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Phenotypic characterization of seven individuals with Marbach–Schaaf neurodevelopmental syndrome

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Abstract

We present the phenotypes of seven previously unreported patients with Marbach–Schaaf neurodevelopmental syndrome, all carrying the same recurrent heterozygous missense variant c.1003C>T (p.Arg335Trp) in *PRKAR1B*. Clinical features of this cohort include global developmental delay and reduced sensitivity to pain, as well as behavioral anomalies. Only one of the seven patients reported here was formally diagnosed with autism spectrum disorder (ASD), while ASD-like features were described in others, overall indicating a lower prevalence of ASD in Marbach–Schaaf neurodevelopmental syndrome than previously assumed. The clinical spectrum of the current cohort is similar to that reported in the initial publication, delineating a complex developmental disorder with behavioral and neurologic features. *PRKAR1B* encodes the regulatory subunit R1β of the protein kinase A complex (PKA), and is

expressed in the adult and embryonal central nervous system in humans. PKA is crucial to a plethora of cellular signaling pathways, and its composition of different regulatory and catalytic subunits is cell-type specific. We discuss potential molecular disease mechanisms underlying the patients' phenotypes with respect to the different known functions of PKA in neurons, and the phenotypes of existing R1β-deficient animal models.

KEYWORDS
autism spectrum disorder, global developmental delay, neurodevelopmental disorder, pain insensitivity, *PRKAR1B*, protein kinase a complex

1 | INTRODUCTION

The genetic landscape of intellectual disability (ID) is diverse and still expanding, reflecting the complex developmental biology of the human central nervous system (CNS). Well over 1500 syndromic and nonsyndromic genetic disorders (with an additional >1250 candidate genes) with ID have been identified as of November 2021 (SysID database; Kochinke et al., 2016). The identification of new candidate genes is largely driven by the broad application of exome/genome sequencing in clinical practice. In 2021, our group characterized a neurodevelopmental disorder (subsequently named Marbach–Schaaf neurodevelopmental syndrome/MASNS, # 619680 by OMIM), reporting de novo missense variants in *PRKAR1B* in six patients, who were mostly recruited from a large cohort of patient/parent trio-exomes.

PRKAR1B encodes the R1β subunit of the protein kinase A (PKA) complex, a heterotetramer of two regulatory and two catalytic subunits. As a principal mediator of cyclic adenosine monophosphate (cAMP)-mediated downstream signaling, PKA is vital for a wide variety of signaling pathways in metazoan cells. While PKA complexes are present in all human tissues, the respective composition of PKA varies across cell types, depending on the expression of different subunits. Regulatory subunits mediate the localization of PKA to a cellular compartment by binding A-kinase-anchoring proteins (AKAPs; Pidoux & Tasken, 2010; Taylor, Keshwani et al., 2012b), and PKA kinase activity by releasing catalytic subunits in the presence of cAMP (Soberg & Skalhegg, 2018; Taylor, Ilouz et al., 2012a). The R1β subunit is highly expressed in the central nervous system (CNS), and expression during embryonal development of the human brain could be demonstrated by RNAscope imaging at Carnergie stage 22 (estimated postfertilization age of 52–55 days). Functional studies found reduced basal PKA kinase activity in HEK293 cells transfected with *PRKAR1B* constructs harboring variants identified in patients from the initial publication (Marbach et al., 2021).

In the initial six patients, symptoms of MASNS included global developmental delay (GDD) and apraxia or clumsiness, without consistent facial dysmorphisms or organ malformations. In the case of patients carrying the recurring NM_001164760.2: c.1003C>T p.(Arg335Trp) variant, insensitivity to pain of varying degree was reported. The patients showed behavioral abnormalities associated with autism spectrum disorder (ASD), such as arm/hand flapping, repetitive, and sensory-seeking behavior (Marbach et al., 2021). ASD was diagnosed based on DSM-5 criteria in all of the patients of that initial cohort, and four patients were diagnosed with attention deficit hyperactivity disorder (ADHD).

Here, we report seven additional patients with MASNS, all carrying the *PRKAR1B* c.1003C>T variant, who were reported to our group since the initial publication.

