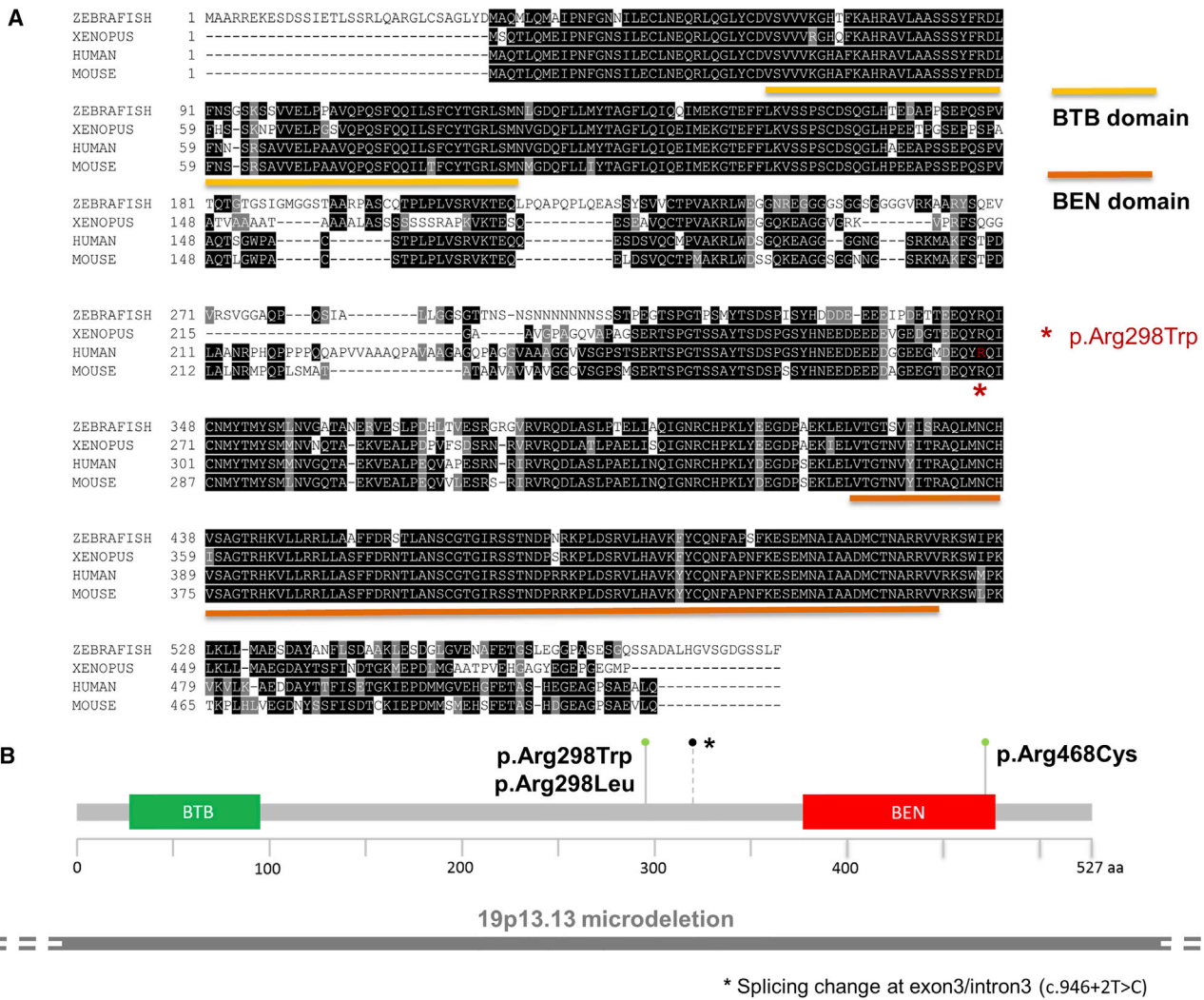


Figure 3. Predicted Protein Change for the De Novo *NAC1* Variant in Context of Protein Domains

(A) Amino acid sequence alignment of human *NAC1* with zebrafish, *Xenopus*, and mouse. The BEN and BTB domains are shown, and the location of the p.Arg298Trp missense change is depicted by a red star. The amino acid residue is conserved in all four species and is within a stretch of identical residues.

