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Expansion of the Genotypic and Phenotypic Spectrum of WASF1-Related Neurodevelopmental Disorder

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Abstract: In humans, de novo truncating variants in WASF1 (Wiskott–Aldrich syndrome protein family member 1) have been linked to presentations of moderate-to-profound intellectual disability (ID), autistic features, and epilepsy. Apart from one case series, there is limited information on the phenotypic spectrum and genetic landscape of WASF1-related neurodevelopmental disorder (NDD). In this report, we describe detailed clinical characteristics of six individuals with WASF1-related NDD. We demonstrate a broader spectrum of neurodevelopmental impairment including more mildly affected individuals. Further, we report new variant types, including a copy number variant (CNV), resulting in the partial deletion of WASF1 in monozygotic twins, and three missense variants, two of which alter the same residue, p.W161. This report adds further evidence that de novo variants in WASF1 cause an autosomal dominant NDD.

Keywords: WASF1; autism; intellectual disability; neurodevelopmental disorder

1. Introduction

WASF1 (Wiskott–Aldrich syndrome protein family member 1)/WAVE1 (WASP-family verprolin homologous protein 1) forms one of the subunits of a protein complex (WAVE Regulatory Complex, WRC) that helps regulate actin remodeling. Within this pathway, binding of the GTPase RAC1 (Ras-related C3 botulinum toxin substrate 1) to distinct sites within the WRC alters the conformation of the complex, promoting accessibility of the VCA/WCA region within the complex to actin and the ARP2/3 (actin-related protein 2/actin-related protein 3) complex, which leads to actin filament assembly [1,2]. This process is necessary for neuronal roles like synaptic plasticity [3–5].
In humans, defects in WASF1 have been associated with a neurodevelopmental disorder (NDD). In 2018, Ito et al. reported a case series of five unrelated individuals with moderate-to-profound intellectual disability (ID), autistic features, and epilepsy who had de novo truncating variants in WASF1. Three individuals shared a recurrent nonsense variant [c.1516C>T (p.R506*)], while the other two individuals had unique variants [c.1558C>T (p.Q520*) and c.1482delinsGCCAGG (p.I494Mfs*23)]. All these variants clustered around the WH2 (WASP homology 2) domain and were predicted to disrupt the C-terminal actin-binding WCA domain. In fibroblast samples from two individuals with the recurrent variant, functional studies revealed decreased production of the WASF1 protein as well as evidence of disrupted actin remodeling [6].

Apart from that case series, there is limited information on the phenotypic spectrum and genetic landscape of WASF1-related NDD. In this report, we describe in detail the developmental, neurological, and systemic findings of six individuals with WASF1-related NDD. We demonstrate a broader spectrum of neurodevelopmental impairment including more mildly affected individuals. Further, we report new variant types, including a copy number variant (CNV), resulting in partial deletion of WASF1 in monozygotic twins, and three missense variants, two of which alter the same residue, p.W161. This report adds further evidence that de novo variants in WASF1 cause an autosomal dominant NDD.

2. Methods

All six patients (P1–P6) underwent clinical evaluations by a pediatric neurologist, medical geneticist, and/or developmental pediatrician. The patients had clinical genetic testing—including chromosomal microarray, exome sequencing, and, in some patients, single gene testing—as indicated based on phenotype.

The monozygotic twin sisters, P1 and P2, received a molecular diagnosis by chromosomal microarray, specifically a targeted oligonucleotide array comparative genomic hybridization (CGH), performed by the DNA Diagnostic Laboratory of Children’s Hospital Boston (Boston, MA, USA). DNA was extracted from peripheral blood, fragmented, labeled, and hybridized to the array CGH. The father and mother underwent targeted testing for the CNV identified in the sisters. P1 and P2 later underwent clinical exome sequencing, performed at GeneDx Laboratory (Gaithersburg, MD, USA), which did not identify the deletion nor any other contributory variants; however, CNV analysis was not performed as part of the exome sequencing analysis.

