Phenotypic expansion of Bosch–Boonstra–Schaaf optic atrophy syndrome and further evidence for genotype–phenotype correlations

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Abstract

Bosch–Boonstra–Schaaf Optic Atrophy Syndrome (BBSOAS) is an autosomal dominant neurodevelopmental disorder caused by loss-of-function variants in NR2F1 and characterized by visual impairment, developmental delay, and intellectual disability. Here we report 18 new cases, provide additional clinical information for 9 previously reported individuals, and review an additional 27 published cases to present a total of 54 patients. Among these are 22 individuals with point mutations or in-frame deletions in the DNA-binding domain (DBD), and 32 individuals with other types of variants including whole-gene deletions, nonsense and frameshift variants, and point mutations outside the DBD. We corroborate previously described clinical characteristics including developmental delay, intellectual disability, autism spectrum disorder diagnoses/features thereof, cognitive/behavioral anomalies, hypotonia, feeding difficulties, abnormal brain MRI findings, and seizures. We also confirm a vision phenotype that includes optic nerve hypoplasia, optic atrophy, and cortical visual impairment. Additionally, we expand the vision phenotype to include alacrima and...
1 | INTRODUCTION

Bosch–Boonstra–Schaff Optic Atrophy Syndrome (BBSOAS; OMIM 615722) is an autosomal dominant disorder characterized by visual impairment due to optic nerve abnormalities and/or cortical visual impairment (CVI), developmental delay (DD), and intellectual disability (ID) (Bosch et al., 2014; Chen et al., 2016). BBSOAS is caused by loss-of-function variants in NR2F1, which encodes for a highly conserved orphan nuclear receptor that regulates transcription (Bosch et al., 2014). As with most nuclear receptors, NR2F1 contains a DNA-binding domain (DBD) formed by two zinc finger domains and a ligand-binding domain (LBD) with two highly conserved regions, though the ligand remains unknown. Given the high degree of similarity to its mouse ortholog, Nr2f1, several studies of Nr2f1 knockout mouse models have been performed. Nr2f1 was shown to be involved in neurogenesis (Faedo et al., 2008), neural differentiation (J.-I. Park, Tsai, & Tsai, 2003), eye and optic nerve development (Bertacchi et al., 2019), cortical patterning (Alfano, Magrinelli, Harb, & Studer, 2014; Armentano et al., 2007; Faedo et al., 2008; Zhou, Tsai, & Tsai, 2001), thalamocortical axon guidance (Zhou et al., 1999), arborization (Qiu et al., 1997), and hippocampal volume and functional organization (Fiore et al., 2017).

The phenotypic consequences of NR2F1 variants in humans have become a topic of study only recently, with the first six individuals with heterozygous missense mutations in or deletions of NR2F1 reported in 2014 (Bosch et al., 2014). These six patients demonstrated optic atrophy and ID, and five also had CVI. As data from in vitro reporter assays showed that missense mutations in the DBD and LBD decreased NR2F1 transcriptional activity, and these mutations led to phenotypic consequences similar to those of deletions, a haploinsufficiency model of disease was proposed.

In 2016, a study of 20 new cases revealed additional clinical characteristics including hypotonia, seizures including infantile spasms (IS), autism spectrum disorder (ASD), oromotor dysfunction, thinning of the corpus callosum, and hearing defects, in addition to visual and cognitive defects (Chen et al.). This study was also the first to propose genotype–phenotype correlations, reporting that missense variants outside of the DBD led to milder phenotypic consequences than variants located elsewhere. Functional data from an in vitro dual-luciferase reporter assay corroborated this finding, as missense mutations in the DBD led to even less luciferase activity (and thus transcriptional activity) than the negative control. This result suggested that DBD variants may lead to a more severe phenotype due to a dominant-negative effect, consistent with the fact that NR2F1 binds to DNA as a homodimer (Chen et al., 2016).

As of January 2020, 36 total cases of BBSOAS caused by loss-of-function NR2F1 variants have been reported in the literature (Al-Kateb et al., 2013; Bertacchi, Parisot, & Studer, 2018; Bojanek et al., 2020; Bosch et al., 2014, 2016; Chen et al., 2016; Dimassi et al., 2016; Hino-Fukuyo et al., 2015, 2017; Hobbs, Wolters, & Rayapati, 2020; Kaiwar et al., 2017; Martín-Hernández et al., 2018; Park et al., 2019). Here, we report 18 new individuals with molecularly confirmed heterozygous variants in NR2F1, provide expanded clinical information on 9 of the previously published individuals, and review the extant literature to bring the total number of individuals to 54. Among these, we present 22 individuals with point mutations or in-frame deletions in the DBD and 32 individuals with mutations elsewhere; the latter group includes 24 individuals with deletions, nonsense variants, and frameshift variants, as well as 8 patients with point mutations outside of the DBD. We also report several newly identified clinical characteristics of BBSOAS and further examine genotype–phenotype correlations.