(B) The Arg298 residue occurs within *NAC1* between the BTB domain and BEN domain, closer to the BEN domain. A single p.Arg468Cys change (gray-green lollipop) has been noted in a large cohort study of intellectual disability,⁶ while the c.946+2T>C (A316 splice) change (black lollipop with asterisk to indicate the position of the splice change) has been noted in a cohort of patients with autism spectrum disorder. Interestingly, a variant involving the same amino acid, converting from Arg to Leu (c.893G>T [p.Arg298Leu]), has been observed once in the ExAC database and in four individuals in gnomAD, with undefined clinical information. Spanning this region is a microdeletion, shown as a gray bar under the gene domain structure.

advanced paternal age was not a common feature of this cohort. Additional variants identified through exome sequencing in these individuals are summarized in Table S2.

Further investigation of this gene in the ExAC database revealed a single loss-of-function (LoF) variant (probability of LoF intolerance [pLI] = 0.96) and fewer than expected number of missense variants ($z = 5.23$) despite relatively good coverage of the gene, suggesting that mutations in *NAC1* are subject to selection and that rare, predicted pathogenic variants may confer risk for human diseases. Additionally, the residual variation intolerance score (RVIS) of *NAC1* is 14, with a raw score of -0.7 ,²¹ indicating that it is a gene that does not tolerate functional variants. The p.Arg298Trp variant is not observed in the ExAC

or gnomAD databases, although a missense change from arginine to leucine of the same amino acid (p.Arg298Leu) has been observed in one individual in the ExAC database and in four individuals in gnomAD.²² This suggests that the p.Arg298Trp change that we report is likely to exert a specific functional effect on the protein and may lead to the distinct disease phenotype presented in this report. Extensive search of somatic databases identified a single case in metastatic breast cancer with the p.Arg298Trp variant.²³ The combined annotation dependent depletion (CADD) score,²⁴ which ranks deleteriousness of single-nucleotide variants within the human genome, is 25.8 for this variant, indicating that it is predicted to be in the top 0.14% most damaging in the genome. The Arg298

residue is extremely conserved from human to zebrafish with a GERP++ RS score of 4.89 (Figure 3A) and this variant is predicted to be deleterious by several prediction algorithms including align GVGD, PolyPhen, SIFT, and MutationTaster. These in silico analysis tools support the contention that the p.Arg298Trp change is likely to exert a specific functional effect on the protein.

Furthermore, we calculated the estimated probability of observing seven de novo *NAC1* variants among the 17,228 individuals who underwent exome sequencing for neurodevelopmental/neurologic disorders across the different clinical testing laboratories and research studies that tested the seven individuals (GeneDx, 6,478; BHCMG, 3,400; Baylor, 6,250; UCLA, 1,100). The significance of this finding was evaluated using a binomial test, with the mutation rate as the probability of success, the number of individuals who underwent exome sequencing as the number of trials, and the number of probands who were found to have this mutation as the number of successes.²⁵ The probability of a chance occurrence is small and remains highly significant when Bonferonni correction is applied for the 3 billion base pairs in the genome ($p = 1.25 \times 10^{-14}$). Thus, our findings implicate *NAC1* as a genome-wide significant disease-associated gene.

Although *NAC1* has not previously been associated with a Mendelian phenotype, a link between *NAC1* and intellectual disability has been suggested, with apparent de novo variants being noted in single individuals within large sequencing studies of intellectual disability^{5,6} and autism spectrum disorder² cohorts (Table S3). One female with intellectual disability (IQ of ~45), autism, and schizo-affective disorder has been noted with a de novo missense allele c.1402C>T (p.Arg468Cys) in *NAC1*, located in a different region of the gene, the BEN domain (Figure 3A).^{5,6} In another exome-sequencing effort from the Simons Simplex Collection, one individual with autism spectrum disorder was identified to have a de novo splicing variant (presumably LoF), c.946+2T>C, in *NAC1*.² These individuals do not appear to have, or at least were not reported as having, other key manifestations seen in our individuals, such as cataracts, epilepsy, irritability, and microcephaly. Contiguous gene deletions involving *NAC1* are seen in 18 individuals in the DECIPHER database, ranging in size from 83 kb to 2.39 Mb, and duplications involving *NAC1* are seen in 12 individuals, ranging in size from 211 kb to 58.83 Mb (Table S3), and thus the relative contribution of the loss of *NAC1* in these individuals is difficult to infer. A known microdeletion/microduplication syndrome involving chromosome 19p13.13 includes most of *NAC1* along with ~16 other genes and is characterized by macrocephaly, overgrowth, and intellectual disability, but it has been speculated that *NFIX* is the critical gene in the region.^{26,27} There are no copy losses involving *NAC1* in non-disease individuals in the Database of Genomic Variants.²⁸ Taken together, none of the previous studies involving *NAC1* aberrations report the syndromic phenotype we delineate here in