The other four patients, P3–P6, received a molecular diagnosis by trio exome sequencing performed in clinical diagnostic laboratories. Sanger sequencing by the diagnostic laboratories confirmed the detected WASF1 variants. For P3–P5, exome sequencing was performed by GeneDx Laboratory (Gaithersburg, MD, USA) with methods as previously reported [7]. For P6, WES was performed using a TruSeq Exome kit (Illumina, San Diego, CA, USA) and sequenced on the Illumina NextSeq 500 (Illumina, San Diego, CA, USA) in Marseille Medical Genetics Center (MMG, Marseille, France). Data were analyzed using Varaft [8].

The patient cohort was assembled via connections through GeneMatcher [9].

3. Results

3.1. Demographics and Genotype

Among the six individuals in this cohort, four were unrelated and two were monozygotic twin sisters. All had de novo variants, consisting of a chromosomal deletion encompassing a portion of the WASF1 gene in the twin sisters and WASF1 single nucleotide variants (SNVs) in \( n = 4 \) (Table 1, Figure 1).
Table 1. Demographic and genetic features of the cohort.

|                | P1 (MZ Twin) | P2 (MZ Twin) | P3              | P4              | P5              | P6              |
|----------------|--------------|--------------|-----------------|-----------------|-----------------|-----------------|
| Sex            | Female       | Female       | Male            | Female          | Female          | Male            |
| Age at last exam| 15 years     | 15 years     | 7 years         | 4 years 7 months| 6 years         | 4 years         |
| WASF1 variant previously reported | No           | No           | Yes             | No              | No              | No              |
| Basis for WASF1 variant discovery | Chromosomal microarray | Partial gene | Trio exome sequencing | Trio exome sequencing | Trio exome sequencing | Trio exome sequencing |
| WASF1 variant * | deletion (exons 8–10) | c.1516C>T | p.R506 * | p.K172E | p.W161C | p.W161R |
| Protein change | N/A          | N/A          | De novo         | De novo         | De novo         | De novo         |
| Inheritance    | De novo      | De novo      | Heterozygous    | Heterozygous    | Heterozygous    | Heterozygous    |
| Zygosity       | Heterozygous | Heterozygous | Normal          | Normal          | Normal          | Normal          |
| Chromosomal microarray | As above | As above | Normal           | Normal           | Normal           | Normal           |
| Consanguinity  | No           | No           | No              | No              | No              | No              |

* All sequence variants are described in reference to RefSeq transcript NM_003931.2. N/A = not applicable; MZ = monozygotic.

Figure 1. Schematics depicting location of WASF1 variants and evidence of pathogenicity of the WASF1 single nucleotide variants (SNVs). (A) Scheme 1 protein and the locations of the SNVs in P3–P6. Blue circles denote missense variants, and the yellow circle denotes the nonsense variant. Abbreviations of domains: WASP homology 1 domain (WH1, in blue), Basic domain (B, in magenta), Proline-rich region (Pro, in salmon pink), WASP homology 2 domain (WH2, in green), Cofilin homology domain (C, in purple), Acidic domain (A, in yellow). (B) Schematic diagram showing the 3′ end of WASF1. Red line indicates the deletion in P1 and P2, which includes exons 8–10 of WASF1 and extends beyond the 3′ end of the gene. (C) Schematic diagram depicting conservation throughout various species of WASF1 amino acids including and surrounding the missense variants identified in this report. (D) Table summarizing protein domains and results of in silico pathogenicity prediction tools for the four single nucleotide variants, which are described in reference to RefSeq transcript NM_003931.2.
The chromosomal copy number variant (CNV) in the twin sisters was a deletion within cytogenetic band 6q21, approximately 180 kb in size, with genomic location as follows: arr[NCBI37/hg19] 6q21(110243269_110423466) × 1. The deleted interval involves two genes: the entire GPR6 gene and exons 8–10 of WASF1.

Among the four patients with SNVs, one individual (P3) had the recurrent nonsense variant c.1516C>T (p.R506*) previously published [6]. The remaining three individuals with SNVs had missense variants: c.514A>G (p.K172E) in P4; c.483G>T (p.W161C) in P5; and c.481T>A (p.W161R) in P6. Notably, two of the missense variants altered the same amino acid residue (p.W161), and all three clustered at or near the end of the WH1 (WASP homology 1) domain. Evidence of pathogenicity of the missense variants is shown in Figure 1. All sequence variants are described in reference to RefSeq transcript NM_003931.2.

