A Recurrent De Novo Variant in *NACC1* 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 (*NACC1*) gene in seven affected individuals. One of the seven individuals is mosaic for this variant. *NACC1* encodes a transcriptional repressor implicated in gene expression and has not previously been associated with germline disorders. The probability of finding the same missense *NACC1* 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 *NACC1* 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 *NACC1*.

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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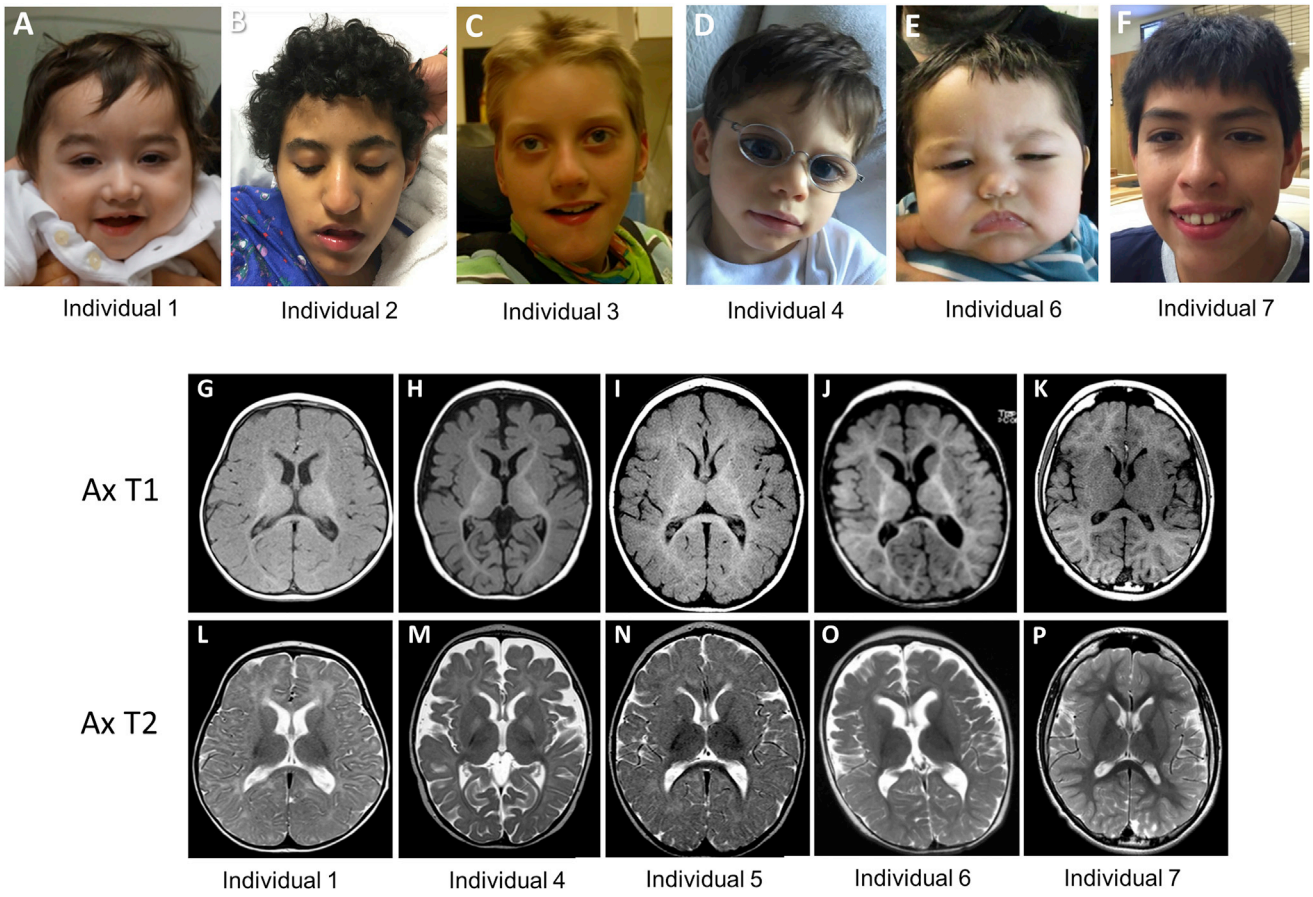

Figure 1. Clinical and Neuroradiologic Characteristics of the Seven Probands
(A) Individual 1 at 20 months of age.
(B) Individual 2 at 12 years of age.
(C) Individual 3 at 9 years of age.
(D) Individual 4 at 3.5 years of age.
(E) Individual 6 at 13 months of age.
(F) Individual 7 at 12 years of age.
(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 (*NACC1* [MIM: 610672]). *NACC1* 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 *NACC1* 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 subspecialized neurologic care. Bilateral cataracts requiring surgical extraction were present in five out of seven

Table 1. Phenotypic Features in Individuals with *NACC1* 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	
NACC1 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

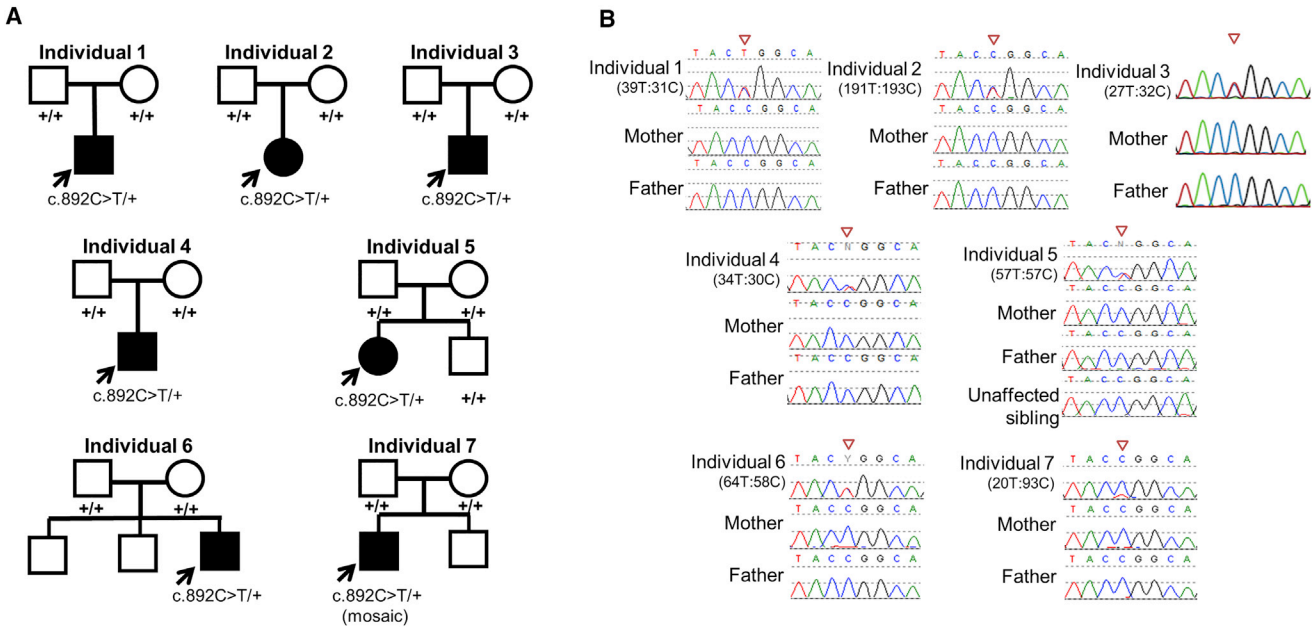

Figure 2. Recurrent De Novo c.892C>T (p.Arg298Trp) Missense Change in *NACC1* Observed in All Seven Probands
(A) Family pedigrees for individuals 1–7 with de novo events in each affected proband. Note that unaffected sibling of individual 5 was also sequenced and found not to have the de novo variant.
(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 reanalyzed 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 *NACC1* (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 *NACC1* 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 *NACC1* 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,

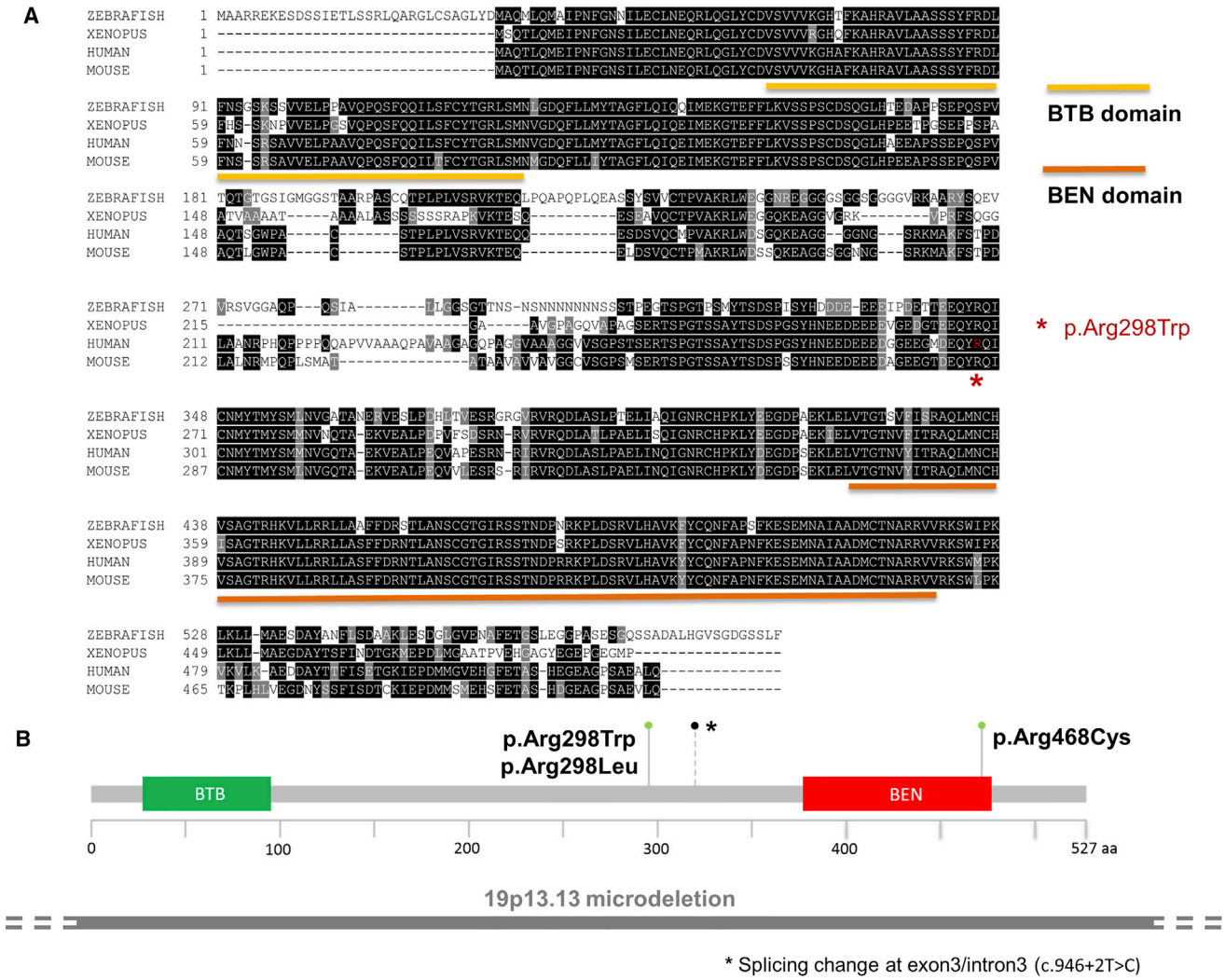

Figure 3. Predicted Protein Change for the De Novo *NACC1* Variant in Context of Protein Domains
(A) Amino acid sequence alignment of human NAC1 with zebrafish, *Xenopus*, and mouse. The BEN and BTB domains are shown, and the location of the p.Arg298Trp missense change is depicted by a red star. The 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 *NACC1* are subject to selection and that rare, predicted pathogenic variants may confer risk for human diseases. Additionally, the residual variation intolerance score (RVIS) of *NACC1* 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 *NACC1* 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 *NACC1* as a genome-wide significant disease-associated gene.

Although *NACC1* has not previously been associated with a Mendelian phenotype, a link between *NACC1* 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 disorder[2] 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 *NACC1*, 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 *NACC1*.[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 *NACC1* are seen in 18 individuals in the DECIPHER database, ranging in size from 83 kb to 2.39 Mb, and duplications involving *NACC1* 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 *NACC1* in these individuals is difficult to infer. A known microdeletion/microduplication syndrome involving chromosome 19p13.13 includes most of *NACC1* 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 *NACC1* in non-disease individuals in the Database of Genomic Variants.[28] Taken together, none of the previous studies involving *NACC1* 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 *NACC1* 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 reported[6] (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 *NACC1* 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 *NACC1* 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 *NACC1* 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/

GVGD, 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/

References

1. Gonzaga-Jauregui, C., Lupski, J.R., and Gibbs, R.A. (2012). Human genome sequencing in health and disease. Annu. Rev. Med. *63*, 35–61.
2. Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., Levy, D., Stessman, H.A., Witherspoon, K.T., Vives, L., Patterson, K.E., et al. (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature *515*, 216–221.
3. Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., et al. (2012). De novo gene disruptions in children on the autistic spectrum. Neuron *74*, 285–299.
4. de Ligt, J., Veltman, J.A., and Vissers, L.E. (2013). Point mutations as a source of de novo genetic disease. Curr. Opin. Genet. Dev. *23*, 257–263.
5. de Ligt, J., Willemsen, M.H., van Bon, B.W., Kleefstra, T., Yntema, H.G., Kroes, T., Vulto-van Silfhout, A.T., Koolen, D.A., de Vries, P., Gilissen, C., et al. (2012). Diagnostic exome sequencing in persons with severe intellectual disability. N. Engl. J. Med. *367*, 1921–1929.
6. Gilissen, C., Hehir-Kwa, J.Y., Thung, D.T., van de Vorst, M., van Bon, B.W., Willemsen, M.H., Kwint, M., Janssen, I.M., Hoischen, A., Schenck, A., et al. (2014). Genome sequencing identifies major causes of severe intellectual disability. Nature *511*, 344–347.
7. Ohba, C., Kato, M., Takahashi, N., Osaka, H., Shiihara, T., Tohyama, J., Nabatame, S., Azuma, J., Fujii, Y., Hara, M., et al. (2015). De novo KCNT1 mutations in early-onset epileptic encephalopathy. Epilepsia *56*, e121–e128.
8. Rivière, J.B., van Bon, B.W., Hoischen, A., Kholmanskikh, S.S., O'Roak, B.J., Gilissen, C., Gijsen, S., Sullivan, C.T., Christian, S.L., Abdul-Rahman, O.A., et al. (2012). De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. Nat. Genet. *44*, 440–444, S1–S2.
9. Veltman, J.A., and Brunner, H.G. (2012). De novo mutations in human genetic disease. Nat. Rev. Genet. *13*, 565–575.
10. Gauthier, J., and Rouleau, G.A. (2012). De novo mutations in neurological and psychiatric disorders: effects, diagnosis and prevention. Genome Med. *4*, 71.
11. White, J., Beck, C.R., Harel, T., Posey, J.E., Jhangiani, S.N., Tang, S., Farwell, K.D., Powis, Z., Mendelsohn, N.J., Baker, J.A., et al. (2016). POGZ truncating alleles cause syndromic intellectual disability. Genome Med. *8*, 3.
12. Korutla, L., Wang, P.J., and Mackler, S.A. (2005). The POZ/BTB protein NAC1 interacts with two different histone deacetylases in neuronal-like cultures. J. Neurochem. *94*, 786–793.
13. Lee, T.I., and Young, R.A. (2013). Transcriptional regulation and its misregulation in disease. Cell *152*, 1237–1251.
14. Chong, J.X., Buckingham, K.J., Jhangiani, S.N., Boehm, C., Sobreira, N., Smith, J.D., Harrell, T.M., McMillin, M.J., Wiszniewski, W., Gambin, T., et al.; Centers for Mendelian Genomics (2015). The genetic basis of Mendelian phenotypes: discoveries, challenges, and opportunities. Am. J. Hum. Genet. *97*, 199–215.
15. Yang, Y., Muzny, D.M., Xia, F., Niu, Z., Person, R., Ding, Y., Ward, P., Braxton, A., Wang, M., Buhay, C., et al. (2014). Molecular findings among patients referred for clinical whole-exome sequencing. JAMA *312*, 1870–1879.
16. Lee, H., Deignan, J.L., Dorrani, N., Strom, S.P., Kantarci, S., Quintero-Rivera, F., Das, K., Toy, T., Harry, B., Yourshaw, M., et al. (2014). Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA *312*, 1880–1887.
17. Sobreira, N., Schiettecatte, F., Valle, D., and Hamosh, A. (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum. Mutat. *36*, 928–930.
18. Krkljus, S., Abernathy, C.R., Johnson, J.S., Williams, C.A., Driscoll, D.J., Zori, R., Stalker, H.J., Rasmussen, S.A., Collins, F.S., Kousseff, B.G., et al. (1998). Analysis of CpG C-to-T mutations in neurofibromatosis type 1. Mutations in brief no. 129. Online. Hum. Mutat. *11*, 411.
19. Francioli, L.C., Polak, P.P., Koren, A., Menelaou, A., Chun, S., Renkens, I., van Duijn, C.M., Swertz, M., Wijmenga, C., van Ommen, G., et al.; Genome of the Netherlands Consortium (2015). Genome-wide patterns and properties of de novo mutations in humans. Nat. Genet. *47*, 822–826.
20. Cooper, D.N., Mort, M., Stenson, P.D., Ball, E.V., and Chuzhanova, N.A. (2010). Methylation-mediated deamination of 5-methylcytosine appears to give rise to mutations causing human inherited disease in CpNpG trinucleotides, as well as in CpG dinucleotides. Hum. Genomics *4*, 406–410.
21. Petrovski, S., Gussow, A.B., Wang, Q., Halvorsen, M., Han, Y., Weir, W.H., Allen, A.S., and Goldstein, D.B. (2015). The intolerance of regulatory sequence to genetic variation predicts gene dosage sensitivity. PLoS Genet. *11*, e1005492.
22. Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., et al.; Exome Aggregation Consortium (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature *536*, 285–291.
23. Robinson, D.R., Wu, Y.M., Vats, P., Su, F., Lonigro, R.J., Cao, X., Kalyana-Sundaram, S., Wang, R., Ning, Y., Hodges, L., et al. (2013). Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat. Genet. *45*, 1446–1451.
24. Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Genet. *46*, 310–315.
25. Allen, A.S., Berkovic, S.F., Cossette, P., Delanty, N., Dlugos, D., Eichler, E.E., Epstein, M.P., Glauser, T., Goldstein, D.B., Han, Y., et al.; Epi4K Consortium; and Epilepsy Phenome/Genome Project (2013). De novo mutations in epileptic encephalopathies. Nature *501*, 217–221.
26. Jorge, R., Silva, C., Águeda, S., Dória, S., and Leão, M. (2015). Intellectual disability and overgrowth-A new case of 19p13.13 microdeletion syndrome with digital abnormalities. Am. J. Med. Genet. A. *167A*, 2839–2843.
27. Nimmakayalu, M., Horton, V.K., Darbro, B., Patil, S.R., Alsayouf, H., Keppler-Noreuil, K., and Shchelochkov, O.A. (2013). Apparent germline mosaicism for a novel 19p13.13 deletion disrupting NFIX and CACNA1A. Am. J. Med. Genet. A. *161A*, 1105–1109.

28. MacDonald, J.R., Ziman, R., Yuen, R.K., Feuk, L., and Scherer, S.W. (2014). The Database of Genomic Variants: a curated collection of structural variation in the human genome. Nucleic Acids Res. *42*, D986–D992.
29. Freed, D., and Pevsner, J. (2016). The contribution of mosaic variants to autism spectrum disorder. PLoS Genet. *12*, e1006245.
30. Acuna-Hidalgo, R., Bo, T., Kwint, M.P., van de Vorst, M., Pinelli, M., Veltman, J.A., Hoischen, A., Vissers, L.E., and Gilissen, C. (2015). Post-zygotic point mutations are an underrecognized source of de novo genomic variation. Am. J. Hum. Genet. *97*, 67–74.
31. Ishikawa, M., Nakayama, K., Yeasmin, S., Katagiri, A., Iida, K., Nakayama, N., and Miyazaki, K. (2010). NAC1, a potential stem cell pluripotency factor expression in normal endometrium, endometrial hyperplasia and endometrial carcinoma. Int. J. Oncol. *36*, 1097–1103.
32. Nakayama, K., Nakayama, N., Davidson, B., Sheu, J.J., Jinawath, N., Santillan, A., Salani, R., Bristow, R.E., Morin, P.J., Kurman, R.J., et al. (2006). A BTB/POZ protein, NAC-1, is related to tumor recurrence and is essential for tumor growth and survival. Proc. Natl. Acad. Sci. USA *103*, 18739–18744.
33. Yap, K.L., Fraley, S.I., Thiaville, M.M., Jinawath, N., Nakayama, K., Wang, J., Wang, T.L., Wirtz, D., and Shih, IeM. (2012). NAC1 is an actin-binding protein that is essential for effective cytokinesis in cancer cells. Cancer Res. *72*, 4085–4096.
34. Ishibashi, M., Nakayama, K., Yeasmin, S., Katagiri, A., Iida, K., Nakayama, N., and Miyazaki, K. (2009). Expression of a BTB/POZ protein, NAC1, is essential for the proliferation of normal cyclic endometrial glandular cells and is up-regulated by estrogen. Clin. Cancer Res. *15*, 804–811.
35. Nakayama, K., Rahman, M.T., Rahman, M., Yeasmin, S., Ishikawa, M., Katagiri, A., Iida, K., Nakayama, N., and Miyazaki, K. (2010). Biological role and prognostic significance of NAC1 in ovarian cancer. Gynecol. Oncol. *119*, 469–478.
36. Shih, IeM., Nakayama, K., Wu, G., Nakayama, N., Zhang, J., and Wang, T.L. (2011). Amplification of the ch19p13.2 NACC1 locus in ovarian high-grade serous carcinoma. Mod. Pathol. *24*, 638–645.
37. Yeasmin, S., Nakayama, K., Rahman, M.T., Rahman, M., Ishikawa, M., Katagiri, A., Iida, K., Nakayama, N., Otuski, Y., Kobayashi, H., et al. (2012). Biological and clinical significance of NAC1 expression in cervical carcinomas: a comparative study between squamous cell carcinomas and adenocarcinomas/adenosquamous carcinomas. Hum. Pathol. *43*, 506–519.
38. Hernández-Porras, I., Schuhmacher, A.J., Garcia-Medina, R., Jiménez, B., Cañamero, M., de Martino, A., and Guerra, C. (2016). K-Ras(V14I) -induced Noonan syndrome predisposes to tumour development in mice. J. Pathol. *239*, 206–217.
39. Stevenson, D.A., Schill, L., Schoyer, L., Andresen, B.S., Bakker, A., Bayrak-Toydemir, P., Burkitt-Wright, E., Chatfield, K., Elefteriou, F., Elgersma, Y., et al. (2016). The Fourth International Symposium on Genetic Disorders of the Ras/MAPK pathway. Am. J. Med. Genet. A. *170*, 1959–1966.
40. Shashi, V., Pena, L.D., Kim, K., Burton, B., Hempel, M., Schoch, K., Walkiewicz, M., McLaughlin, H.M., Cho, M., Stong, N., et al.; Undiagnosed Diseases Network (2016). De novo truncating variants in ASXL2 are associated with a unique and recognizable clinical phenotype. Am. J. Hum. Genet. *99*, 991–999.
41. Korutla, L., Wang, P., Jackson, T.G., and Mackler, S.A. (2009). NAC1, a POZ/BTB protein that functions as a corepressor. Neurochem. Int. *54*, 245–252.
42. Blanchard, M.G., Willemsen, M.H., Walker, J.B., Dib-Hajj, S.D., Waxman, S.G., Jongmans, M.C., Kleefstra, T., van de Warrenburg, B.P., Praamstra, P., Nicolai, J., et al. (2015). De novo gain-of-function and loss-of-function mutations of SCN8A in patients with intellectual disabilities and epilepsy. J. Med. Genet. *52*, 330–337.
43. Endele, S., Rosenberger, G., Geider, K., Popp, B., Tamer, C., Stefanova, I., Milh, M., Kortüm, F., Fritsch, A., Pientka, F.K., et al. (2010). Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat. Genet. *42*, 1021–1026.
44. Yap, K.L., Sysa-Shah, P., Bolon, B., Wu, R.C., Gao, M., Herlinger, A.L., Wang, F., Faiola, F., Huso, D., Gabrielson, K., et al. (2013). Loss of NAC1 expression is associated with defective bony patterning in the murine vertebral axis. PLoS ONE *8*, e69099.
45. Korutla, L., Furlong, H.A., 4th, and Mackler, S.A. (2014). NAC1, A POZ/BTB protein interacts with Parkin and may contribute to Parkinson's disease. Neuroscience *257*, 86–95.
46. Scofield, M.D., Korutla, L., Jackson, T.G., Kalivas, P.W., and Mackler, S.A. (2012). Nucleus Accumbens 1, a Pox virus and Zinc finger/Bric-a-brac Tramtrack Broad protein binds to TAR DNA-binding protein 43 and has a potential role in amyotrophic lateral sclerosis. Neuroscience *227*, 44–54.

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Supplemental Data

A Recurrent De Novo Variant in *NACC1* Causes a Syndrome Characterized by Infantile Epilepsy, Cataracts, and Profound Developmental Delay

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Supplementary Data

Case Reports

Individual 1: A 20-month-old Caucasian male was evaluated through the Undiagnosed Diseases Network for infantile spasms, acquired microcephaly, lamellar cataracts, failure to thrive, and global developmental delays.

He was born after an uneventful pregnancy at 39 weeks to a 34-year-old G_1P_0 mother and a 32-year-old father, with a birth weight of 3.473 kg (60^{th} percentile), a birth length of 48.3 cm (20^{th} percentile), and an OFC of 34 cm (30^{th} percentile). The neonatal course was complicated by difficulty latching to the breast requiring formula supplementation. A consistent left lateral gaze was also noted.

Developmental concerns were first noted at 4 months of age, when he was not rolling over, had poor head control, did not visually track and did not babble. Developmental regression was noted at 9 months with onset of seizures. Currently he is profoundly delayed with poor head control and does not sit independently, intentionally grasp objects or consistently track.

He was clinically diagnosed with infantile spasms at 9 months, confirmed by EEG showing hypsarrhythmia at 11 months old, at which time a 2-week course of ACTH was instituted with poor response. Seizures have been managed with Clonazepam and Topamax. Ketogenic diet was initiated at 19 months old due to recurrence of infantile spasms, and he has been seizure free since two weeks after diet was begun.

He was diagnosed with bilateral lamellar cataracts, without prominent subcapsular or nuclear component, at 7 months of age and underwent removal of these at 10 months of age.

A brain MRI at 1 year of age showed mildly delayed myelination within the peripheral white matter, and brain volume was mildly decreased with slight enlargement of ventricles. Brainstem auditory evoked response (BAER) at 10 months old revealed adequate hearing for speech development. A gastrostomy tube was placed at 11 months due to failure to thrive. Since infancy he has been difficult to soothe.

On exam at 20 months, he was small for his age, with the height at 76cm ($<1^{st}$ percentile), weight at 9.8 kg (3^{rd} percentile) and OFC at 42 cm ($<0.01^{th}$ percentile). He had deep set eyes, depressed nasal bridge, slightly upturned nose, midface hypoplasia, and thick eyebrows. He had bilateral fifth finger clinodactyly, single palmar creases, small fifth toenails and dysplastic right toenail. He had a repetitive movement involving his upper extremities, in which he pulled the middle top part of his shirt downward with both hands.

Chromosome microarray was normal. A comprehensive epilepsy panel revealed a novel, heterozygous and maternally inherited *SCN2A* variant of unknown significance. Clinical WES (GeneDx) identified a novel heterozygous and maternally inherited *CRYGD* variant of unknown significance associated with autosomal dominant cataracts. The individual's maternal grandmother was also found to have both of these variants, and both the mother and maternal grandmother are neurologically normal with no history of cataracts. Family history was noncontributory.

Individual 2: A 12-year-old female presented to neurology clinic with epilepsy, microcephaly, cataracts, failure to thrive and severe developmental delay with regression. Her medical care had previously been managed in Saudi Arabia, so access to her full records was limited.

She was born at 36 weeks following induction of labor, but the reasons for this are unclear. By parental report she was small for gestational age and oligohydramnios was noted in the third trimester. She was admitted to the NICU for 7 days due to low weight and weakness.

The parents report normal development (she sat with support, rolled over) until 7-10 months of age when she began to lose motor skills, developed seizures, and was noted to be microcephalic. She began having difficulty feeding, lost weight and was irritable with increased crying. Currently all areas of development are profoundly impaired. She does not sit unsupported or speak. She does not have purposeful hand movement. She cries when she's hungry, and eats and drinks by mouth although there is concern with swallowing liquids.

She began having abnormal movements at 7 months of age which worsened by 10 months, described by parents as looking like seizures. At 11 months she was started on 3 AEDs during admission at a Saudi hospital, one of which was Clonazepam, but these records were unattainable. Her seizures have been managed solely with Clonazepam since 9 years old (duration of 3 years), although she continues to have seizures when doses of medication are missed. Seizures are described as paroxysmal quick jerk of arm upwards and head tilting to same side one time and then gradual relaxation. Seizures never last more than a few seconds, but can happen 100 times per day when off medications. She has never had a grand mal seizure. EEG at 12 years old showed only intermittent bifrontal slowing with no epileptiform discharges.

Her first cataract was diagnosed at 10 years of age, and the second at 11 years old. She has malformed globes.

A brain MRI at 17 months reportedly showed brain atrophy and fluid accumulation. Repeat imaging at 12 years old showed slight prominence of the extra-axial CSF spaces and the supratentorial ventricular system, arachnoid cyst in the left middle cranial fossa and left posterior parasagittal region, and developmental venous anomaly in the left centrum semiovale, in addition to the malformed globes. Spectroscopy revealed a slight decrease in NAA in the basal ganglia.

She has chronic anemia and also has had a reaction to sedation in the past that required CPR, intubation, and 1 week of ventilatory support.

On exam at 12 years of age, she was small with weight at 27.5 kg (<5 percentile), OFC at 46.5 cm (<2nd percentile). She was mildly dysmorphic, had bilateral cataracts and was hypertonic when awake. She did not react to visual stimuli although was responsive to touch and sound. She had Rett's-like hand automatisms.

Prior diagnostic workup included metabolic screening [serum amino acids (slightly elevated alanine), acylcarnitine profile, ammonia, lactate/pyruvate, lysosomal studies, very long chain fatty acids], CSF studies [neurotransmitters (slightly low 5-methyltetrahydrofolate), CSF amino acids and CSF lactate/pyruvate]. She also has a heterozygous pathogenic, maternally inherited variant in ***F11*** that was thought to explain her anemia, and a maternally inherited variant of uncertain significance in *G6PD* identified on WES. She has two healthy siblings who are neurologically normal.

Individual 3: An 18-1/2-year-old Caucasian male was evaluated for bilateral congenital cataracts, seizures, hypotonia since infancy, microcephaly, failure to thrive and profound developmental delay.

He was born at term via vaginal delivery to a 41-year-old G_3P_2 mother and a 37-year-old father, both of Norwegian ancestry. His mother noted less movement during the pregnancy as compared to previous pregnancies. Birth weight was 4100 grams and birth length was 52 cm. Apgar score was 9. Hypotonia was noted at birth.

Developmental concerns were first noted at 8 weeks of age. He has had intensive psycho-motoric training, Domain patterning therapy from 14 months to 3 years of age, and Bobat neuro-developmental training. Although he was able to stand with support during early childhood, he has never achieved ambulation. From age 14 he was unable to sit without support due to spasticity. He has no language but does make sounds and can express his likes and dislikes. He recognizes his parents and siblings but has had long-standing tactile aversion leading to strong dislike of being touched or held. This issue has gradually improved in adolescence.

First seizure activity was noted at 6 months and he has had a long-standing history of epilepsy. Since 2 years of age he had tonic seizures. EEG at 5 ½ years of age showed increased activity, especially in the frontal lobe, related to his tonic-clonic seizures. Lamotrigine was initiated, which had no effect on the seizures and was discontinued after one year. Valproate therapy was effective, but EEG at 10 years of age still showed localized focal epileptic activity.

Bilateral congenital cataracts were diagnosed at 6 weeks of age, and he underwent surgery on the right eye at 1 year of age, and on the left eye at 2 years of age. He developed post-surgery cataracts and underwent laser surgery on the right lens at 2 years of age and left lens at 6 years of age.

MRI at 1 year of age showed reduced white matter and incomplete subcortical myelination of brain stem, thalamus and corpus callosum. MRI at 2 years of age showed increased myelination

as compared to the first imaging although still somewhat reduced related to age, reduced white matter and a hypoplastic corpus callosum with temporal lobes most affected. MRI at 11 years of age showed increased subarachnoid fluid over the frontal cerebral convexities, over both lateral fossae and along the interhemispheric fissures, as well as hyperintense foci in both hippocampi consistent with hippocampal sulcus remnant and myelinated but thin corpus callosum. The findings indicated generalized atrophy and mildly decreased brain volume. MR spectroscopy was notable for lower NAA levels left central hemisphere.

Constipation and gastroesophageal reflux noted at 5 years of age. Due to low weight, reduced growth and feeding difficulties, percutaneous endoscopic gastrostomy was performed at 6 ½ years of age; however at 18 years of age he continues to eat oral meals every day. Due to spasticity he has developed contractures of the feet, knees and hips, which have been treated with orthopedic interventions including triple arthrodesis, bilateral adductor tendonectomy, and right side femur/pelvic osteotomy to correct hip subluxation and stabilize the hips. His knee contractures have been managed with repeated Botulinum injections. He has developed thoracolumbar scoliosis.

Since early infancy he has had bouts of inconsolability and screaming for days at a time. Melatonin has been used for sleep disturbances. He has had Rett-like stereotypic hand movements since early childhood.

On exam at 6 ½ years of age he was microcephalic with OFC 49 cm (3 cm below 2.5 percentile). He had hyperreflexic patellar tendons, normal Achilles tendon reflexes and downturned plantar reflexes. He had increased muscle tonus upper extremities. At 18 ½ years of age he had profound intellectual disability and stereotypic hand movements.

Previous testing included chromosomal microarray and Sanger sequencing for all known and available Rett/Angelman-like genes. Family history is noncontributory; he has two healthy siblings.

Individual 4: A 3-year, 7-month-old Caucasian male was evaluated for seizures, hypotonia, microcephaly, bilateral cataracts and glaucoma, bilateral sensorineural hearing loss (SNHL) and severe developmental delay. He was diagnosed with Stickler syndrome at 3 years of age by WES upon identification of a pathogenic *COL11A1* variant. His mother, who also has congenital SNHL and glaucoma, was found to have the same variant. However, this diagnosis did not explain his complete presentation.

He was born to a 30-year-old G_1P_0 mother at 39 weeks gestation and weighed 7 pounds, 6 ounces. The pregnancy was complicated by gestational diabetes at 28 weeks, requiring insulin at 36 weeks. Labor was induced at 39 weeks gestation for maternal hypertension. The delivery was uncomplicated.

He began exhibiting developmental delays and feeding difficulties within the first month of life. Currently is able to roll over but cannot sit unassisted. He does not reach for or transfer toys.

Seizure activity was first observed at 4 months old; infantile spasms were diagnosed by EEG at 6 months and responded well to a 6 week course of ACTH. Levetiracetam was then initiated. He has been seizure free without AEDs since 2 years old (duration 17 months), and EEGs have been normal. He continues to have frequent sleep myoclonus.

Bilateral cataracts were diagnosed at birth and removed at 4 months of age. He has undergone bilateral goniotomy multiple times.

Brain MRI at 4 months showed mild delay in myelination and at 7 months showed significant interval cerebral volume loss. Brain imaging at 2 years, 2 months showed microcephaly and slight delay in myelination with considerable progression compared to previous imaging.

He has tracheomalacia, gastroesophageal reflux disease and feeding intolerance and disinterest which resulted in failure to thrive. Oral feeding improved during his ACTH treatment, however decreased significantly since completing therapy. Gastrostomy tube was placed at 9 months. Mild hearing loss with normal tympanic membranes was found on ABR at 16 months.

He has periods of being irritable which last hours to days at a time where he has inconsolable crying. These are sometimes associated with constipation. He has sleep difficulties. He has a history of breath-holding spells which occurred several times a day prior to ACTH therapy; they seemed unrelated to seizures and cardiology evaluation was normal. The frequency of breath-holding spells has decreased significantly, and they now occur only when he is upset.

On exam at 3 years, 7 months he had diffuse hypotonia. Previous testing included chromosome microarray and CDG. His mother also has congenital hearing impairment and left sided glaucoma secondary to an eye injury; remaining family history was noncontributory.

Individual 5: A 9-year-old Caucasian female had been followed since age 8 months by medical genetics for epilepsy, microcephaly, bilateral cataracts, failure to thrive and severe developmental delay. Prior to WES she had a diagnosis of mitochondrial disease based on muscle biopsy that showed a reduction in several respiratory chain complexes.

She was born via full-term normal spontaneous vaginal delivery to a 30-year-old G_1P_0 mother and a 33-year-old father, with a birth weight of 7 pounds 10 ounces. The neonatal course was uncomplicated.

Early developmental milestones were normal (good head control, rolled at 2 months of age, pushed to a prone position at 2 to 3 months of age). She had a social smile and tracked visually at approximately 2 months, although she never made eye contact. Currently she is profoundly impaired with no speech, no visual tracking and limited purposeful movement.

Myoclonic seizures began at 5 months of age consisting of myotonic jerking of her upper extremities, and a diagnosis of infantile spasms was tentatively made. An EEG showed hypsarhythmia during sleep and overall was consistent with diffuse epileptic encephalopathy. She was put on Zonisamide for presumed infantile spasms but continued to have clustering of seizures consisting of myotonic jerks as well as dozens of tonic jerks per day. At 10 months the Zonisamide was discontinued and her seizures doubled in frequency. Levetiracetam was started but discontinued after 2 weeks due to extreme irritability. Valproic acid was then initiated until an EEG at 11 months confirmed infantile spasms. ACTH was started at a low dose but spasms increased in intensity. Multiples AEDs were then attempted but did not result in improvement or had undesirable side effects, including Felbamate, Vigabatrin, Topamax, Lamotrigine and the ketogenic diet. Banzel (Rufinamide) was initiated at 2-½ years old, resulting in the first major improvement in her seizures. Seizures are currently well managed with Banzel, although there have been signs of breakthrough seizures with onset of puberty.

Nuclear cataracts were first noted in the right eye at 11 months and left eye at 12 months. She has had both cataracts as well as secondary cataracts removed during a total of four procedures.

A brain MRI at 5 months showed demyelination of the deep white matter. MR spectroscopy was normal.

She had feeding difficulties and formula intolerance requiring gastrostomy tube placement at 2-½ years of age, which was replaced 5 weeks later with a gastro-jejunal tube due to chronic vomiting. She has constipation and urine retention which has resulted in several UTIs. She has signs of precocious puberty since 5 years of age, and at 8 years of age hormonal changes were seen. She has had a single episode of difficulty waking up from anesthesia (following muscle biopsy at 2-½ years old).

She was a colicky baby and has had bouts of extreme irritability during childhood. At 2-½ years of age she began having choreic movements and increased sleep disturbance. Despite trials of Zolpidem, Lorazepam, Diazapam, Trazadone, Lortab, Gabapentin and Methadone, sleep patterns could not be stabilized. Around 5 years of age, an improvement was seen when calorie intake was lowered with a change to PurAmino formula, although she continues to have shorter bouts of extreme irritability. She has had breath-holding spells.

Previous diagnostic tests included a chromosome microarray, sequencing of the *MECP2*, *CDKL5* and *ARX* genes, enzyme testing for Neuronal Ceroid Lipofuscinosis types 1 and 2, metabolic screens [plasma amino acids (very mild elevation of alanine), homocysteine, acylcarnitine profile,

lactate, urine and plasma creatine studies, urine polyol studies (tenfold elevated ribitol), urine purine studies, urine organic acids (elevated lactate)]. CSF neurotransmitters and amino acids were normal. She had a muscle biopsy that showed a reduction in several respiratory chain complexes including complex 1 and complex 4, fulfilling minor criteria of the modified Walker criteria. Mitochondrial copy number was normal in the muscle sample and sequencing of the mitochondrial genome was likewise normal. She is the only child to her parents. Family history includes a paternal first cousin with seizures.

Individual 6: A 13-month-old male of Mexican ancestry was evaluated for developmental delay, hypotonia, infantile spasms, and bilateral sensory hearing loss.

He was born at 37 weeks gestation to a 31-year-old mother through induced vaginal birth after uneventful pregnancy and a 34-year-old father. The birth weight was 3.203 kg (30^{th} percentile) and length was 48 cm (20^{th} percentile). Slow heart rate was noted during birth.

Developmental concerns started at 4 months of age, when parents noticed that he would not laugh or look at them. At 6 months of age he failed a hearing and vision screen, and further evaluation identified bilateral sensorineural hearing loss and low vison, poor visual response, lack of tracking with both eyes, delayed visual maturation and bilateral hyperopia with astigmatism. At 9 months of age global developmental delay and hypotonia were noted. He began smiling and rolling over at this age. At 10 months old, breath holding spells with body stiffening was reported, followed by weakness. He had difficulty swallowing, and choked on thin liquids. He was not able to sit on his own, did not crawl, and had poor head control.

At 11 months old the first tonic seizure was observed. Video electroencephalogram (vEEG) indicated generalized hypsarrhythmia, frequent multifocal independent spike-wave discharges along with frequent biposterior or biparietal and occasionally generalized PFA. He completed a course of prednisolone which resolved his spasms. Zonisamide and clonazepam were initiated to control tonic seizures. The age of onset of seizures could not be determined, as they may have been present from birth but unrecognized. An MRI was performed at 12 months which was reported as unremarkable, with no gross structural anomaly and no acute intracranial findings; upon close review by an outside neuroradiologist volume loss was noted.

On physical examination at 12 months of age, he was noted to have central hypotonia, with weight of 7.95kg (4^{th} percentile), height of 73cm (12^{th} percentile), and a head circumference of 45.5cm (33^{rd} percentile). He had a high arched palate, protuberant ears, hyperpigmented nevus, and bilateral, overlapping first, second, and third toes. He continues to have poor head control.

Chromosomal microarray, creatine phosphokinase (CK), thyroid stimulating hormone (TSH) and acylcarnitine profile were normal. Family history includes a 33-year-old maternal aunt who has a seizure disorder with onset at age 15.

Individual 7: A 12-year-old male was evaluated for developmental delay, seizure disorder, and cortical dysplasia, with prior medical care managed in Columbia.

He was born at 40 weeks gestation via C-section for a cephalopelvic disproportion from a 29-year-old primigravida mother and a 32-year –old-father.

Developmental concerns were noted shortly after birth with poor suck. He had prolonged crying episodes with right arm posturing similar to dystonic posture. At three months persistent startle and lack of interaction were noted. At 6 months he was unable to roll over. At 12 months he was diagnosed with cerebral palsy. He passed vision and hearing tests at 16 months. First words were spoken at age 2, and by age 10 his vocabulary totaled 20-40 words. Currently he has severe speech delay but is beginning to put two words together. He continues to have extensive drooling. He has autistic behaviors including hand flapping. He is able to walk but has an abnormal gait and requires leg braces for hyperextensibility. He startles easily, and episodes of startle lead to him falling over. He continues to make developmental progress.

At two years of age he had a single febrile seizure possibly due to influenza that included prolonged body stiffening. At 10 years of age he was noted to have multiple night time seizures with cyanotic, whole body convulsions. EEG confirmed electrical status epilepticus of sleep (ESES), which was well controlled with diazepam and most recent vEEG at age 10 showed no evidence of ESES. Currently he is not taking any medications. The timing of seizures suggested that additional episodes may have been missed over his childhood due to their night time occurrence.

MRI at 10 months of age showed an arachnoid cyst but no other abnormalities. MRI at age 2 was negative for intracranial injuries but showed enlarged adenoid tissue. The most recent MRI, at age 10 years, showed scattered areas of subcortical white matter T2/FLAIR hyperintensity involving the left superior frontal gyrus, posterior aspect of the left cingulate gyrus, left inferior frontal gyrus, and left supramarginal gyrus.

At eight months he was diagnosed with gastrointestinal reflux disease (GERD). He has a history obstructive sleep apnea. Concerns for his vision at age ten resulted in the prescription for hyperopic correction lenses, although no strabismus was observed.

On exam at 12 years of age, weight was 53kg (61st percentile), height was 152cm (66th percentile), and his head circumference was 53cm (52nd percentile). He had myopathic face, midface hypoplasia, flat nasal bridge, high arched palate, protuberant ears, and low anterior hairline. He had a cafe au lait spot on right neck and multiple nevi. He continued to hold his mouth open and drooled. He continues to have problems with balance and falls frequently.

Previous metabolic testing included urine organic acids, plasma amino acids and urine mucopolysaccharides. Chromosomal microarray results were normal and genetic testing for TSC1/2, Fragile X, and X-linked Mental retardation (XLMR) panel were negative. The parents are of Colombian ancestry and consanguinity was denied. The individual has a younger brother with no medical condition. Paternal aunt had a history of seizures.

Table S1 Summary of exome sequencing platforms and coverage in seven unrelated individuals reported here with de novo *NACC1* p.Arg298Trp variant

	Individual 1	**Individual 2**	**Individual 3**	**Individual 4**	**Individual 5**	**Individual 6**	**Individual 7**
Sequencing laboratory	GeneDx	GeneDx	Baylor Hopkins Centers for Mendelian Genomics	Baylor Genetics	Baylor Genetics	UCLA	UCLA
Sequencing type	Trio exome sequencing	Trio exome sequencing	Proband exome sequencing (Sanger sequencing of NACC1 in proband and parents)	Proband exome sequencing (Sanger sequencing of NACC1 in proband and parents)	Proband exome sequencing (Sanger sequencing of NACC1 in proband and parents)	Trio exome sequencing	Trio exome sequencing
Capture and library construction	Agilent Clinical Research Exome Kit	Agilent Clinical Research Exome Kit	6plex using VCRome + PKv2	Biotin-labeled VCRome 2.1 in-solution Exome probes	Biotin-labeled VCRome 2.1 in-solution Exome probes	Agilent Clinical Research Exome Kit	Agilent Clinical Research Exome Kit
Sequencing platform	Illumina HiSeq2000	Illumina HiSeq2000	Illumina HiSeq2000	Illumina HiSeq2500	Illumina HiSeq2500	Illumina HiSeq2500	Illumina HiSeq2500
Average depth of targeted bases	73x	167x	84X	135x	235x	94x	78x
Percentage of bases covered >10 x	95.1%	97.5%	98.0%	98.3%	99.0%	96.50%	96.20%

Table S2 Additional variants detected by exome sequencing in seven individuals with a *NACC1 de novo* p.Arg298Trp variant.

	Gene	**Disease**	**Inheritance pattern**	**HGVS**	**Classification**	**Inherited from**
Individual 1	*CRYGD*	Cataract 4, multiple types (MIM: 115700)	AD	c.118A>T (p.S40C) heterozygous	VUS	Mother
Individual 2	*F11*	Factor XI deficiency (MIM: 612416)	AD/AR	c.403G>T (p.E135X) heterozygous	pathogenic	Mother
	G6PD	Favism (MIM: 134700); Hemolytic anemia due to G6PD deficiency (MIM: 300908)	XL	c.1003G>A (p.A335T) heterozygous	VUS	Mother
Individual 3	*ABCB6*	Microphthalmia, isolated, with coloboma 7 (MIM: 614497)	AD	c.1A>G (p.M1?) heterozygous	Likely pathogenic	Unknown
Individual 4	*COL11A1*	Fibrochondrogenesis (MIM: 228520); Marshall syndrome (MIM: 154780); Stickerler syndrome, type II (MIM: 604841)	AD/AR	c.4084C>T (p.R1362X) heterozygous	Pathogenic	Mother
	MYH9	Macrothrombocytopenia and progressive sensorineural deafness [MIM:600208]; May-Hegglin anomaly [MIM:155100]; Sebastian syndrome [MIM:605249]	AD	c.5338C>T (p.R1780W) heterozygous	VUS	Mother
	DYRK1A	Mental retardation, autosomal dominant 7 [MIM:614104]	AD	c.1789G>A (p.A597T) heterozygous	VUS	Father
Individual	*ANKRD*	KBG syndrome	AD	c.5230C>	VUS	Father

5	*11*	(MIM: 148050)		G (p.H1744 D) heterozygo us		
	ANKRD 11	KBG syndrome (MIM: 148050)	AD	c.3019C> G (p.R1007G) heterozygo us	VUS	Father
	UPF3B	Mental retardation, X-linked, syndromic 14 (MIM: 300676)	XL	c.1061G> A (p.R354Q) heterozygo us	VUS	Mother
Individual 6	No additional variant reported					
Individual 7	No additional variant reported					

Only heterozygous variants in known disease genes with autosomal dominant (AD) or X-linked (XL) inheritance and biallelic variants in known autosomal recessive (AR) disease genes are included here, and only for diseases where there is phenotypic overlap with the individuals' phenotypes.

Table S3 Summary of cases with *de novo NACC1* variants or deletions involving the *NACC1* from literature reports and DECIPHER database.

