De novo variants of NR4A2 are associated with neurodevelopmental disorder and epilepsy

Sakshi Singh  
*University Medical Centre Utrecht*

Rachel Slaugh  
*Washington University School of Medicine in St. Louis*

Jorge Granadillo  
*Washington University School of Medicine in St. Louis*

et al.

Follow this and additional works at: [https://digitalcommons.wustl.edu/oa_4](https://digitalcommons.wustl.edu/oa_4)

© Part of the Medicine and Health Sciences Commons

Please let us know how this document benefits you.

**Recommended Citation**

Singh, Sakshi; Slaugh, Rachel; Granadillo, Jorge; and et al., "De novo variants of NR4A2 are associated with neurodevelopmental disorder and epilepsy." *Genetics in Medicine*. 22, 8. 1413 - 1417. (2020).  
[https://digitalcommons.wustl.edu/oa_4/529](https://digitalcommons.wustl.edu/oa_4/529)

This Open Access Publication is brought to you for free and open access by the Open Access Publications at Digital Commons@Becker. It has been accepted for inclusion in 2020-Current year OA Pubs by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
De novo variants of NR4A2 are associated with neurodevelopmental disorder and epilepsy

Sakshi Singh, PhD1, Aditi Gupta, PhD2,3, Michael Zech, MD4,5, Ashley N. Sigafos, BS2,6, Karl J. Clark, PhD2,6, Yasemin Dincer, MS7,8, Matias Wagner, MD4,5, Jennifer B. Humberson, MD9, Sarah Green, MS10, Koen van Gassen, PhD1, Tracy Brandt, PhD FACMG11, Rhonda E. Schnur, MD FACMG11, Francisca Millan, MD FACMG11, Yue Si, MD PhD11, Volker Mall, MD7,12, Juliane Winkelmann, MD4,5,13,14, Ralitza H. Gavrilova, MD2,15,16, Eric W. Klee, PhD2,3,15, Kendra Engleman, MS17, Nicole P. Safina, MD17, Rachel Slaugh, MS18, Emily M. Bryant, MS19, Wen-Hann Tan, BMBS20, Jorge Granadillo, MD18, Sunita N. Misra, MD PhD19, G. Bradley Schaefer, MD10, Shelley Towner, MS9, Eva H. Brilstra, MD PhD1 and Bobby P. C. Koeleman, PhD1

**Purpose:** This study characterizes the clinical and genetic features of nine unrelated patients with de novo variants in the NR4A2 gene.

**Methods:** Variants were identified and de novo origins were confirmed through trio exome sequencing in all but one patient. Targeted RNA sequencing was performed for one variant to confirm its splicing effect. Independent discoveries were shared through GeneMatcher.

**Results:** Missense and loss-of-function variants in NR4A2 were identified in patients from eight unrelated families. One patient carried a larger deletion including adjacent genes. The cases presented with developmental delay, hypotonia (six cases), and epilepsy (six cases). De novo status was confirmed for eight patients. One variant was demonstrated to affect splicing and result in expression of abnormal transcripts likely subject to nonsense-mediated decay.

**Conclusion:** Our study underscores the importance of NR4A2 as a disease gene for neurodevelopmental disorders and epilepsy. The identified variants are likely causative of the seizures and additional developmental phenotypes in these patients.

**Keywords:** NR4A2; epilepsy; seizures; neurodevelopmental disorder; developmental disorder

**INTRODUCTION**

The NR4A2 gene encodes a steroid–thyroid hormone–retinoid receptor that acts as a nuclear receptor (NR) transcription factor. The NR transcription factors play a regulatory role in various aspects of mammalian physiology such as neuronal development, inflammation, carcinogenesis, and memory formation. NR4A2 is required for development, function, and neurotransmission of dopaminergic neurons.