3.2. Neurodevelopmental Features

All individuals in this cohort had global developmental delay (GDD) or ID (Table 2). One of the youngest individuals in the cohort (P4) had a diagnosis of GDD, though her visual-motor and language abilities were in the low-to-average range. She underwent a developmental assessment at age 32 months using the Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS) [10], which demonstrated a visual motor/problem-solving developmental quotient of 77% and a language developmental quotient of 72%. At this age, she was able to jargon and use 50–100 words as well as two-word phrases. Among the remaining five participants, best estimate of ID severity (based on factors such as adaptive skills) ranged from moderate ID in n = 1 to severe-to-profound ID in n = 4. None of these five individuals had spontaneous, specific spoken words. Notably, there was no history of developmental regression in any of the participants.

Behavioral challenges in the cohort included a nearly universal presence of autism spectrum disorder (ASD). Half (n = 3/6) exhibited repetitive hand movements, including midline hand stereotypies and hand-wringing behaviors. These behaviors prompted consideration of Rett syndrome, specifically MECP2 gene sequencing in P1, P2, and P5; and CDKL5 gene sequencing in P5; prior to exome sequencing, the results were normal. Anxiety was noted in n = 1/6, and aggressive behaviors occurred in n = 3/6.

Motor challenges were common in this cohort. All individuals had hypotonia, and one individual had spasticity and dystonia. From a functional motor standpoint, the most severely affected individual was P6, who was only just starting to crawl at age 4 years. The twin sisters also had severe motor limitations, as neither could walk independently for long distances. By comparison, the other three individuals were able to walk independently, but age of achievement of this milestone was delayed, ranging from 2 to 3 years.

3.3. Neurological Features

Among neurological features, epilepsy affected n = 5/6 individuals. Seizure types included generalized tonic clonic seizures (n = 1), tonic seizures (n = 2), focal seizures (n = 1), reflex seizures (n = 1), and myoclonic seizures (n = 1). Infantile spasms occurred in P6. Among the individuals with epilepsy in the cohort, electroencephalogram (EEG) showed evidence of slowing, as well as focal and generalized epileptiform activity. Brain MRIs were normal in n = 3 patients and showed non-specific abnormalities in n = 3 patients.

3.4. Systemic Features

None of the patients had congenital anomalies or consistent dysmorphic features (Table 3). Some had variable additional systemic manifestations. Notable growth issues included failure to thrive (n = 2/6) and short stature (n = 2/6). Endocrine features consisted of growth hormone deficiency (n = 1/6), hypothyroidism (n = 1/6), and precocious puberty (n = 1/6). Strabismus was present in 3/6 of the individuals, including both twin sisters, one of whom also had optic atrophy. Vasomotor instability occurred in the twin sisters. Except for the twin sisters who were born at 26 weeks gestation, the perinatal histories of the affected individuals were overall uncomplicated.
Table 2. Neurological and developmental features of the cohort.

| Language/Cognitive | P1 (MZ Twin)       | P2 (MZ Twin)       | P3                | P4                      | P5                         | P6                         |
|--------------------|--------------------|--------------------|-------------------|-------------------------|----------------------------|-----------------------------|
| Current best language abilities | Babbling          | Babbling          | Non-specific vocalizations | 50–100 words; two-word phrases | Non-specific vocalizations | Non-verbal                  |
| Age of saying first word besides mama/dada | N/A               | N/A               | N/A               | 17 months              | N/A                        | N/A                         |
| Age of speaking full sentences | N/A               | N/A               | N/A               | 3–4 years              | N/A                        | N/A                         |
| Global delay (GDD)/intellectual disability (ID) | Yes (ID)          | Yes (ID)          | Yes (ID)          | Yes (GDD)              | Yes (ID)                   | Yes (ID)                    |
| IQ estimate | Profound ID        | Profound ID        | Moderate ID        | Low-average (CAT/C:AMS at 32 months: language DQ 72%, visual motor/cognitive DQ 77%) | Severe ID                   | Profound ID                  |