2 | SUBJECTS AND METHODS

2.1 | Subjects

As of July 2019, approximately 117 individuals with variants of NR2F1 have been identified through genome sequencing, whole exome sequencing (WES), or chromosome microarray (CMA) (coordinates based upon build hg19) and reported to Baylor College of Medicine in Houston, Texas via genetic counselors and physicians or via families’ self-referral to Dr. Schaaf’s lab. From this cohort, families for whom contact information was available were notified of an opportunity to participate in a clinical research study as part of an inaugural family conference for the syndrome. The conference and study opportunity were also announced in the closed Facebook group for BBSOAS (see Section 5). Inclusion criteria were the presence of a deletion of or likely pathogenic variant in NR2F1, and the ability to travel to Houston, Texas. The exclusion criterion was an inability to travel to Houston. Written informed consent was obtained as manifest latent nystagmus (fusional maldevelopment), and we broaden the behavioral phenotypic spectrum to include a love of music, an unusually good long-term memory, sleep difficulties, a high pain tolerance, and touch sensitivity. Furthermore, we provide additional evidence for genome–phenotype correlations, specifically supporting a more severe phenotype associated with DBD variants.

KEYWORDS

BBSOAS, developmental delay, intellectual disability, NR2F1, optic atrophy
approved by the Baylor College of Medicine Institutional Review Board.

Twenty-seven participants were enrolled in the study and clinically evaluated as part of the conference; 9 of these individuals had been described in prior studies, while 18 had not been previously reported. Data from this cohort of 27 individuals was combined with the 27 previously published individuals who were not part of the present clinical study, for a total of 54 patients presented here. The average age of the entire group presented here is 12.4 years (SD: 10.4 years) and includes 27 males and 26 females; the sex and age of one patient was not available. Patients 33, 34, and 38 harbor additional genetic variants but were nonetheless included (see Table S1).

2.2 Methods

The 27 study participants, both new and previously reported, underwent a clinical assessment by a geneticist at Texas Children’s Hospital in April 2018, which included a structured parent/guardian interview based on a predetermined list of questions (Web Resource 1). Chart review was performed for any missing information.

Twenty-six of the 27 participants also received ophthalmology exams by pediatric neuro-ophthalmologists at Texas Children’s Hospital. Visits included an evaluation of optic atrophy and optic nerve hypoplasia, with photography of the optic nerves and optical coherence tomography for participants who were able to cooperate. In addition, patients were clinically assessed for CVI, broadly defined here as bilateral visual impairment due to a nonocular cause (i.e., based in the brain) in the presence of normal pupil reactivity (Good et al., 1994; Good, Jan, Burden, Skoczenski, & Candy, 2001). While eye anomalies such as optic nerve abnormalities, strabismus, and occasionally nystagmus may be present, in cases of CVI the degree of visual impairment exceeds that which would be expected from these eye anomalies alone (Hoyt, 2003; Huo, Burden, Hoyt, & Good, 1999). In this cohort, CVI was suspected when a patient’s visual acuity and function seemed to be worse than the appearance of the optic nerves would suggest (Huo et al., 1999). Poor visual acuity and visual field abnormalities supported the presence of CVI (Good et al., 1994). Clinical assessment was corroborated by a parent-report survey of behavioral characteristics of CVI including variable visual functioning (Good et al., 1994), visual latency, difficulty with distance viewing, preference for movement, difficulty with visual complexity, color preference, light-gazing, visual field preference, impaired reflex blink to visual threat, preference for familiar objects, and absence of visually guided reach (Jan, Groenveld, Sykanda, & Hoyt, 1987; Roman-Lantzy, 2007). An overview of the eye/vision phenotype is presented here, with more detailed findings reported separately.

In addition, a retrospective evaluation of brain magnetic resonance imaging, when available, was performed by a neuro-radiologist at Texas Children’s Hospital; these findings will also be reported separately.

Phenotypic data acquired for the previously published participants who were seen by Dr. Schaaf corroborated, corrected, and/or supplemented data from the extant literature. This data was then compiled with phenotypic information for previously described patients not evaluated as part of this study, to present the most complete possible phenotypic spectrum of BBSOAS.

The relationship between genotype and phenotype was assessed using two groups of patients: those with missense variants and in-frame deletions in the DBD (n = 22, 13 previously described), and those with all other variants (n = 32; 23 previously described). To test for categorical variable differences between groups, a Fisher’s Exact test was used to account for small sample sizes. Categorical phenotypic variables include all those listed in Table 1. Each item was coded as “yes” or “no,” instances of the full phenotype not being met are indicated with an asterisk in Table S1 but were coded as “yes” for the purpose of statistical analysis given that statistical power was already limited by the sample sizes. Between-group differences in continuous variables, specifically motor and speech milestones (measured in months; listed in Table 2), were evaluated using Student’s t-tests. A statistically significant difference between groups for each item was determined by a p-value of <.05. All analyses were conducted using Stata 14.