The NR4A2 protein consists of two main domains: a DNA binding domain (DBD) and a ligand binding domain (LBD). The DBD is a highly conserved domain containing two C4 type zinc fingers that bind to specific motifs in DNA hormone response elements, and is connected to the C-terminal LBD via a linker region. The NR4A2 protein functions by binding small molecule ligands within conserved ligand binding patches located in the hydrophobic core of the LBD. Ligand binding induces a conformational change in the LBD leading to changes in interaction of nuclear receptor coregulators and other proteins. This alters chromatin structure and gene expression and, therefore, up and down regulation of target genes. A mutated gene may encode for a misfolded protein,

1Department of Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands; 2Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA; 3Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA; 4Institut für Neurogenomik, Helmholtz Zentrum München, Munich, Germany; 5Institut für Humangenetik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; 6Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA; 7Lehrstuhl für Sozialpädiatrie, Technische Universität München, Munich, Germany; 8Zentrum für Humangenetik und Laboratoriumsdiagnostik (MVZ), Martinsried, Germany; 9Department of Pediatrics, University of Virginia, Charlottesville, VA, USA; 10Department of Pediatrics, University of Virginia, Charlottesville, VA, USA; 11Division of Genetics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO, USA; 12Ann & Robert H. Lurie Children’s Hospital, Epilepsy Center, Chicago, IL, USA; 13Division of Genetics and Genomics, Department of Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, MA, USA; 14GeneDx, for Systems Neurology, SyNergy, Munich, Germany; 15Departments of Clinical Genomics and Neurology, Mayo Clinic, Rochester, MN, USA; 16Department of Neurology, Mayo Clinic, Rochester, MN, USA; 17Division of Clinical Genetics, Children’s Mercy Kansas City, University of Missouri Kansas City School of Medicine, Kansas City, MO, USA; 18Division of Genetics in Medicine, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO, USA; 19Department of Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands; 20Section of Genetics and Metabolism, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO, USA; 21Department of Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands; 22Division of Genetics and Genomics, Department of Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, MA, USA. Correspondence: Bobby P. C. Koeleman (b.p.c.koeleman@umcutrecht.nl)

Submitted 4 December 2019; revised 16 April 2020; accepted: 17 April 2020

Published online: 5 May 2020
dysfunctional ligand binding pocket, or dysfunctional DNA binding.

Haploinsufficiency of the NR4A2 gene caused by heterozygous chromosomal deletions was previously associated with a neurodevelopmental disorder with high penetrance, suggesting that heterozygous loss of NR4A2 is autosomal dominant. To our knowledge, there is only one previous report of a variant of NR4A2 (NM_006186.3: c.327dup, p.S110Vfs*2) associated with epilepsy.

Here we report nine patients with variants in NR4A2 and developmental delay/intellectual disability with or without epilepsy.

MATERIALS AND METHODS
Informed consent for genetic testing was obtained from all patients and parents included in the present study. The consent and protocols were approved by the respective institutional ethical review boards (University Medical Centre Utrecht, Mayo Clinic, Children’s Mercy Kansas City, Technical University of Munich, University of Arkansas for Medical Sciences, University of Virginia, Washington University School of Medicine, Boston Children’s Hospital, Ann & Robert H. Lurie Children’s Hospital). All patients underwent exome sequencing; however, the variant identified in patient 2 was detected using a targeted exome analysis for neurodevelopmental genes. The deletion observed in patient 9 was detected by comparative genome hybridization using an Agilent 180K oligoarray. The microdeletion was verified by fluorescence in situ hybridization (FISH). All other cases were analyzed using the complete exome (Supplementary information). By sharing through GeneMatcher, we discovered other patients harboring variants in the NR4A2 gene. Five patients had missense variants, one had a microdeletion, and three had nonsense or frameshift variants.

RESULTS
We report a case series of nine patients with novel variants in NR4A2, including eight patients with a confirmed de novo variant, and one patient with a larger deletion encompassing NR4A2. The patients (five females, four males; mean age 12.4 years, age range 2–43 years, at the time of inclusion in current study) show heterogeneous phenotypes (detailed phenotypes are given in online Supplementary information). Their neurodevelopmental phenotypes are characterized by delayed psychomotor development (9/9), which was initially normal in two patients. Individuals presented with varying levels (mild to severe) of intellectual disability (ID)/developmental delay (DD). Other features include epilepsy (6/9), speech/language impairment (5/9), behavioral problems (5/9), and movement disorder/hypotonia (8/9). Patients presented with variable epilepsy phenotype, including rolandic epilepsy, generalized encephalopathy, West syndrome, and infantile spasms. One patient showed epileptoform activity and photosensitivity on electroencephalogram (EEG). Seizure type included tonic clonic, generalized, absence, and focal seizures. Seizures remained refractory in two patients and the remaining four became seizure-free on appropriate antiepileptic drugs. Behavioral problems included autism, attention deficit–hyperactivity disorder, hyperactivity, anxiety, and hyposensitivity. Two patients had ataxia. There was no apparent genotype–phenotype correlation.