2 | METHODS

2.1 | Ethics and data collection

Data collection was performed as part of human research study H-34578 (understanding the molecular causes of neuropsychiatric disease), which was approved by the Baylor College of Medicine Institutional Review Board (IRB). Written informed consent for publication of medical data and images was obtained from the parents or legal guardians of the respective individuals as required by the IRB. Initial contact with our group was usually was established by a patient's physician, who received the consent form and a comprehensive questionnaire (see Supporting Information Data). The physicians informed the patient's caretakers of the study and collected all clinical data presented in this publication.

2.2 | Molecular and cytogenetic diagnostics, segregation

Single exome analyses were performed on different platforms in patients #1, #3, and #5, while single genome analysis was performed in patient #4. De novo status of the respective *PRKAR1B* variants was determined by sanger sequencing of patient and parental samples. Trio-exome sequencing was performed in in the case of patient #2, #6, and #7.

Prior to exome or genome sequencing, patients #2–7 received chromosomal microarray analysis (CMA), and chromosome analysis was performed in patients #4 and #7. Additionally, molecular genetic testing for Myotonic dystrophy, Spinal muscular atrophy, and Prader–Willi syndrome was performed in patient #5 due to muscular

TABLE 1 Developmental milestones and the neurologic phenotypes

Individual #	1	2	3	4	5	6	7
Sex	M	F	M	F	M	F	F
Age	3 years 1 months	5 years 8 months	15 years 2 months	9 years 1 month	6 years 6 months	3 years 2 months	16 years 7 months
PRKAR1B Variant (NM_001164760) and mode of inheritance	c.1003C>T, p.(Arg335Trp) de novo	c.1003C>T, p.(Arg335Trp) de novo	c.1003C>T, p.(Arg335Trp) unknown[a]	c.1003C>T, p.(Arg335Trp) de novo	c.1003C>T, p.(Arg335Trp) de novo	c.1003C>T, p.(Arg335Trp) de novo	c.1003C>T, p.(Arg335Trp) de novo
Neonatal period							
Hypotonia	No	No	No	Yes (mild)	No	Yes	No
Feeding difficulties	No	No	No	Poor suck (initially)	No	Poor suck (mild)	No
Other abnormalities	No	No	No	No	No	No	No
Developmental milestones							
Sitting without support	1 year 6 months	11 months	5 months	1 year 2 months	1 year 3 months	10 months	<9 months
Crawling	21 months	Patient did not crawl	12 months	Patient did not crawl	Patient did not crawl	13 months	n.s.
Walking	4 years	1 year 8 months	1 year 8 months	2 years	2 years 2 months	19 months	14 months
Motor skill regression?	Yes (noted at age 6 m)	No	No	No	No	No	No
Age of 1st words (months)	1 year 6 months	None	2 years	1 year	2 years	2 years 5 months	>3 years
Age of combining words	No	No	5 years	4 years	4 years	>2 years 5 months	4 years
Fluent language	No	No	No	Yes	3-word sentences, not fluent	4–5 word sentences, not fluent	n.s.
Language regression?	Yes (noted at age 6 months)	n.a.	No	No	No	No	No
Neurologic examination							
Pain tolerance	High	High	Normal	Rather high (subjective observation by parents)	High	High	Normal
Somatosensory system	Hypersensitivity[b]	Normal	Normal	n.s.	Normal	n.s.	Normal
Temperature perception	Normal	Normal	Normal	n.s.	Normal	Normal	Normal
Gross motor skills	Delayed, only crawling	Normal	Normal	Delayed	Delayed	Delayed	Normal
Fine motor skills	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed
Dyspraxia/Apraxia	No	No	Yes	No	No, but notable clumsiness	No	Yes

(Continues)

TABLE 1 (Continued)

Individual #	1	2	3	4	5	6	7
Hypotonia or otherwise abnormal muscular strength	Yes	No	No	No	Yes	No, but low muscle strength during the first 2 years.	No
Other abnormalities	No	No	No	Stereotypic movements of hands and feet	No	Mildly ataxic, wide-based gait	No
History of seizures	No	No	No	No	No	No	No
Hearing impairment	No	No	No	No	No	No	No
Vision impairment	Myopia −0.5 dpt Strabismus (left eye)	No	No	Strabismus	No	No	No
Sleep abnormalities	No	No	Abnormal circadian rhythm	Mild sleep apnea	Yes, treated with melatonin	Difficulties initiating sleep, possible sleep apnea (not tested)	No
Autism							
Any formal testing (ADOS, ADRI)	ADOS	No	n.s.	No	No	No	No
Meets diagnostic criteria of autism	Yes	No	n.s.	n.s.	No	No	No
Autistic features (without formal testing)	No	Stereotypic movement, flapping when happy, prefers to be alone	n.s.	Stereotypes, inflexibility to routine changes, social withdrawal	No	No	No
ADHD							
Attention deficit	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hyperactivity	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Diagnosed ADHD	No	No	n.s.	Yes	No	No	No
Cognitive evaluation							
Formal cognitive evaluation (IQ or DQs)	No	Brunet-Lézine R at 3 years 6 months: QIT 35 (motricity 20m, QD: 47.8; ocular coordination 13 months 18 days; QD: 32; language, 11 months, QD: 26.3; Sociability 15 months 5 days, QD: 37)	DD/moderate ID	K-ABC-II, SON-R 2½–7 (nonverbal intelligence evaluation)—moderate intellectual disability (IQ 49), speech and motor delay	Vineland-3 showed weakness in all domains	No	No
Special education?	Yes	Yes	Yes	Yes	Yes	Yes	Yes

TABLE 1 (Continued)

Individual #	1	2	3	4	5	6	7
Laboratory anomalies, other genetic test results							
	No	No	CMA: Duplication of 431.5 kb within 12q13.12 (49488125–49919743) (hg19), maternally inherited. The variant was classified as VUS.	Elevated CK, TruSight One (Illumina) multi-gene panel analysis: 2 heterozygous pathogenic *CLCN1* variants in *cis*, likely contributing to CK anomalies. Likely pathogenic *FOXH1* variant, no cardiac anomalies.	No	CMA: One duplication of at least 794 kb within 5q35.3, and another duplication of at least 118 kb within 8p23.3. Both variants were classified as VUS.	No

Abbreviations: n.s., not specified; n.a., not applicable.

[a]The mother was tested negative; the father is deceased.

[b]The patient shows discomfort while nail clipping, when his head is touched, and with some fabrics of clothes.

hypotonia. Structural anomalies and additional sequence variants identified during diagnostic workup of patients #1–7 are listed in Table 1.

3 | RESULTS

3.1 | Patient cohort

We received clinical information of a total of seven patients (three males and four females), who were then included in our study. De novo status of the c.1003C>T variant could be established in all but one individual (patient #3, for whom one parent was deceased and the other was tested negative). All patients were delivered at term after uneventful pregnancies, with normal anthropometric measurements. The neonatal period was mostly uncomplicated, although muscular hypotonia and poor suck were noted in patients #4 and #6. The average age at the time of data collection was 8.5 years (SD 60 months), with an age range of 3–16 years. Two patients were taller than their peers (patients #1 and #6), while the others were within the normal height range at the time of last measurement. The body mass index of two patients (#2 and #5) was at or above the 97th percentile, and a lack of satiety was reported in patient #5. The occipitofrontal circumference of patient #4 was at the 1st percentile. A compilation of the patient's anthropometric data is shown in Table S1.

3.2 | Physical appearance

As in the first cohort of patients reported in 2021, the individuals of this study did not show a consistent pattern of physical malformations or facial dysmorphisms. A short or upturned nose was noted in four, and a short philtrum was seen in four patients. Four patients had thin upper lips or small lips in general, and two had (subtle) epicanthal folds. No congenital organ malformations were reported. Patient #3 had developed scoliosis and patient #4 had pes adductus. A compilation of patient images is shown in Figure 1.

3.3 | Developmental milestones

Language and motor development were generally delayed: Free sitting was achieved at an average age of 11.7 months (*SD* 4 months), and independent walking at an average age of 24.4 months (*SD* 10.3 months). Among the six verbal patients, first words were spoken at an average age of 23.8 months (SD 7.6 months), and patient #4 and #7 acquired fluent speech. Regression of motor skills was observed in one male patient (patient #1) beginning at the age of 6 months, which may resemble earlier reports of two male patients of the first cohort who lost previously acquired skills (Marbach et al., 2021). Among the two adolescent patients #3 and #7, the onset of puberty was delayed in patient #3 and normal in patient #7.

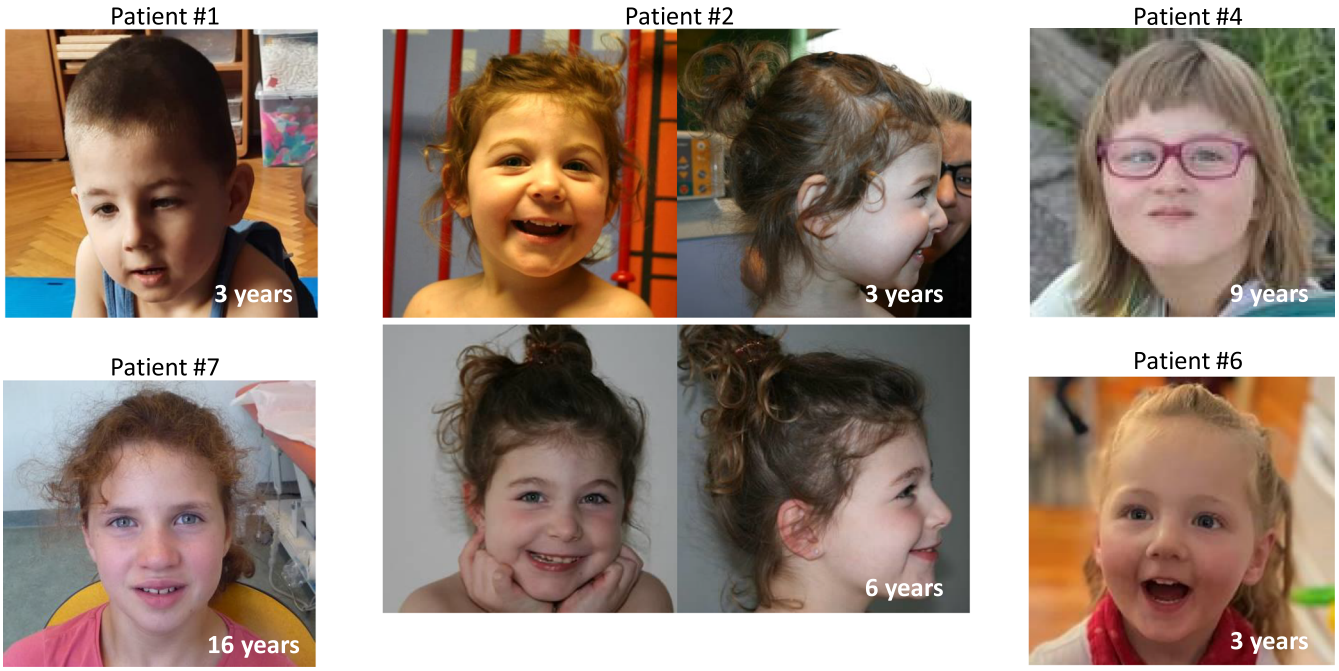

FIGURE 1 Facial phenotypes of patients #1, #2, #4, #6, and #7. The patient's age at the time of the photograph is indicated in the respective picture

3.4 | Neurologic phenotype

All patients showed impaired coordination of physical movements to some extent: gross motor skills were delayed in four of the seven patients and all patients showed delayed development of fine motor skills, such as joining puzzle pieces or drawing. However, dyspraxia was diagnosed in only one patient. Hearing and vision were mostly normal, albeit the diagnoses of myopia and strabismus in one, and strabismus in another patient. None of the patients had experienced seizures.

A distinctive feature of individuals carrying the c.1003C>T variant reported in the first publication was high pain tolerance, which was noted to a variable extent in all but one patient. Patient #1 did not react when hitting himself during activities at home, and grasped hot beverages without expression of discomfort. Patient #4 did not respond with pain when blood was drawn, or when she had blisters or small injuries, and instead showed distress when experiencing minor pressure such as handholding or hair combing, occasionally—despite the unpainful situation—responding with "ouch." Patient #6 was described by her parents as almost unresponsive to pain during the first 18 months of life. She slowly acquired an appropriate pain response from the age of 18 months onwards, with pain tolerance remaining subjectively high. It should be noted that no formal evaluation of pain sensitivity was performed, meaning that these reports reflect subjective observations of the patient's parents and/or physicians.

While most patients showed higher-than-normal pain tolerance, temperature perception was reported as normal in most patients. Magnetic resonance imaging of the head revealed structurally normal brains in most patients, although cisternomegaly was noted in patient #5, and white matter anomalies of the periventricular region were seen in patient #6 shortly after birth. Electroencephalograms, if performed, were normal or inconclusive. Formal cognitive evaluation was performed in four patients, which, depending on the type of test, revealed below average performance in most tested domains, or moderate ID (patient #3). All patients required some form of special education. A compilation of developmental milestones and the neurologic phenotypes is shown in Table 1.

3.5 | Behavioral profile

ASD was diagnosed using the Autism Diagnostic Observation Schedule 2 (ADOS-2) only in patient #1 of the current cohort, and none of the other patients had received formal testing for ASD. Symptoms associated with ASD, such as stereotypic movements and hand flapping were reported in two additional patients. Formal evaluation of ASD was pending in the case of patient #2, and autism was suspected by the parents of patient #4, although her stereotypies and inflexibility to routine changes were classified in the context of ID rather than ASD by the child's psychologist. While displaying features of ASD, patient #2 was also described as social and affectionate, and as investing strongly in interpersonal relations. Patient #6 was also described as social and interactive. All patients displayed hyperactivity and restlessness, which was reported as challenging for parents and caregivers, and patient #4 was formally diagnosed with ADHD. Awareness of danger was reduced in most patients. Heightened anxiety was reported in four patients and social withdrawal in three. Table 2 summarizes the behavioral profile of individuals #1–7.

TABLE 2 Behavioral profile

Trait (1 = not present; 2 = rarely present, 3 = sometimes present, 4 = frequently present, 5 = always present)							
Individual #	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Hyperactivity	5	5	5	5	5	5	<5 (decreased with age)
Underactivity	1	1	2	1	2	1	1
Stubbornness	4	4	5	3 (5 without medication)	2	4	2 (decreased with age)
Temper tantrums	1	3	5	4	4	3	1
Aggression	1	1	2	4	3	4	1
Controlling and manipulative behavior	3	1	3	1	2	4	1
Compulsivity	1	3	2	4	4	5	1
Anxiety	1	2	4	4	4	3	1
Social withdrawal	3	1	4	4	3	1	1
Difficulty with change in routine	1	3	4	5	4	4	1
Absent awareness of danger	5	5	3	5	5	3	2 (decreased with age)
Self-harm	1	1	1	1	5	2	1
Other traits	Cheerful, smiling, always happy.	Really social child, invests strongly in relations, very affectionate and sensitive			Smiling, cooperative, hyperactive	Bangs head when falling asleep	Echolalia

4 | DISCUSSION

Including the six individuals published in the original article in April 2021, we have collected clinical information of a total of 13 patients carrying missense variants in *PRKAR1B*. The variants (NM_001164760.2) c.586G>A (p.Glu196Lys) and c.500_501inv (p.Gln167Leu) were each reported once, while the variant c.1003C>T (p.Arg335Trp) was found a total of 11 individuals. All patients within the combined cohorts show GDD with speech delay. Five (38%) patients have been diagnosed with ADHD, and seven (54%) have been formally diagnosed with ASD. Muscular hypotonia was noted in 6 (46%) patients, and 9 of the 11 patients (82%) with the c.1003C>T variant (69% of all patients) have abnormally high pain tolerance. An overview of all individuals with de novo *PRKAR1B* variants can be found in Table S2.

A notable difference between the first cohort of North American patients, and the second cohort of predominantly European patients, is the low frequency of formally diagnosed ASD in the latter. The disparity between both cohorts might be coincidental and explained by a higher variability of ASD among individuals with MASNS than the initial study suggested. On the other hand, only one of the European patients had received formal psychological assessment of ASD. The disparity might therefore also represent a lower clinical threshold to apply DSM V criteria in the United States, and a shift in the diagnostic categorization of patients with ID and ASD in favor of ASD (Polyak et al., 2015). More uniform assessment criteria for ASD exist in the United States in the form of DSM V, when compared to historically different assessment procedures in European countries, which may contribute to varying prevalence estimates for ASD across Europe (Chiarotti & Venerosi, 2020; Garcia-Primo et al., 2014). While ASD-associated features such as motor stereotypies are frequent among patients with MASNS, the generally social and contact-seeking behavior of some patients suggest that ASD should be regarded as one of the more variable features of MASNS, regardless of possible biases in assessment.

Rare germline variants of *PRKAR1B* have previously been observed in individuals with adrenal abnormalities: the variant c.200C>T, p.(A67V) was found in a patient with adrenal lesions and adrenocorticotropic hormone-independent Cushing syndrome, who was also diagnosed with Beckwith–Wiedemann syndrome, and the variant c.898G>A, p.(A300T) was found in a patient with Cushing syndrome, micronodular adrenocortical disease, and liver focal nodular hyperplasia (Drougat et al., 2020). Both patients became symptomatic in childhood and underwent bilateral adrenalectomy at the age of 8 months and 9 years, respectively. Additionally, variants of *PRKAR1A*, which encodes the regulatory PKA subunit R1α, have been implicated in carney complex (# 160980; Kirschner et al., 2000), and primary pigmented nodular adrenocortical disease (# 610489; Groussin et al., 2002), where both disorders showed autosomal-dominant inheritance. None of the 13 MASNS patients has a clinical history of Cushing syndrome, adrenal tumors, or any other tumors. However, the patients are quite young (aged 3–16 years, with an average age of ~8.5 years), meaning that symptoms of adrenocortical disease could yet manifest. We therefore recommend a low clinical threshold for assessment by an endocrinologist and abdominal ultrasound in case of potential symptoms of adrenal disease.

It should be noted that the phenotypes of R1β-deficient (biallelic knockout) mice and rats seem to approximate some aspects of the phenotype of patients carrying monoallelic *PRKAR1B* missense-variants, such as deficits in spatial learning and memory formation in rats, and abnormal nociception in both species (Hoang Trung et al., 2021; Malmberg et al., 1997). As previously demonstrated in mice (Brandon et al., 1995), impaired long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse was also observed in hippocampal slices of *PRKAR1B*-deficient rats. *PRKAR1B*-deficient animals also displayed longer latency to react when exposed to a painful stimulus (hot plate test), had impaired fear conditioning, and showed increased durations of vertical activity (i.e., exploratory behavior) and time spent in the center in an open field test, when compared to wild-type rats. The authors hypothesized that some of the behavioral abnormalities were linked to the deficits in LTP at the Schaffer collateral pathway, resulting in impaired spatial learning and contextual conditioning (Hoang Trung et al., 2021). These observations would be in line with reduced sensitivity to pain and GDD in patients with MASNS—although the underlying genetic changes differ (absence of functional R1β in the animal model vs. co-occurrence of wild type and mutant R1β in humans).

The majority of patients with MASNS and all individuals reported in this study carry the recurring c.1003C>T missense variant. On the molecular level, the variant leads to the substitution of an arginine residue by a tryptophan residue within one of the two cAMP-binding-domains of R1β (Figure S1) (Ilouz et al., 2012), which might impair the cAMP-sensing property of the mutant protein. If this holds true, PKA complexes containing mutant R1β would be less sensitive to rising cAMP concentrations, affecting cellular signaling downstream of PKA in *PRKAR1B*-expressing cells of the CNS. This theory would be consistent with the observation of reduced cAMP-stimulated (total) PKA activity in HEK293 cells transfected with a *PRKAR1B* p.Arg335Trp expression construct, compared to cells transfected with the wild type *PRKAR1B* construct, although this reduction was not statistically significant ($p = 0.06$; Marbach et al., 2021).

Possible disturbances of several PKA-dependent functions could contribute to the reduced sensitivity to pain in most patients carrying the p.(Arg335Trp) variant, although it is currently unknown whether these pathways obligatory require the R1β subunit. For example, Gs alpha subunit ($G\alpha_s$) signaling via adenylyl cyclase/cAMP/PKA in response to pain, itch, and inflammation could be impaired, resulting in reduced sensitization of transient receptor potential channels (TRP) and reduced excitability of primary sensory nerves (Geppetti et al., 2015). PKA is also involved in spinal cord central sensitization in response to repeated nociceptive stimuli through phosphorylation of NMDA (N-methyl-D-aspartic acid) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, recruitment of AMPAR to the cell membrane, and persistent strengthening of synapses by

phosphorylation of the transcription factor CREB (cAMP response element-binding protein; Basbaum et al., 2009; Latremoliere & Woolf, 2009). *PRKAR1B* mRNA expression has been detected in human dorsal root ganglia (Hall et al., 2022), and R1β protein expression has been demonstrated in the spinal cord of mice (Cadd & McKnight, 1989). While molecular and cellular disease mechanisms of MASNS remain to be elucidated, the Arg 335 residue is emerging as a "hot-spot" for pathogenic missense variant(s) in the *PRKAR1B* gene.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SysID Database. Retrieved from https://www.sysid.dbmr.unibe.ch/.

AlphaFold Protein Structure Database. Retrieved from https://alphafold.ebi.ac.uk/.

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