3 RESULTS

The most common clinical features across the sample of 54 patients as a whole are DD, speech delay, vision impairment, and hypotonia, all of which are present in at least 89% of individuals for whom information was available. The present study also confirmed a high prevalence of ASD or features thereof (present in 80% of individuals), cognitive/behavioral anomalies (78%) and feeding difficulties including oromotor dysfunction (70%) and mouth-stuffing (84%). The eye/vision phenotype was clarified and expanded to include optic atrophy or optic disc pallor (82%), optic nerve hypoplasia or a small optic nerve (49%), CVI (68%), manifest latent nystagmus/fusion maldevelopment (52%), and alacrima (78%). The behavioral phenotype was also further broadened by parent report to include a love of music (present in 100% of individuals surveyed), an unusually good long-term memory (76%), a high pain tolerance (78%), sleep difficulties (61%), and touch sensitivity (59%). Results of genotype-phenotype correlation analyses for patients’ physical, cognitive, and behavioral characteristics are available in Table 1; analysis of patients’ developmental milestone attainment can be found in Table 2. Each individual patient’s phenotypic and developmental data are provided in Table S1 and Table S2, respectively.

3.1 Developmental delay and intellectual disability

DD, defined here as a delay in milestone acquisition in at least one domain, is present in 42/47 individuals in the cohort (89%). Speech
and motor delays are both prominent, affecting 38/42 (91%), and 33/41 (83%) of individuals, respectively, although the degree of delay is variable. Though typically children are able to sit by 6 months, crawl by 12 months, and walk by 18 months (Dosman, Andrews, & Goulden, 2012), children with BBSOAS achieved these respective milestones at an average age of 14 months (range: 6–36 months, SD: 8 months, 1/36 not yet achieved at age 4 years), 16 months (range: 9–48 months, SD: 9 months, 4/31 not yet achieved with the oldest age 8 years), and 33 months (range: 11–240, SD: 41 months, 9/42 not yet achieved with the oldest age 22 years). Likewise, while children typically speak their first words by the age of 14 months and combine words by the age of 24 months (Dosman et al., 2012), among children with BBSOAS these milestones were respectively reached at an average age of 32 months (range: 11–72 months, SD: 18, range: 11–72 months, 12/42 not yet achieved with the oldest being 8 years of age) and 47 months (range: 18–84 months, SD: 21, not yet: 17/37 with the oldest being 23 years of age). Eighteen of 43 individuals (42%) are considered nonverbal (the oldest being 23 years of age).

### TABLE 1

| Phenotypic consequences | Overall (N = 51) | Patients with missense mutations or in-frame deletions in the DNA binding domain (N = 22) | Patients with all other variants (N = 29) | p-value |
|-------------------------|------------------|------------------------------------------------------------------------------------------|------------------------------------------|---------|
| **Development/behavior** |                  |                                                                                         |                                          |         |
| Developmental delay     | 42/47 (89%)      | 20/20 (100%)                                                                             | 22/27 (82%)                              | .063    |
| Speech delay            | 38/42 (91%)      | 18/18 (100%)                                                                             | 20/24 (83%)                              | .12     |
| Motor delay             | 33/41 (81%)      | 17/17 (100%)                                                                             | 16/24 (67%)                              | .013    |
| Autism spectrum disorder or features | 37/46 (80%) | 17/19 (89%)                                                                             | 20/27 (74%)                              | .27     |
| Cognitive/behavioral anomalies | 36/46 (78%) | 14/19 (74%)                                                                             | 22/27 (82%)                              | .72     |
| Nonverbal               | 18/43 (42%)      | 13/18 (72%)                                                                              | 5/25 (20%)                               | .001    |
| Unable to walk independently | 9/42 (21%) | 9/17 (53%)                                                                              | 0/25 (0%)                                | <.001   |
| **Vision**              |                  |                                                                                         |                                          |         |
| Vision impairment (general) | 47/52 (90%) | 20/21 (95%)                                                                             | 27/31 (87%)                              | .64     |
| Optic atrophy or optic disc pallor | 41/50 (82%) | 19/21 (91%)                                                                             | 22/29 (76%)                              | .27     |
| Alacrima                | 21/27 (78%)      | 10/12 (83%)                                                                              | 11/15 (73%)                              | .662    |
| Cortical visual impairment | 27/40 (68%) | 14/20 (70%)                                                                             | 13/20 (65%)                              | >.99    |
| Manifest latent nystagmus/fusional Maldevelopment | 15/29 (52%) | 6/12 (50%)                                                                             | 9/17 (53%)                               | >.99    |
| Optic nerve hypoplasia or small optic nerve | 19/39 (49%) | 8/17 (47%)                                                                              | 11/22 (50%)                              | >.99    |
| **Other**               |                  |                                                                                         |                                          |         |
| Love of music           | 27/27 (100%)     | 12/12 (100%)                                                                             | 15/15 (100%)                             | >.99    |
| Hypotonia               | 39/43 (91%)      | 16/17 (94%)                                                                              | 23/26 (89%)                              | >.99    |
| Mouth-stuffing          | 21/25 (84%)      | 7/8 (88%)                                                                               | 14/17 (82%)                              | >.99    |
| Unusually good long-term memory | 19/25 (76%) | 6/9 (67%)                                                                               | 13/16 (81%)                              | .63     |
| High pain tolerance     | 21/27 (78%)      | 8/12 (67%)                                                                               | 13/15 (87%)                              | .36     |
| Feeding problems (any)  | 30/43 (70%)      | 14/18 (78%)                                                                              | 16/25 (64%)                              | .50     |
| Sleep difficulties       | 17/28 (61%)      | 9/13 (69%)                                                                               | 8/15 (53%)                               | .46     |
| Touch sensitivity        | 16/27 (59%)      | 11/12 (92%)                                                                              | 5/15 (33%)                               | .0047   |
| Abnormal brain MRI      | 25/42 (60%)      | 16/21 (76%)                                                                             | 9/21 (43%)                               | .058    |
| Seizures                | 24/46 (52%)      | 14/19 (74%)                                                                              | 10/27 (37%)                              | .019    |
| Abnormal temperature regulation | 11/27 (41%) | 4/12 (33%)                                                                              | 7/15 (47%)                               | .70     |
| Abnormal hearing         | 14/43 (33%)      | 4/18 (22%)                                                                               | 10/25 (40%)                              | .33     |
| Short stature            | 6/47 (13%)       | 4/20 (20%)                                                                               | 2/27 (7%)                                | .38     |