**Behavioral/Mood**

| Autistic   | Yes | Yes | Yes | Yes | Yes | No |
|------------|-----|-----|-----|-----|-----|----|
| Repetitive hand movements | Yes | No  | Yes | No  | No  | No |
| Midline hand stereotypies | Yes | Yes | No  | No  | No  | No |
| Head banging, hitting | Yes | No  | Yes | No  | No  | Yes (when frustrated) |
| Anxiety    | No  | Yes | No  | No  | No  | No |
| Aggression | Yes | No  | Yes | No  | No  | No |

**Motor**

| Current best motor abilities | Taking steps with support | Walking without support for a short number of steps | Walking independently | Walking independently | Climbing up steps with alternating feet | Starting to crawl |
|------------------------------|---------------------------|------------------------------------------------------|------------------------|-----------------------|----------------------------------------|------------------|
| Age of walking independently | N/A                       | N/A                                                  | 2 years                | 2 years 1 month       | 3 years                                | N/A              |
| Axial hypotonia              | Yes                       | Yes                                                  | Yes                    | Yes                   | Yes                                    | Yes              |
| Appendicular hypertonia      | Yes (spasticity/dystonia) | No                                                   | No                     | No                    | No                                     | No               |
| Neurological                            | P1 (MZ Twin) | P2 (MZ Twin) | P3                  | P4                  | P5                  | P6                  |
|----------------------------------------|--------------|--------------|---------------------|---------------------|---------------------|---------------------|
| Microcephaly                           | Yes          | Yes          | No                  | Borderline (6th percentile) | No                  | Yes                 |
| Cortical visual impairment             | Yes          | Yes          | No                  | No                  | Yes (improving)     | Yes                 |
| Epilepsy                               | Yes          | Yes          | Yes                 | No                  | Yes                 | Yes                 |
| Seizure types                          | Generalized tonic seizure | Tonic seizures with atonic components | Focal seizures, reflex seizures | N/A | Myoclonic seizures | Infantile spasms, tonic seizures, hypermotor seizures with dystonic postures |
| Disrupted sleep                        | Yes          | Yes          | No                  | No                  | Yes (resolved)      | Yes                 |
| Dysphagia                              | No           | No           | No                  | Yes                 | Yes                 | Yes                 |
| Age of latest MRI brain                | 15 months    | 3 years      | 3 years             | 2 years 3 months    | 4 years             | 18 months           |
| Brain MRI findings                     | Porencephalic cyst (sequela of prior intraventricular and intraparenchymal hemorrhage) | Normal | Mild, stable ventriculomegaly | Normal | Thickened anterior corpus callosum | Normal |
| Age of latest EEG                      | 15 years     | 14 years     | Moderate slowing (bilateral midline and central regions, maximal left); focal epileptiform discharges (left midline, central head regions) | 7 years 1 years 10 months | Diffuse slowing; generalized interictal epileptiform discharges (bifrontal predominance and associated frontal slowing); frequent myoclonic seizures (head jerks) | 3 years |
| EEG findings                           | Diffuse slowing | Diffuse slowing | Frontal spikes in sleep | Normal | Slowing; slow paroxysmal abnormalities (bi-occipital location) | Normal |

N/A = not applicable; MZ = monozygotic; DQ = developmental quotient; MRI = magnetic resonance imaging; EEG = electroencephalogram.
Table 3. Perinatal and systemic features of the cohort.