the seven individuals carrying a de novo p.Arg298Trp variant, strengthening the phenotypic association specific to this missense change. Individual 7 had a milder clinical presentation compared to the other individuals reported here; at 12 years of age he had not developed cataracts, had normal tone and head size, and was ambulatory with limited speech. This may be explained by the likely mosaicism of the recurrent c.892C>T variant in peripheral blood, demonstrated by atypical allele balance (Figures 2A and 2B). Although the exact contribution of the mosaic *NAC1* variant to the individual's milder phenotype is difficult to ascertain from exome data alone and requires further functional studies, previous studies suggest that mosaic mutations frequently occur in individuals diagnosed with intellectual disability and autism spectrum disorders.^{29,30}

NAC1 has a documented role in cancer. *NAC1* knockdown or overexpression of an *NAC1* mutant containing only the BTB/POZ domain promotes cell apoptosis and senescence, inhibits cytokinesis, and prevents tumor formation.^{31–33} *NAC1* is upregulated in several types of neoplasms, especially ovarian serous carcinomas, either at the transcriptional level or through DNA amplification and overexpression. This is associated with cell proliferation, migration and invasion, chemotherapy resistance, tumor recurrence, and poor prognosis.^{31,32,34–37} While an oncogenic role has been proposed for *NAC1*'s involvement in cancers,³² we do not currently know whether or not our individuals with the recurrent p.Arg298Trp variant are at risk for cancers. Certainly, there is precedence for many genes in which germline variants cause neurodevelopmental disorders and somatic variants can be associated with cancers.^{38–40}

Previous studies have shown that *NAC1* forms a homodimer or heterodimers with other binding partners through the BTB/POZ domain and functions as a transcriptional repressor through recruitment of histone deacetylase.^{12,41} The p.Arg298Trp variant is located outside the BTB/POZ domain that is important for cancer progression and the BEN domain in which one missense variant associated with intellectual disability has been reported⁶ (Figure 3B). A dominant-negative function (especially since the protein dimerizes) or a gain-of-function (GoF) is possible for the p.Arg298Trp variant, since the prior reported LoF alleles in *NAC1* result in an overlapping but less severe phenotype of intellectual disability and autism,^{2,6} and it is well known that LoF and GoF variants in the same gene can result in heterogeneous phenotypes with overlapping features.^{25,42,43} Future studies are required to elucidate the mechanism of disease related to this *NAC1* variant.

NAC1 is known to have other biological functions, including involvement in psychomotor response to cocaine administration in rats,^{30–32} vertebral patterning in mice,⁴⁴ interaction with Parkin suggestive of a role in Parkinson disease,⁴⁵ and involvement with TDP-43, which is implicated in individuals with amyotrophic lateral sclerosis.⁴⁶ The role of *NAC1* in normal neurologic function

is highlighted by its role in mitigating protein turnover in dendritic cells and in maintaining synaptic plasticity.⁴⁰ As a transcriptional repressor and protein binding factor, NAC1 probably functions in regulating neural development and network establishment by altering expression, localization, and degradation of many downstream nervous system genes. Given the characteristic phenotype associated with the p.Arg298Trp variant, future transcriptional investigations may further delineate the impact of this rare, recurrent, highly penetrant allele and could potentially lead to targeted therapies.

The constellation of cataracts, severe epilepsy, profound intellectual disability, irritability, microcephaly, and stereotypical movements is unusual and given the consistent occurrence of these phenotypes across our seven individuals, the allele-specific variant that we report in *NAC1* should be considered strongly in individuals with similar features.

Supplemental Data

Supplemental Data include case reports and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.12.013>.

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Web Resources

CADD, <http://cadd.gs.washington.edu/>
ClinVar, <https://www.ncbi.nlm.nih.gov/clinvar/>
DECIPHER, <http://decipher.sanger.ac.uk/>
ExAC Browser, <http://exac.broadinstitute.org/>
GenBank, <http://www.ncbi.nlm.nih.gov/genbank/>
GeneMatcher, <https://genematcher.org/>
gnomAD Browser, <http://gnomad.broadinstitute.org/>

GVDG, http://agvgd.hci.utah.edu/agvgd_input.php
 Human Phenotype Ontology (HPO), <http://www.human-phenotype-ontology.org/>
 MutationTaster, <http://www.mutationtaster.org/>
 OMIM, <http://www.omim.org/>
 PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>
 SIFT, <http://sift.bii.a-star.edu.sg/>
 The Human Protein Atlas, <http://www.proteinatlas.org/>

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