Note: Bolded p-values, p < .05; underlined p-values, p > .05 but difference is of clinical relevance.
23 years of age resides in an assisted living facility. Similarly, Patient 1 was clinically estimated to have a DQ <25, indicating profound ID, and at the age of 22 is cared for at home by his parents. In addition, at the age of 15 years, Patient 19 had reached the developmental level of 6–13 months of age. In contrast, three individuals received IQ scores ranging from 35 to 49, consistent with moderate ID, and six individuals scored between 50 and 69, indicating mild ID. Several individuals showed discrepancies between verbal and nonverbal abilities. Patient 40’s verbal and performance IQ scores fell in the moderate and mild range, respectively; in contrast, Patient 30 reported a verbal IQ score of 96 and a performance IQ score of 70, and Patient 50 reported a full-scale IQ score of 94 but a performance IQ score of 54. Strikingly, Patient 43’s verbal and performance scores were 141 and 63, respectively; at the age of 23 this individual had graduated from college, had been employed, and lived alone with support. Additionally, Patient 45’s IQ was 77–80, and Patient 49’s full-scale IQ score was 80 (though she still requires special education services), reflecting borderline intellectual functioning. An additional seven children have not been formally tested but have received special education services, two are supported by classroom aids, one receives accommodations, and two attend regular education classes supplemented by pull-out instruction.

### 3.2 | ASD and behavior

Features of autism are likewise prominent among individuals with BBSOAS, though the true prevalence of the disorder is difficult to ascertain. Twelve individuals were diagnosed with ASD following formal evaluation, three were provisionally noted to have ASD based on clinical observation, three were diagnosed with PDD-NOS, and 10 demonstrated ASD-related features but had not been formally evaluated; an additional nine were designated as having ASD in prior publications though additional information was unavailable. Of note, two individuals were formally assessed and found not to meet criteria for ASD, and an additional seven had not been tested because features were not present.

Though a distinct behavioral phenotype did not emerge, 36/46 individuals (78%) display behavioral anomalies, including OCD, ADHD, stereotypies such as head-banging and self-injurious behaviors, and engagement in activities associated with restricted interests (such as cutting paper and organizing objects according to size). However, the extent to which some of these behaviors arise due to autism and/or visual impairment is unclear. Also of note, two patients experience auditory hallucinations, one of whom also exhibits paranoid delusions.

### 3.3 | Vision impairment

Vision impairment continues to be a primary characteristic of BBSOAS, evident in 47/52 (90%) of individuals. Though the full vision phenotype will be reported separately, it is important to note that vision challenges extend beyond optic atrophy (OA), as the syndrome name would suggest, to also include optic nerve hypoplasia (ONH) as well as CVI. Though 41/50 individuals (82%) have OA or optic nerve pallor, 19/39 (49%) have ONH or small optic discs, and 27/40 (68%) have CVI, with many individuals exhibiting more than one of the above.

Interestingly, 21/27 individuals (78%) experience absent or decreased reflex tears (indicated as alacrima in Table 1 and Table S1), and 15/29 (52%) demonstrate manifest latent nystagmus/fusion mal-development (indicated as MLN in Table 1 and Table S1).