| Perinatal Features                  | P1 (MZ Twin)                                      | P2 (MZ Twin)                                      | P3            | P4            | P5                        | P6            |
|-------------------------------------|--------------------------------------------------|--------------------------------------------------|---------------|---------------|----------------------------|---------------|
| Method of conception                | In vitro fertilization                           | In vitro fertilization                           | Unassisted    | Unassisted    | Unassisted Single umbilical | Unassisted    |
| Pregnancy complications             | Concern for twin-twin transfusion                | Concern for twin-twin transfusion                | None          | None          | None                       | None          |
| Gestational age                     | 26 weeks                                         | 26 weeks                                         | 41 1/2 weeks  | 38 weeks      | 39 weeks Concern for small | 40 weeks      |
| Delivery method                     | C-section                                        | C-section                                        | C-section     | Vaginal       | C-section Vaginal          | Vaginal       |
| NICU stay?                          | Yes                                              | Yes                                              | No            | No            | No                         | No            |
| Perinatal complications             | Neonatal depression, intraventricular hemorrhage, | Intraparenchymal                                   | None          | None          | None                       | Difficulty breastfeeding |
| Birth weight                        | 645 g                                            | Unknown                                          | 4100 g        | 3260 g        | 3005 g                     | 3690 g        |
| Birth head circumference            | Unknown                                          | Unknown                                          | 36 cm         | 34 cm         | Unknown                     | 36 cm         |
| Birth length                        | Unknown                                          | Unknown                                          | 53.3 cm       | 50 cm         | 44.5 cm                     | 49 cm         |
| Systemic Features                   |                                                  |                                                  |               |               |                            |               |
| Dysmorphisms                        | Triangular face, midface hypoplasia, upslanting   | Triangular face, midface hypoplasia, upslanting   | Long face,    | None          | Frontal bossing, broad     | None          |
|                                    | palpebral fissures, pointed chin                 | palpebral fissures                                | simple ears   |               | forehead, normal nasal     |               |
|                                    |                                                   |                                                  |               |               | bridge with squared tip    |               |
| Growth                              | Normal                                           | Normal                                           | Failure to thrive | None        | Short stature (parents    | None          |
|                                    |                                                   |                                                  |               |               | had short stature)         |               |
| Endocrine                           | None                                             | None                                             | None          | None          | Hypothyroidism, growth     | None          |
|                                    |                                                   |                                                  |               |               | hormone deficiency         |               |
| Ophthalmological                    | Strabismus, optic atrophy                        | Strabismus                                       | None          | None          | None                       | None          |
|                                    |                                                   |                                                  |               |               | Exotropia                  | None          |
| Gastrointestinal                    | None                                             | None                                             | Constipation, failure to thrive | None | None | Gastrostomy at 3 years old |
|                                    |                                                   |                                                  |               |               |                           |               |
| Musculoskeletal                     | Camptodactyly                                     | None                                             | Pes planus    | None          | In-toeing, tight heel      | None          |
|                                    | Café au lait macules, lentiginous compound nevus | None                                             |               | None          | cords                     |               |
| Dermatological                      | None                                             | None                                             | None          | None          | None                       | None          |
| Autonomic                           | Vasomotor instability                             | Vasomotor instability                             | None          | None          | None                       | None          |

MZ = monozygotic.
4. Discussion

In this work, we expanded the genetic landscape of WASF1-related NDD to include de novo missense variants and a partial gene deletion. We report one patient with the recurrent p.R506* variant, bringing the number of published patients with this specific variant to four. We also broadened the phenotypic spectrum to include more mildly affected individuals.

Multiple lines of evidence support our assertion that the three missense variants are causative of the patients’ phenotype, even though functional studies were not performed to confirm their pathogenicity and determine their mechanism of action (i.e., loss of function vs. gain of function vs. dominant-negative action). These variants are absent from general population databases, alter highly conserved amino acid residues, and are predicted by in silico tools to be deleterious. These variants are clustered in a known functional domain; two of the variants alter the same amino acid residue (p.W161), while the remaining variant (p.K172E) affects a residue that is nearby. Furthermore, the individuals with these variants in our cohort have a shared phenotype consistent with WASF1-related NDD (GDD/ID and seizures without congenital anomalies or major dysmorphic features).

We report a CNV causing WASF1-related NDD. The CNV is a deletion that affects exons 8–10 of WASF1, likely disrupting the C-terminal actin-binding WCA domain, which is also affected by the recurrent p.R506* variant. Of note, the CNV deletion affects not only exons 8–10 of WASF1, but also the entirety of GPR6, which encodes G protein-coupled receptor 6. Variants in GPR6 have not been reported in association with human disorder to date. Based on gnomAD (https://gnomad.broadinstitute.org/, accessed on 27 May 2021) constraint metrics, GPR6 is relatively tolerant to loss-of-function variation as evidenced by the probability of a loss-of-function intolerance (pLI) score of 0.07, which suggests that GPR6 is likely tolerant to loss-of-function variation such as a heterozygous deletion [11]. In contrast, the pLI for WASF1 is 1, indicating that it is highly intolerant to loss-of-function variation. The twin sisters’ phenotype is consistent with WASF1-related NDD, so most likely the partial deletion of WASF1 accounts for their full phenotype, even though we cannot exclude the possibility that haploinsufficiency of GPR6 is contributory.