Genetic results
We identified eight patients with intragenic NR4A2 variants and one patient carrying a larger deletion including NR4A2. Variants were not present in ExAC and gnomAD. De novo occurrence of the variants was confirmed for eight of these patients. The origin of the variant in patient 5 could not be confirmed due to unavailability of the father, but it was not maternal. Five patients had missense variants, one had a microdeletion, and three had nonsense or frameshift variants leading to a premature stop codon (Table 1, Fig. 1). Four missense variants and one splice-acceptor site variant (c.839G>A, p.C280Y; c.914G>A, p.C305Y; c.857T>C, p.F286S; c.968G>T, p.C323F; c.865-1_865delGCinsAAAAAG-GAGT) were located in the DBD of the protein that may affect DNA binding of the transcription factor. Patient 4 carried a missense variant (c.1175A>G, p.D392G) affecting a hinge region without any secondary structure in the LBD. Patient 5 had a nonsense variant (c.1576G>T, p.E526*) in the
LBD, introducing premature termination. All missense variants were located in a gene region that is enriched for pathogenic variants across the NR4A2 gene family (see Supplementary Figure S4).

Patient 6 had a frameshift variant (c.325dup) in N-terminal regulatory domain introducing premature termination that is predicted to lead to nonsense-mediated decay (NMD) and LoF, similar to the previously published epilepsy patient (c.327dup).7 Patient 9 carried a chromosomal microdeletion arr[GRCh37]2q23.3q24.1(154790212_158488241)x1 of size >3.6 Mb (3698029 bp) encompassing the NR4A2 gene. The deletion also covered ten flanking genes (KCNJ3, GPD2, GALNT5, ERMN, CYTIP, ACVR1C, ACVR1, UPP2, CCDC148, and PKP4).

Various in silico tools predicted that the variant in patient 2 affects splicing that would lead to LoF. This was confirmed by RT-PCR (see Supplementary Figure S2), which revealed altered splicing leading to aberrant transcripts with an out of frame skipping of exon 4 (130 nucleotides), which will potentially cause truncation and LoF through NMD.

All variants had high predictive scores for a detrimental effect as predicted by SIFT, PolyPhen-2, and CADD scores. The NR4A2 gene appears to be under a high selective strain and extremely intolerant to LoF variation as evidenced from its high probability of being LoF intolerant (pLI) score (1.0) and lower than expected missense variant counts (Z-score = 2.24), as observed in both ExAC and gnomAD. The haploinsufficiency score (HI score = 1.28%) shows this gene to be highly dosage sensitive. Therefore, intolerance to LoF can play an important role in the development of pathogenic phenotypes in patients.3,10,11

We report nine patients with early onset epilepsy and/or a developmental disorder, of whom eight carried intragenic variants and on a larger deletion including NR4A2. Six of these patients with de novo NR4A2 variants had epilepsy. The apparent intolerance to LoF and missense variation of NR4A2 suggests that these variants are causing the phenotype in patients.3,10,11 In previous studies, haploinsufficiency of NR4A2 has been implicated in a neurodevelopmental phenotype, including significant language impairment11,12 and ID.2,7 These symptoms overlap with those observed in the patients studied here. We also observed language impairment in five of nine patients, which may be linked to the more prominent expression of NR4A2 in the superior temporal gyrus (STG), a brain region linked to language development.13

Regardless of the similarities among these nine patients, the underlying explanations for the phenotypes of patient 4 and patient 9 may be different. Patient 4 had a deceased sibling with similar phenotype. Therefore, the de novo variant detected in patient 4 may not explain the full phenotype, and another cause, as well as germline mosaicism, should be considered. For patient 9 the microdeletion also affected ten other genes that could have contributed to the clinical features. For example, KCNJ3, also deleted in this patient, encodes for subunit G-protein activated inward rectifier potassium channel 1. Alterations in the function of this potassium channel subunit have been associated with epilepsy.14

It remains unclear how these variants of NR4A2 contribute to the epileptogenesis. However, the physiologic role of