### 3.4 | Hypotonia and feeding difficulties

Hypotonia is present in 39/43 individuals (91%), often noticeable both in infancy and as children get older.
Feeding challenges are present in 30/43 individuals (70%) and include difficulty latching and poor suck, as well as trouble chewing and swallowing, leading some individuals to use a nasogastric tube or gastrostomy tube for all or part of their nutrition. Those who eat by mouth often exhibit mouth over-stuffing (21/25; 84%) as well as food-pocketing.

### 3.5 Music, long-term memory, high pain tolerance, and sleep disturbance

Several additional common yet previously unidentified characteristics emerged across variant types. Though these features were identified via parent report and are challenging to rigorously quantify, they nevertheless serve to further expand the phenotype. In particular, 27/27 individuals assessed have a love of music. One individual has been described as “living for music,” and several families have identified which music genre or artist their children prefer. Also of note, 19/25 individuals (76%) were reported to have unusually good long-term memory. For example, Patient 12 reportedly speaks two languages fluently and can remember dates of birth, with an IQ of 52 (Bosch et al., 2014; Chen et al., 2019). Interestingly, 21/27 patients (78%) also reported a high pain tolerance, evidenced by a lack of reaction to blood draws, vaccinations and other situations that would ordinarily provoke a response; this feature has likewise not been described in previous reports of the syndrome. Sleep difficulties, including difficulty falling and staying asleep as well as early awakening, also emerged as a newly identified feature, present in 17/28 individuals (61%) based on parent report. Six patient families reported administering medication such as melatonin, clonazepam, trazadone, and clonidine to address sleep problems.

### 3.6 Genotype–phenotype correlations

Given prior findings suggesting a more severe phenotype associated with mutations in the DBD of NR2F1, here we also assess genotype–phenotype correlations. Specifically, we compare the clinical

**FIGURE 1** Graphical representation of the location of translation initiation, missense, nonsense, and frameshift variants and in-frame deletions along the NR2F1 protein. DBD, DNA binding domain. LBD, ligand binding domain. Bolded variants correspond with patients who have not been previously reported.

**FIGURE 2** Graphical representation of the size and location of deletions encompassing NR2F1. Bolded coordinates correspond to patients who have not been previously reported.
characteristics of two groups of patients: those with missense mutations and in-frame deletions in the DBD (n = 22), and those with any other type of variant (n = 32). The latter group includes those with whole-gene deletions as well as translation initiation, nonsense, and frameshift mutations (n = 24), and those with missense mutations in the LBD (n = 5) and elsewhere outside of the DBD (n = 2). The distribution of missense, nonsense, and frameshift variants as well as in-frame deletions is depicted in Figure 1; the sizes and locations of whole-gene deletions are shown in Figure 2.

Regarding development, patients with missense variants and in-frame deletions in the DBD show a greater prevalence of inability to communicate verbally and to walk (as well as motor delay more generally). Specifically, 72% of patients with DBD variants are nonverbal, compared to 20% of individuals with variants elsewhere (p = .001). Similarly, 53% of individuals with DBD variants are unable to walk unassisted, while all 25 assessed individuals with other variants have attained this skill (p < .001). In addition, among individuals who have met each motor and language milestone, those with DBD variants tended to attain skills later than those with variants elsewhere; however, these differences did not reach statistical significance except in the case of sitting upright (mean ± SD = 19 ± 9 months versus 11 ± 5 months; p = .02). Also of clinical though not statistical significance, the DBD variant group showed a greater prevalence of DD than did the non-DBD variant group (20/20 vs. 22/27; p = .063).

The two groups also differ in seizure prevalence (74% of patients with DBD variants versus 37% of patients with other variants; p = .019). Across the cohort, seizure types include focal, absence, myoclonic, atonic, and generalized tonic–clonic seizures. Interestingly, IS with and without hypersynchrony have thus far only been reported in patients with DBD variants, further distinguishing the consequences of this genotypic group.

The difference in prevalence of abnormal brain MRI findings (76% of those with DBD variants versus 43% of those with other mutations; p = .058) did not reach statistical significance, yet it is clinically relevant. A complete characterization of findings will be reported separately, though common features include thinning of the corpus callosum, thin optic nerves and a small optic chiasm, delayed myelination, and white matter loss.

Among parent-reported behavioral characteristics, touch sensitivity also emerged as both a newly identified clinical feature and one whose prevalence differs between the two variant groups (92% for the DBD variant group vs. 33% for the non-DBD variant group; p = .0047). Among those with DBD variants, a preference for deep pressure and dislike of light touch were most often reported; aversion to the hands being touched was also frequently mentioned. Patients in this group were described as being “proprionception-seeking,” liking stimulation, and enjoying rough-housing.

4 | DISCUSSION

Here, we present 22 patients with DBD variants and 32 patients with variants elsewhere to corroborate and quantify these relationships. It was found that variants in the DBD are associated with a higher prevalence of several characteristics, namely, motor delay, the inability to walk unassisted, the absence of speech, seizures, and touch sensitivity, compared to other types of variants. Differences in phenotype of BBSOAS and further elucidate genotype-phenotype correlations. Consistent with previously reported findings, the present study confirms characteristic clinical features of hypotonia, DD, and vision impairment, along with a high prevalence of ASD or features thereof, cognitive/behavioral anomalies, feeding difficulties, seizures, and abnormal brain MRI findings. Importantly, we also expand the vision phenotype to include alacrima and manifest latent nystagmus/fusional maldevelopment, and broaden the behavioral phenotype to involve a love of music, an unusually good long-term memory, a high pain tolerance, touch sensitivity, and sleep difficulties. The enrichment of pathogenic mutations in the DBD and LBD further supports the importance of these highly conserved functional domains in NR2F1, and the consistency of clinical features across all variant types confirms the pathogenicity of these variants. At the same time, the increased prevalence of several features among the DBD variant group strengthens the association between these variants and a more severe phenotype.

4.1 | The molecular basis of Genotype-phenotype correlations

Extant literature has proposed genotype-phenotype correlations and potential variant type pathomechanisms (Bertacchi et al., 2018; Bosch et al., 2014; Chen et al., 2016; Kaiwar et al., 2017). Specifically, given that NR2F1 binds to DNA as a homodimer, that DBD variants reduce luciferase activity (indicative of transcriptional activity) beyond that of even a negative control, and that DBD variants have been associated with more severe phenotypes, a dominant-negative effect has been proposed (Chen et al., 2016). The more severe phenotype may also be due to impaired binding of homodimerized NR2F1 to crucial NR2F1 targets when a missense variant in the DBD is present.

In contrast, luciferase reporter assay data has shown that missense variants in the LBD and other regions outside of the DBD diminish but do not completely abolish transcriptional activity; these hypomorphic variants have previously been associated with comparably mild observed clinical features (Chen et al., 2016). Haploinsufficiency due to nonsense and frameshift variants as well as whole-gene deletions of NR2F1 have also been associated with more mild features (Chen et al., 2016). Additionally, among patients with translation initiation variants, although the third codon of NR2F1 (ATG) could potentially serve as an alternative start codon, fibroblast data showed decreased NR2F1 protein levels (by 60%) and NR2F1 mRNA levels (by 45%); these findings suggest alterations in both translation and transcription and support a mechanism of haploinsufficiency for these variants leading to a milder phenotype (Chen et al., 2016).

Here, we present 22 patients with DBD variants and 32 patients with variants elsewhere to corroborate and quantify these relationships. It was found that variants in the DBD are associated with a higher prevalence of several characteristics, namely, motor delay, the inability to walk unassisted, the absence of speech, seizures, and touch sensitivity, compared to other types of variants. Differences in
DD and abnormal brain MRI findings are of clinical interest though not statistically significant.

### 4.2 Developmental delay and intellectual disability

Regarding development, variants both within and outside of the DBD are associated with DD and ID. However, a significantly larger proportion of patients with DBD variants experience motor delays and have not yet achieved the ability to walk independently or communicate verbally, indicative of more severe delays among this group. Additionally, all motor and language milestones were achieved later on average by individuals with DBD variants compared to variants elsewhere, though this difference reached statistical significance only in the case of the ability to sit without support. Also of clinical though not statistical significance, the only five individuals (of 47) who do not demonstrate DD all have variants outside of the DBD.

Of note, though statistical analysis of cognitive impairment was not possible due to variability in assessment methodology, it appears that DBD variants are also associated with a greater degree of ID; all individuals with severe ID have mutations in this genotypic group. However, it is unclear the extent to which this more substantial cognitive impairment is a direct result of variant type versus a consequence of other characteristics more common in those with DBD variants, such as IS, further discussed below.

### 4.3 Autism spectrum disorder and behavior

ASD diagnoses or features are prominent among patients with both variant types, with no statistically significant difference in prevalence between groups. It is possible that severity of autism/features could distinguish the two groups, but severity was inconsistently reported and could not be assessed here. Moreover, families of some children anecdotally have pursued and advocated for an autism diagnosis even in the context of mild/borderline symptoms, given the additional support and therapies (such as applied behavior analysis) made available with a diagnosis. Future systematic unbiased assessment of the cognitive and behavioral phenotype of BBSOAS would be of benefit.

No consistent cognitive/behavioral profile emerged, though many individuals reportedly demonstrate anomalies. Given the high prevalence of ASD and its association with restricted and repetitive behaviors and interests (Watt, Wetherby, Barber, & Morgan, 2008), it is possible that these behaviors represent features of autism rather than a separate phenotypic characteristic. It is also conceivable that certain behaviors such as head-banging could be related to visual impairment, as previous studies have indicated that such behaviors among those with visual impairment could serve to obtain caregiver attention, self-soothe, and increase stimulation (Molloy & Rowe, 2011).

Regarding additional anomalies, it is notable that Patient 42 (newly reported) and Patient 44 (Hobbs et al., 2020) also experience auditory hallucinations. As Hobbs and colleagues note, although psychosis is associated with epilepsy as well as visual impairment (Clancy, Clarke, Connor, Cannon, & Cotter, 2014; Menon, Rahman, Menon, & Dutton, 2003), Patient 44’s hallucinations persisted in the absence of seizures and were more distressing than those typically reported in the context of impaired vision. While this evidence may point to a potential association between BBSOAS and psychosis, such speculation remains premature in the absence of additional assessment.

### 4.4 Vision impairment

Though the full phenotypic spectrum of visual impairment in BBSOAS will be reported separately, it is worth noting that vision challenges may include optic atrophy/optic nerve pallor, optic nerve hypoplasia/decreased optic nerve size, CVI, and/or manifest latent nystagmus (fusional maldevelopment). No differences in prevalence of these vision-related conditions were seen between genotypic groups, though differences in severity were not assessed.

It is also worth mentioning that although underdevelopment of the optic nerve combined with structural abnormalities of the brain and other developmental anomalies may initially be suggestive of septo-optic dysplasia (SOD), the features of BBSOAS are distinct. In particular, though dysgenesis/thinning of the corpus callosum is present among BBSOAS patients, the absent septum pellucidum characteristic of SOD is not seen in BBSOAS (Rush & Bajandas, 1978). Likewise, although hypopituitarism is a main feature of SOD, endocrine abnormalities are not widespread in BBSOAS; in particular, while growth hormone deficiency is the most common manifestation of hypopituitarism in SOD (Webb & Dattani, 2010), short stature is present in just 13% of those with BBSOAS.

BBSOAS is also distinguished by the strikingly common (81%) presence of alacrima (operationalized here as absent or decreased reflex tear production, assessed by parent report), representing a distinct and particularly relevant expansion of the phenotype. Though phenotype-first diagnosis of BBSOAS is unlikely and molecular confirmation via genetic testing remains important, given the small number of disorders associated with alacrima and DD, it is conceivable that alacrima could serve as a salient “clue” in constructing a differential diagnosis (Adams & Schaaf, 2018; Brodsky & Tusa, 2004).

### 4.5 Hypotonia and feeding difficulties

Hypotonia is present at a high prevalence among patients with variants both in and outside of the DBD; it is often first noticed neonatally but persists as children get older. Interestingly, prior groups have proposed potential mitochondrial involvement in the phenotype, as muscle biopsy revealed a mitochondrial complex IV deficiency in Patient 5 (Martín-Hernández et al., 2018) and a complex I deficiency in Patient 44 (Hobbs et al., 2020); however, a systematic assessment of mitochondrial function would be necessary before drawing definitive conclusions regarding a potential pathomechanism.
Patients in both genotypic groups often have feeding difficulties that arise in infancy but continue to have challenges beyond this period. Here, we present newly identified challenges of mouth over-stuffing and food pocketing, though other difficulties including poor latch/suck and trouble chewing and swallowing have been previously described (Martín-Hernández et al., 2018). Feeding difficulties may arise from oromotor dysfunction associated with defects of the glossopharyngeal nerve, which provides sensory and motor innervation to the pharynx and tongue (Qiu et al., 1997).

Such neural aberrations are seen in Nr2f1-null mice, who have impaired suckling and swallowing which often leads to starvation, dehydration, and death in the first few days of life (Qiu et al., 1997); however, it is important to bear in mind that all human BBSOAS patients have heterozygous rather than homozygous pathogenic NR2F1 variants.

4.6 | Seizures

Though seizures are present among patients in both genotypic groups, the prevalence is significantly greater among those with DBD variants. Moreover, all members of the present cohort with a history of IS have DBD variants. Typically characterized by brief flexions or extensions of the limbs, neck and trunk accompanied by distinct electroencephalography (EEG) patterns with an onset in the first year of life, IS is considered one of the “catastrophic childhood epilepsies” due to its refractoriness and association with poor neurodevelopmental outcomes (Shields, 2006); therefore, the high prevalence of IS among those with DBD variants represents a notable distinguishing consequence of these variants. However, it is important to recognize that the extent of impairment resulting from IS depends on the consequences of both the seizures themselves as well as the underlying etiology (Michaud et al., 2014), and while IS in general carries a substantial risk of morbidity and mortality, there is currently no evidence to suggest BBSOAS is associated with a significantly shortened lifespan even in the context of IS. Though it is unclear to what extent the consequences of IS may compound the effects of a DBD variant on development and cognition, and certainly early treatment response and cessation of IS is desirable, the (comparatively) good long-term prognosis has important implications for the counseling of patient families. Moreover, because multiple BBSOAS diagnoses were made through studies involving WES of patients with IS, these findings have implications for the diagnostic evaluation and prognosis of those with IS more broadly.

4.7 | Brain MRI anomalies

Generally, abnormal brain MRI findings are more common among those with DBD variants compared to those with variants elsewhere, though such anomalies are present among both groups and the difference does not reach statistical significance. A comprehensive and systematic analysis of brain MRI findings is forthcoming, but it is apparent that hypoplasia/dysplasia of the corpus callosum as well as hypoplasia of the optic nerves and optic chiasm are common, and delayed myelination is also occasionally seen. Similarly, Nr2f1-null mice show abnormalities in the development of the corpus callosum (Armentano, Filosa, Andolfi, & Studer, 2006) and delayed and reduced myelination (Yamaguchi et al., 2004), as well as altered thalamocortical connections (Zhou et al., 2011).

4.8 | Hearing loss

Interestingly, hearing loss continues to be a relatively uncommon phenotype (affecting 33% of individuals, with no statistically significant difference in prevalence between the two genotypic groups), despite clear defects in the cochlea (and vestibular system) of Nr2f1-null mice (Tang, Alger, & Pereira, 2006), as well as hearing defects in heterozygous knockout mice (F. Pereira, personal communication, April 28, 2018).

4.9 | Music, long-term memory, high pain tolerance, and sleep disturbance

A love of music, unusual long-term memory capabilities, a high pain tolerance, sensitivity to touch, and sleep difficulties emerged as newly identified characteristics of BBSOAS, though these features were not statistically significant difference in prevalence between the two genotypic groups). While enjoyment of music, good long-term memory, elevated pain tolerance, and sleep challenges are experienced by a large percentage of both genotypic groups, the prevalence of touch sensitivity is significantly higher among those with DBD variants compared to those with other variants.

Though the etiology of these characteristics in the context of BBSOAS remains to be determined, several have previously been reported to be associated with ASD (Allen, Hill, & Heaton, 2009; Grandin, 1992; Williams, Sears, & Allard, 2004). In particular, sensory perception abnormalities are present in 69–95% of individuals with ASD, and a study comparing pain sensitivity between those with ASD and controls found that those with ASD were hypo-sensitive to subjective pain intensity, but did not differ in pain detection threshold or pain tolerance compared to controls (Allely, 2013, Yasuda et al., 2016). Also of note, the prevalence of sleep problems among children with ASD is 40–80%, compared to 25–40% among typically developing children (Cohen, Conduit, Lockley, Rajaratnam, & Cornish, 2014).

Regarding a potential etiology of the memory findings, this phenotype recapitulated in an Nr2f1 heterozygous mouse model, which demonstrated altered fear memory extinction (Chen et al., 2019). An investigation of possible mechanisms revealed impaired hippocampal synaptic plasticity, evidenced by deficits in hippocampal long-term potentiation and long-term depression (Chen et al., 2019). The mice also showed a smaller hippocampal volume (Chen et al., 2019).
CONCLUSION

In summary, here we corroborate previously described characteristics of BBSOAS including DD encompassing a nonverbal phenotype and an inability to walk, ID, ASD, and features thereof, cognitive/behavioral anomalies, vision impairment including OA, ONH, and CVI, hypotonia, feeding difficulties, an abnormal brain MRI, and seizures. We also further expand the vision phenotype to include alacrima and manifest latent nystagmus/fusion maldevelopment. In addition, we broaden the behavioral phenotypic spectrum to encompass an unusually good long-term memory, a high pain tolerance, a love of music, sleep difficulties, and touch sensitivity. We provide further evidence for genotype–phenotype correlations, specifically supporting a more severe phenotype associated with DBD variants. Continued analyses are needed to further elucidate and confirm this relationship. Finally, we continue to show an enrichment of pathogenic mutations in the DBD and LBD, further emphasizing the importance of these highly conserved functional domains in NR2F1.

As the BBSOAS community continues to grow, families are connecting with one another through social media (https://www.facebook.com/groups/NR2F1/). The NR2F1 Foundation (https://nr2f1.org/) has also recently been established to support and empower those who have newly received a diagnosis.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Megan E. Rech enrolled patients, collected and analyzed data, and wrote the manuscript. John M. McCarthy enrolled patients and collected data. Chun-An Chen wrote parts of the manuscript. Jane C. Edmond, Veeral S. Shah, Daniëlle G. M. Bosch, Gerard T. Berry, Linford Williams, Dmitriy Niyazov, and Charles Shaw-Smith evaluated patients and provided clinical data. Erin M. Kovar and Philip J. Lupo performed statistical analyses. Christian P. Schaaf conceptualized and planned the study, evaluated patients and provided clinical data, supervised the analysis, and edited the manuscript.

DATA AVAILABILITY STATEMENT

Each individual patient’s phenotypic and developmental data are provided in Supporting Information Table S1 and S2, respectively.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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