We broadened the severity spectrum of WASF1-related NDD to include individuals with milder cognitive impairment without epilepsy. All five individuals in the Ito cohort were severely affected: the highest level of intellectual functioning among them was moderate to severe ID (seen in n = 2), and the majority (n = 3) had severe to profound ID; moreover, all but one had epilepsy [6]. In contrast, one individual in our cohort (P4) was mildly affected in terms of cognitive impairment. On developmental testing at 32 months of age, her language and visual motor/cognitive developmental quotients were both above 70%. Assuming a continuation of this current developmental trajectory, these scores may suggest a trajectory towards mild ID or low-to-average IQ, rather than moderate-to-profound ID seen with the others in our cohort and in previously published cohort. Intriguingly, this patient has no current evidence of seizures, including normal EEG, though larger numbers of individuals are needed to determine if the presence of epilepsy within WASF1-related NDD confers a higher likelihood of more severely affected cognitive outcomes.

Our report confirms that WASF1-related NDD is primarily associated with neurodevelopmental impairment but not major congenital anomalies or consistent dysmorphic features. Three of individuals in our cohort were non-dysmorphic, while the others had variable minor dysmorphisms. Relatively more common systemic manifestations included strabismus and growth/endocrine abnormalities (short stature, failure to thrive, precocious puberty), but none of the individuals presented with major structural anomalies.

Rett syndrome-like features, particularly midline hand stereotypies/hand-wrining behavior, were present in half the cohort. Ito et al. referred to specific gene testing conducted among their cohort prior to the WASF1-related diagnosis, and MECP2, CDKL5, and FOXX1 were among these considerations [6]. Altogether, these data suggest that WASF1-related NDD may be considered in the differential of individuals with Rett syndrome-like presentation, broadening the number of genes that can present as Rett mimics [12] and
raising the idea that WASF1 could be considered for inclusion in Rett syndrome gene sequencing panels.

Motor impairment, including hypotonia, was universal in the cohort. In fact, half the cohort was unable to walk independently, while the other half had significant delays in achievement of this milestone (ranging from 2–3 years of age). These findings are similar to those seen in in the Ito cohort, in which age at walking ranged from 25 months to 10 years, and one individual was non-ambulatory at age 23 years [6].

In fact, the diagnosis of cerebral palsy (CP) could be applied to several of the reported patients with WASF1-related NDD, particularly the hypertonic CP subtype. This label, which has elicited controversy as a subtype of CP, refers to CP in which the predominant motor feature is hypotonia [13]. Broadly, CP is defined as a group of disorders of the development of movement and posture due to non-progressive injury to the developing brain [14]. The diagnosis of CP is agnostic to the etiology, which could include genetic and/or non-genetic factors. Notably, the twin sisters had several risk factors for CP including prematurity at 26 weeks and intraventricular hemorrhage, which could lead one to conclude that their impairments were due to perinatal brain injury. However, several characteristics prompted consideration of a genetic etiology: dysmorphisms, Rett-like features, and normal brain MRI in one of the sisters. The presence of a genetic disorder as the etiologic diagnosis should not preclude or negate a clinical diagnosis of CP, because a diagnosis of CP is based on clinical features not underlying etiology [15]. Genetic etiologies should be considered for patients with CP when certain indicators are present, such as lack of history of perinatal brain injury, presence of dysmorphic features or congenital anomalies, or mismatch in clinical presentation with what would be expected based on brain MRI and/or perinatal history [16].

5. Conclusions

In sum, WASF1-related NDD can present as a broad phenotypic spectrum ranging from mild-to-profound GDD/ID with variable features of ASD, epilepsy, and motor impairment. WASF1-related NDD may be caused by different variant types including missense variants and partial gene deletion. Further study is needed to delineate functional impact of missense variants. The small number of cases limits our ability to make genotype–phenotype correlations.

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Informed Consent Statement: Informed consent was obtained from the parents or legal guardians of all subjects involved in this study, including written consent to publish this paper.

Data Availability Statement: The data presented in this study is contained within the article.

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