Patient	**Affected position**	**Sex**	**Size of deleted interval**	**Inheritance**	**Phenotype**	**Reference**
Trio78	*NACC1* c.1402C>T p.R468C	Female	1 nt	*de novo*	delayed developmental development, IQ level of about 45, autistic features and schizo-affective disorder	PMID 24896178, 23033978
Pt13060	*NACC1* c.946+2T>C	Male	1 nt	*de novo*	autism spectrum disorder	PMID 25363768
Dolan et al, 2010, patient 1	deletion, chr19:12498237–13126508	Female	680 kb	*de novo*	Developmental delay, IQ49, overgrowth, macrocephaly, hypotonia, esotropia, nystagmus, poor fixation	PMID 20613546
Dolan et al, 2010, patient 1	deletion, chr19:12536641–13794080	Female	1318 kb	*de novo*	Developmental and speech delay, overgrowth, macrocephaly, Optic nerve hypoplasia, exotropia	PMID 20613546
Dolan et al, 2010, patient 2	deletion, chr19:12793474–13104643	Male	327 kb	*de novo*	Severe speech delay, overgrowth, macrocephaly, seizures, hypotonia, optic atrophy, exotropia	PMID 20613546
Dolan et al, 2010, patient 3	deletion, chr19:12411017–13120904	Female	715 kb	*de novo*	Developmental and speech delay, overgrowth, macrocephaly, seizures, hypotonia, Chiari I malformation with syrinx, Optic nerve hypoplasia, exotropia, nystagmus	PMID 20613546
Auvin et al, 2009	deletion, chr19:12615927–13280259	Male	665 kb	not known	Global developmental delay, overgrowth with advanced bone age, macrocephaly, focal onset seizures on EEG, hypotonia, normal ophthalmologic exam.	PMID 19874387
Lysy et al, 2010	deletion, chr19:10246651–	Female	3033 kb	*de novo*	Non-verbal, global developmental delay, hypotonia, ventriculo-	PMID 19842200

	13280203				megaly, strabismus, proptosis	
Nimmakayalu et al., 2012, patient 1	deletion, chr19:13016005–13415043	Female	399 kb	*de novo*	Significant developmental delay, moderate intellectual disability, generalized hypotonia, epilepsy, progressive unsteady gait. Tall stature and macrocephaly. MRI showed thinning of corpus callosum	PMID 23495138
Nimmakayalu et al., 2012, patient 2	deletion, chr19:13016005–13415043	Female	399 kb	*de novo*	Intellectual disability, macrocephaly, strabismus	PMID 23495138
Bonaglia et al, patient 3	deletion, chr19:12875220–14480616	Male	1605 kb	Maternal	intellectual disability, ADHD, seizures, hypotonia, normal eye exam	PMID 20648052
Jorge et al., 2015	deletion, chr19:12756718–13388309	Female	631 kb	not known	Intellectual disability, overgrowth with advanced bone age, hypotonia, ataxia, ventriculo-megaly, strabismus, nystagmus	PMID 26338046
294330	duplication, chr19:12344536-14642947	Male	2.30 Mb	Unknown	Global developmental delay	DECIPHER
2539	deletion, chr19:13161358-13265508	Male	104.15 kb	*de novo* constitutive		DECIPHER
3820	deletion, chr19:13081794-13265508	unknown	183.72 kb	Unknown	Intellectual disability, Strabismus, Tall stature	DECIPHER
250047	deletion, chr19:12754927-13419259	Male	664.33 kb	*de novo* constitutive		DECIPHER
251206	deletion, chr19:12819000-13609000	Female	790.00 kb	*de novo* constitutive	Ataxia, Intellectual disability, Tall stature, Visual impairment	DECIPHER
253429	deletion, chr19:13076503-	Female	1.60 Mb	*de novo* constit		DECIPHER

	14674918			utive		
253780	deletion, chr19:13068773-13528878	Female	460.11 kb	Unknown		DECIPHER
256712	deletion, chr19:13046572-14958141	Female	1.91 Mb	*de novo* constitutive		DECIPHER
269163	duplication, chr19:11101053-13435131	Male	2.33 Mb	*de novo* constitutive	Abnormality of the central nervous system, Abnormality of the cornea, Atopic dermatitis, Delayed skeletal maturation, Intellectual disability, Microcephaly, Recurrent infections, Short stature, Wide mouth	DECIPHER
2359	duplication, chr19:12242867-14862610	Female	2.62 Mb	Unknown	Clinodactyly of the 5th finger, Hypertelorism, Intellectual disability, Macrodontia, Microcephaly, Spastic diplegia	DECIPHER
262646	deletion, chr19:11892746-13611147	Male	1.72 Mb	*de novo* constitutive	Hydrocephalus, Intellectual disability, Patent ductus arteriosus	DECIPHER
264317	deletion, chr19:13182191-13842254	Male	660.06 kb	*de novo* constitutive	Intellectual disability, Macrocephaly	DECIPHER
265328	deletion, chr19:12592419-13270578	Male	678.16 kb	Unknown	Hearing impairment, Intellectual disability, Macrocephaly, Pectus carinatum, Recurrent fractures, Scoliosis, Tall stature, Visual impairment	DECIPHER
292057	duplication, chr19:13182420-13394529	Male	212.11 kb	Unknown		DECIPHER
267428	deletion, chr19:12745203-13376358	Male	631.16 kb	*de novo* constitutive	Deep palmar crease, Intellectual disability, moderate, Large for gestational age, Macrocephaly at birth,	DECIPHER

					Overgrowth, Prominent forehead, Sparse scalp hair, Spasticity	
257523	duplication, chr19:12841373-15929820	Male	3.09 Mb	*de novo* constitutive	Intellectual disability, Proportionate short stature	DECIPHER
275609	duplication, chr19:11574852-15028946	Male	3.45 Mb	Unknown	Growth hormone deficiency, Microcephaly, Moderate global developmental delay	DECIPHER
265393	duplication, chr19:10950425-14847355	Male	3.90 Mb	*de novo* constitutive		DECIPHER
279720	deletion, chr19:13243584-15633140	Female	2.39 Mb	Unknown	Hypoplasia of the corpus callosum	DECIPHER
280489	deletion, chr19:13081594-13270748	Male	189.16 kb	*de novo* constitutive	Global developmental delay	DECIPHER
301615	duplication, chr19:12997640-13476228	Female	478.59 kb	Unknown	Intrauterine growth retardation, Lymphedema	DECIPHER
285391	deletion, chr19:11338618-13327698	Male	1.99 Mb	*de novo* constitutive	Abnormality of the helix, Aplasia/hypoplasia of the corpus callosum, Dental crowding, High forehead, Hypoplasia of the optic tract, Intellectual disability, profound, Pectus excavatum, Pointed chin, Prominent ears, Scoliosis, Short nasal bridge, Ureteropelvic junction obstruction, Wide mouth	DECIPHER
275388	duplication, chr19:266117-59092570	Male	58.83 Mb	Unknown		DECIPHER
292233	deletion, chr19:13224132-13307044	Female	82.91 kb	Unknown		DECIPHER

258888	duplication, chr19:12997640-13611006	Female	613.37 kb	*de novo* constitutive		DECIPHER
299787	deletion, chr19:12433780-13419259	unknown	985.48 kb	*de novo* constitutive	Delayed speech and language development, Generalized neonatal hypotonia, Global developmental delay, Intellectual disability, mild, Nystagmus, Pectus excavatum of inferior sternum, Plagiocephaly, Triangular face	DECIPHER
305287	duplication, chr19:12880402-13497160	Female	616.76 kb	*de novo* constitutive	Epicanthus, High palate, Intrauterine growth retardation, Iron deficiency anemia, Postnatal microcephaly, Postnatal microcephaly, Short stature, Thin upper lip vermilion	DECIPHER
302023	deletion, chr19:12527157-13476228	Female	949.07 kb	*de novo* constitutive		DECIPHER
284902	duplication, chr19:12744531-13538042	Female	793.51 kb	*de novo* constitutive	Hyperactivity, Intellectual disability	DECIPHER
331245	deletion, chr19:12441723-13662245	Male	1.22 Mb	*de novo* constitutive	Aqueductal stenosis, Developmental stagnation, Generalized hypotonia, Global developmental delay, Hydrocephalus	DECIPHER