Fig. 1 Schematic view of the distribution of pathogenic variants in NR4A2. (a) Transcript description and locations (marked by circles) of the variants found in NR4A2 gene. (b) Predicted effects (blue dots) of the pathogenic variants on NR4A2 protein sequence. The c.327dup,p.S110Vfs*2 variant was published previously.7.
Table 1 Clinical phenotypes of patients with heterozygous de novo and putative de novo NR4A2 variants.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Variant (NM_006186.3)</th>
<th>Inheritance</th>
<th>Protein domain/region</th>
<th>Seizures</th>
<th>Age, years/seizures at seizure onset</th>
<th>Developmental delay</th>
<th>Speech and language impairment</th>
<th>Motor delay</th>
<th>Intellectual disability</th>
<th>Behavioral problems</th>
<th>MRI findings</th>
<th>Neurologic examination findings</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.839G&gt;A, p.C280Y</td>
<td>De novo</td>
<td>ZnF_C4 domain, DNA binding domain</td>
<td>Yes</td>
<td>15/10</td>
<td>Global</td>
<td>NA</td>
<td>NA</td>
<td>Severe</td>
<td>Autism</td>
<td>Normal</td>
<td>Normal</td>
<td>Mild</td>
</tr>
<tr>
<td>2</td>
<td>c.865_1.865delGCGAAGATCT</td>
<td>De novo</td>
<td>ZnF_C4 domain, DNA binding domain</td>
<td>Yes</td>
<td>12/10</td>
<td>Global</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Anxiety</td>
<td>Normal</td>
<td>Mild hypotonia</td>
<td>EDS</td>
</tr>
<tr>
<td>3</td>
<td>c.914G&gt;A, p.C305Y</td>
<td>De novo</td>
<td>ZnF_C4 domain, DNA binding domain</td>
<td>5</td>
<td>MRI</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Severe</td>
<td>No</td>
<td>Moderate</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>c.1175A&gt;G, p.D392G</td>
<td>De novo</td>
<td>Hinge region</td>
<td>Yes</td>
<td>3/5</td>
<td>Global</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>c.1576G&gt;T, p.E526sp</td>
<td>NA</td>
<td>HOLI ligand binding domain</td>
<td>No</td>
<td>5/NI</td>
<td>Global</td>
<td>Yes</td>
<td>Yes</td>
<td>Mild</td>
<td>Attachment disorder</td>
<td>Normal</td>
<td>NA</td>
<td>Movement disorder</td>
</tr>
<tr>
<td>6</td>
<td>c.325dupC, p.Q109Pfs*</td>
<td>De novo</td>
<td>N-terminal regulatory domain</td>
<td>Yes</td>
<td>2/6/4</td>
<td>Global</td>
<td>Yes</td>
<td>No</td>
<td>Sensory sensitivity</td>
<td>Hypersensitivity</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>c.857T&gt;C, p.F286S</td>
<td>De novo</td>
<td>ZnF_C4 domain, DNA binding domain</td>
<td>No</td>
<td>4/NI</td>
<td>Global</td>
<td>Yes</td>
<td>No</td>
<td>Moderate</td>
<td>Type 1 diabetes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>c.325dupC, p.Q109Pfs*</td>
<td>De novo</td>
<td>N-terminal regulatory domain</td>
<td>Yes</td>
<td>19/NI</td>
<td>Global</td>
<td>Yes</td>
<td>No</td>
<td>Moderate</td>
<td>Type 2 diabetes</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>arr[GRCh37]2q23.3q24.1</td>
<td>De novo</td>
<td>NA</td>
<td>yes</td>
<td>43/17</td>
<td>Global</td>
<td>NA</td>
<td>NA</td>
<td>Moderate</td>
<td>Hypersensitivity</td>
<td>NA</td>
<td>Progressive ataxia in adulthood</td>
<td>No</td>
</tr>
</tbody>
</table>

AED antiepileptic drug, EDS Ehlers-Danlos syndrome, EEG electroencephalogram, F female, M male, MRI magnetic resonance image, NA not assessed.
revealing its involvement in respiratory abnormality and lack of response to hypoxia. NR4A2 is encoded by immediate early genes and has a significant role in development, neuroprotection, learning, and memory formation, presenting a reasonable explanation for the associated ID among these patients. These studies indicate that variants in NR4A2 can impair its various functions, and plausibly contribute to the seizure, neurodevelopmental, and global developmental phenotype observed in the described patients.

SUPPLEMENTARY INFORMATION
The online version of this article (https://doi.org/10.1038/s41436-020-0815-4) contains supplementary material, which is available to authorized users.

ACKNOWLEDGEMENTS
This study was supported by the "friends of UMC & Willemina Kinder Ziekenhuis" MING funds.

DISCLOSURE
T.B., R.E.S., F.M., and Y.S. are employees of GeneDx. The other authors declare no conflicts of interest.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES