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KBG Syndrome: Review of the Literature

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KBG syndrome (KBGS) is a multisystem disorder characterized by short stature, distinctive facial features including macrodontia of upper central permanent incisors, and developmental/cognitive delay. It is caused by variants or deletion of Ankyrin Repeat Domain 11 (*ANKRD11*) located in chromosome 16q24.3. Since its initial report in 1975, KBG syndrome has been recognized as an exceedingly rare disorder. However, recent advancements in genetic diagnostic techniques have led to an increase in both the diagnosis rate and the number of reported cases, contributing to a rapid increase in its global prevalence. We review the clinical aspects of KBGS, including previously reported and newly reported cases, as well as the related genetic patterns discovered so far.

Key words: KBG syndrome, ANKRD11, Ankyrin Repeat Domain 11, Chromosome 16

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INTRODUCTION

KBG syndrome (KBGS) (OMIM#148050) is a multisystem disorder characterized by short stature with growth retardation, distinctive facial features including macrodontia of upper central permanent incisors, and developmental/cognitive delay [1,2]. KBGS arises from variants or deletions, in Ankyrin Repeat Domain 11 (*ANKRD11*), which is situated on chromosome 16q24.3. *ANKRD11* is categorized as one of the ankyrin repeat-containing co-factors [2-4]. Since 1975, when the first KBGS patient was reported, more than 300 cases have been reported [1,3]. Until recent years, KBGS was widely recognized as an exceptionally rare genetic disease. However, with the advancement of genetic diagnostic techniques, there has been a notable escalation in both the diagnosis rate and the number of reported cases, thereby contributing to a rapid increase in its global prevalence. Here, we will review the previously reported and newly reported clinical aspects of KBGS and the related genetic patterns revealed so far are reviewed.

CLINICAL ASPECTS

Although the correlation of genotype-phenotype has not been clarified, it has been suggested that the *ANKRD11* variant may be associated with a wider spectrum of clinical features (Table 1).

Major features (over 50% in patients)

In more than 90% of individuals with KBG syndrome, there are documented reports of a varying level of intellectual disability (ID) and developmental delay (DD), particularly being prominent features [5,6]. Cognitive skills vary in individuals during childhood, with most experiencing developmental delays, especially

Table 1. Clinical features of KBGS

Major features (≥50%)	
Intellectual disability and developmental delay	>90%
Macrodontia of upper central incisors	>60%
Distinctive craniofacial findings[a]	>60%
Post natal short stature	>50%
Variable skeletal anomalies[b]	>75%
EEG abnormalities	=50%
Minor features (<50%)	
Cryptorchidism in males	
Hearing loss	
Feeding problems, palatal abnormalities	
Brain malformations	
Behavior problems	
Cardiac defect	

[a]Broad triangular face, short neck, synophrys, hypertelorism, prominent ears or dysplastic helices, bulbous nasal tip, prominent nasal bridge, long and smooth philtrum, and thin upper lip.
[b]Costovertebral abnormalities, large anterior fontanelle with delayed closure, short and webbed neck, abnormal ribs, brachydactyly, clinodactyly, syndactyly of toes, kyphosis, scoliosis, hip dysplasia or Perthes disease, sternum abnormalities, wormian bones in the skull, clavicular pseudoarthrosis, and osteopenia.

in speech. During adulthood, the intelligence of individuals with KBG syndrome spans from moderate impairment to typical levels, with the majority experiencing a mild intellectual disability. While some achieve independence, many require support in daily tasks [1,6-8]. Over 60% of KBGS patients commonly show macrodontia of upper central permanent incisors, distinctive craniofacial findings such as a broad triangular face, short neck, often with synophrys, hypertelorism, prominent ears or dysplastic helices, bulbous nasal tip, prominent nasal bridge, long and smooth philtrum, and thin upper lip [1,6]. Despite presenting with normal growth parameters at birth, over 50% of KBGS patients experience postnatal growth stunting, leading to short stature [1]. Variable skeletal anomalies are common in affected individuals, occurring in 75% of cases. The observed anomalies encompass a wide range of features, such as costovertebral abnormalities, delayed closure of a large anterior fontanelle, a neck that is both short and webbed, abnormal ribs, brachydactyly, clinodactyly, syndactyly of the toes, kyphosis, scoliosis, hip dysplasia or Perthes disease, abnormalities of the sternum, the presence of wormian bones in the skull, clavicular pseudoarthrosis, and osteopenia [1,6,9,10]. Approximately 50% of affected individuals with KBGS exhibit EEG abnormalities, with or without seizures. Seizures may commence anywhere between infancy and adolescence, displaying diverse types of epilepsy. Although tonic-clonic seizures are the most common, there is no exclusive association of a specific epilepsy type with KBGS [1,9].

Minor features (under 50% in patients)

Other known features are cryptorchidism in males, hearing loss, feeding problems, palatal abnormalities, brain malformations, behavior problems such as attention deficit hyperactivity disorder, autism spectrum disorder, anxiety and shyness and cardiac defect [1,6,7].

Possible new clinical features

In a recent case report, the prevalence of strabismus among enrolled 43 participants with KBGS was found to be 23.3%. Additionally, astigmatism, myopia, and hyperopia were reported in 27.9%, 16.3%, and 20.9% of the participants, respectively [11]. In addition, in recent case reports, congenital vaginal agenesis combined with cervical aplasia was reported in a 12-year-old female patient with KBGS, and a patient diagnosed with congenital aganglionic megacolon was also reported [12,13]. Also, in a few case reports, central precocious puberty in KBGS was reported [7,14,15]. In another recent study, in the constitutive *ANKRD11* gene knockout mice, abnormal embryo development and pre-weaning lethality were reported [16]. Ola et al. conducted a study on 42 patients with KBGS, finding higher rates of miscarriage, C-section, premature birth, small gestational age, and NICU admission [16,17].

DIAGNOSTIC METHODS

Genetic diagnostic methods employed for establishing a diagnosis of KBGS encompass targeted sequencing of the *ANKRD11* gene, whole exome sequencing (WES), gene panel testing, and whole genome sequencing. In some cases, chromosomal microarray analysis or comparative genomic hybridization can be done to detect microdeletion of 16q24.3. However, based on numerous case reports and related studies, the diagnosis of KBGS was typically not made through a targeted genetic test specifically for the *ANKRD11* gene variant. Instead, patients presenting with ID or DD underwent comprehensive genetic testing, such as WES or gene panel testing, when there were clinical manifestations of suspected genetic disorders. In many cases, these tests revealed variant in the *ANKRD11* gene, thereby confirming the diagnosis of KBGS. Although there is a lack of universally accepted clinical diagnostic criteria for KBGS, various authors have proposed clinical criteria that can be used for diagnosis. Based on previous studies, if an individual pres-

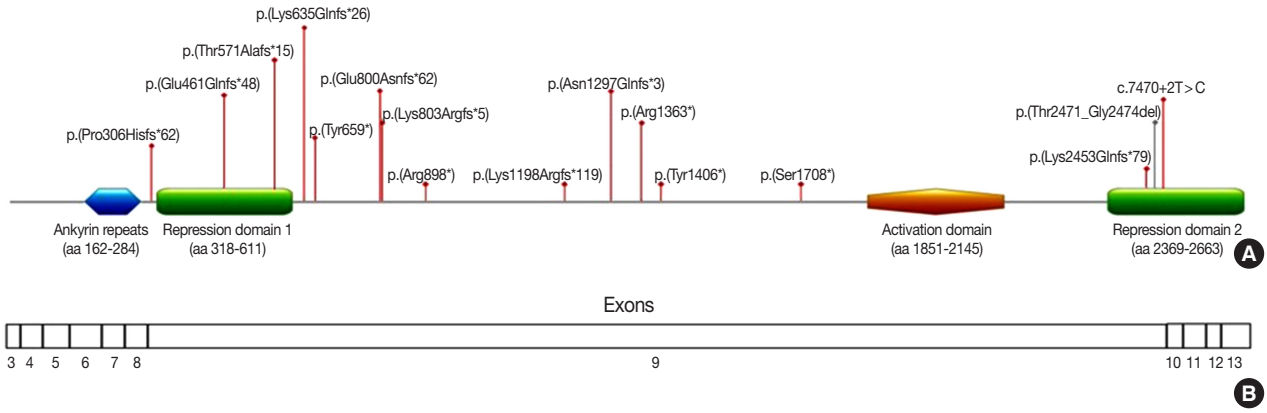


Fig. 1. ANKRD11 variants at the protein and DNA levels. (A) Diagram showing ANKRD11 protein domains and variant positions. Ankyrin repeats (blue), repression domains (green), and activation domain (orange). Loss-of-function mutations (red) and in-frame deletion (gray). (B) Diagram of ANKRD11 exons coding sequence. Exons 6-8 for ankyrin domain, exon 9 for activation and first repression domains, and exons 9-13 for second repression domain. Copy right all reserved at Clinical Genetics, Volume: 100, Issue: 2, Pages: 187-200, First published: 05 May 2021, DOI: (10.1111/cge.13977).

ents with ID or DD along with macrodontia of the upper central incisors or the characteristic facial features mentioned above and postnatal short stature, diagnosing KBGS should be considered [1,3,6].

GENETIC ASPECTS

ANKRD11 gene

ANKRD11 located in 16q24.3, the pathogenic gene responsible for the main phenotype of KBGS, encodes crucial chromatin co-regulator proteins which control histone acetylation and gene expression during neural development (Fig. 1) [3,5]. It functions as chromatin remodelers by engaging with distinct transcriptional repressors or activators situated at both the N- and C-terminals [3,5]. Asli et al. provided evidence showing that *ANKRD11* primarily localizes to neuronal nuclei and is involved in modulating neural plasticity [18]. Their investigation revealed that the N-terminal domain is implicated in protein interactions and homodimer synthesis, while the C-terminal domain plays a crucial role in facilitating the degradation of the *ANKRD11* protein [3,17]. The exact role of the *ANKRD11* in cellular processes and development is not fully understood, but it is believed to play important roles in gene regulation, chromatin remodeling, and protein-protein interactions. In this aspect, Cornelia de Lange syndrome (CdLS; OMIM#122470), Coffin-Siris syndrome (CSS; OMIM#135900), CHOP syndrome (CHOPS; OMIM#616368), and KBGS share certain facial similarities including ID and DD, short stature, and typical facial dysmorphisms due to underlying genetic factors and molecular pathways involved in the developmental process [5,14].

Classification of gene variants

Over 75% of variants in the *ANKRD11* causing KBGS are frameshift and nonsense variants [3]. The majority of variants are of *de novo* origin, with approximately 30% being inherited [19]. Also, deletions of 16q24.3 or parts of *ANKRD11* have been frequently reported [5]. In contrast, reports of missense variant have been relatively infrequent [3,5]. In previous studies, variants such as frame shift and nonsense variant tend to be associated with more severe phenotypic presentations of KBGS [1,3-5,7]. Specifically, it is known that a group having a nonsense or a frameshift variant shows more DD, ID or learning difficulties than a group having a missense variant [3]. However, interestingly, in a study conducted on 29 patients with missense variants in the *ANKRD11* gene, it was found that the clinical characteristics of patients with missense variants were not significantly different from the typical clinical features observed in patients with nonsense or frameshift variants. They suggested that it was associated with disrupted transrepression capacity and reduced protein stability, and that these missense variants had similar *ANKRD11* haploinsufficiency, which has been known as the loss-of-function, as the *ANKRD11* gene that causes KBGS [20]. However, the available literature on missense variants is currently limited, with a scarcity of functional and laboratory-based studies conducted on missense variant.

CONCLUSION

This review focused on the clinical and genetic characteristics of rare KBGS. Since the phenotype of KBGS is variable and non-specific even though in same family, clinical diagnosis of

KBGS is not easy and could be missed [5]. Moreover, it might be difficult to suspect KBGS based only on the clinical features or clinical findings and proceed with the target gene test since some syndromes share clinical features including ID and DD, short stature, and typical facial dysmorphisms [5,13]. However, early diagnosis and timely management of KBGS could be crucial to improve its prognosis. Thus, genetic analysis for *ANKRD11* gene could be considered the patient who has short stature with ID and DD and further study about its variable clinical multi organ manifestations is needed to identify and diagnose more KBGS patients. In addition, subsequent molecular investigations and studies regarding chromatin co-regulation and the expression of the *ANKRD11* gene in various organs are needed.

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CONFLICTS OF INTEREST

The authors have no potential conflicts of interest to disclose.

REFERENCES

1. Low K, Ashraf T, Canham N, Clayton-Smith J, Deshpande C, Donaldson A, et al. Clinical and genetic aspects of KBG syndrome. Am J Med Genet A 2016;170:2835-46. doi: 10.1002/ajmg.a.37842.
2. de Boer E, Ockeloen CW, Kampen RA, Hampstead JE, Dingemans AJM, Rots D, et al. Missense variants in ANKRD11 cause KBG syndrome by impairment of stability or transcriptional activity of the encoded protein. Genet Med 2022;24:2051-64. doi: 10.1016/j.gim.2022.06.007.
3. Gao F, Zhao X, Cao B, Fan X, Li X, Li L, et al. Genetic and phenotypic spectrum of KBG Syndrome: a report of 13 new Chinese cases and a review of the Literature. J Pers Med 2022;12:407. doi: 10.3390/jpm12030407.
4. Nardello R, Mangano GD, Antona V, Fontana A, Striano P, Giorgio E, et al. Electroclinical features and outcome of ANKRD11-related KBG syndrome: a novel report and literature review. Seizure 2021;85:151-4. doi: 10.1016/j.seizure.2020.12.017.
5. Bestetti I, Crippa M, Sironi A, Tumiatti F, Masciadri M, Smeland MF, et al. Expanding the molecular spectrum of ANKRD11 gene defects in 33 patients with a clinical presentation of KBG Syndrome. Int J Mol Sci 2022;23:5912. doi: 10.3390/ijms23115912.
6. Brancati F, Sarkozy A, Dallapiccola B. KBG syndrome. Orphanet J Rare Dis 2006;1:50. doi: 10.1186/1750-1172-1-50.
7. Goldenberg A, Riccardi F, Tessier A, Pfundt R, Busa T, Cacciagli P, et al. Clinical and molecular findings in 39 patients with KBG syndrome caused by deletion or mutation of ANKRD11. Am J Med Genet A 2016;170:2847-59. doi: 10.1002/ajmg.a.37878.
8. van Dongen LCM, Wingbermühle E, Oomens W, Bos-Roubos AG, Ockeloen CW, Kleefstra T, et al. Intellectual profiles in KBG-syndrome: a Wechsler based case-control study. Front Behav Neurosci 2017;11:248. doi: 10.3389/fnbeh.2017.00248.
9. Skjei KL, Martin MM, Slavotinek AM. KBG syndrome: report of twins, neurological characteristics, and delineation of diagnostic criteria. Am J Med Genet A 2007;143A:292-300. doi: 10.1002/ajmg.a.31597.
10. Murray N, Burgess B, Hay R, Colley A, Rajagopalan S, McGaughran J, et al. KBG syndrome: an Australian experience. Am J Med Genet A 2017;173:1866-77. doi: 10.1002/ajmg.a.38121.
11. Carter DC, Kierzkowska O, Sarino K, Guo L, Marchi E, Lyon GJ. Ocular manifestations in a Cohort of 43 patients with KBG syndrome. medRxiv 2023:2023.05.31.23290743. doi: 10.1101/2023.05.31.23290743.
12. Bonsergent SA, Rojo G, Graziani P, Azula ME, Othatz L, Fernie L, et al. Clinical features and desicion making of congenital vaginal agenesis combined with cervical aplasia: case report and literature reviews. Journal of Pediatric and Adolescent Gynecology 2023;36:257-8. doi: 10.1016/j.jpag.2023.01.209.
13. Choi Y, Choi J, Do H, Hwang S, Seo GH, Choi IH, et al. KBG syndrome: clinical features and molecular findings in seven unrelated Korean families with a review of the literature. Mol Genet Genomic Med 2023;11:e2127. doi: 10.1002/mgg3.2127.
14. Kim SJ, Yang A, Park JS, Kwon DG, Lee JS, Kwon YS, et al. Two novel mutations of ANKRD11 gene and wide clinical spectrum in KBG syndrome: case reports and literature review. Front Genet 2020;11:579805. doi: 10.3389/fgene.2020.579805.
15. Scarano E, Tassone M, Graziano C, Gibertoni D, Tamburrino F, Perri A, et al. Novel mutations and unreported clinical features in KBG syndrome. Mol Syndromol 2019;10:130-38. doi: 10.1159/000496172.
16. Groza T, Gomez FL, Mashhadi HH, Munoz-Fuentes V, Gunes O, Wilson R, et al. The International Mouse Phenotyping Consortium: comprehensive knockout phenotyping underpinning the study of human disease. Nucleic Acids Res 2023;51(D1):D1038-D45. doi: 10.1093/nar/gkac972.
17. Kierzkowska O, Sarino K, Carter D, Guo L, Marchi E, Voronova A, et al. Documentation and prevalence of prenatal and neonatal outcomes in a cohort of individuals with KBG syndrome. Am J Med Genet A 2023;191:2364-75. doi: 10.1002/ajmg.a.63311 [published Online First: 20230525]
18. Sirmaci A, Spiliopoulos M, Brancati F, Powell E, Duman D, Abrams A, et al. Mutations in ANKRD11 cause KBG syndrome, characterized by intellectual disability, skeletal malformations, and macrodontia. Am J Hum Genet 2011;89:289-94. doi: 10.1016/j.ajhg.2011.06.007.
19. Awamleh Z, Choufani S, Cytrynbaum C, Alkuraya FS, Scherer S, Fernandes S, et al. ANKRD11 pathogenic variants and 16q24.3 microdeletions share an altered DNA methylation signature in patients with KBG syndrome. Hum Mol Genet 2023;32:1429-

38. doi: 10.1093/hmg/ddac289.
20. Zhang T, Yang Y, Yin X, Wang X, Ni J, Dong Z, et al. Two loss-of-function ANKRD11 variants in Chinese patients with short stature and a possible molecular pathway. Am J Med Genet A 2021; 185:710-18. doi: 10.1002/ajmg.a.62024.

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Clinical and Laboratory Features to Consider Genetic Evaluation among Children and Adolescents with Short Stature

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Conventional evaluation method for identifying the organic cause of short stature has a low detection rate. If an infant who is small for gestational age manifests postnatal growth deterioration, triangular face, relative macrocephaly, and protruding forehead, a genetic testing of *IGF2, H19, GRB10, MEST, CDKN1, CUL7, OBSL1*, and *CCDC9* should be considered to determine the presence of Silver–Russell syndrome and 3-M syndrome. If a short patient with prenatal growth failure also exhibits postnatal growth failure, microcephaly, low IGF-1 levels, sensorineural deafness, or impaired intellectual development, genetic testing of *IGF1* and *IGFALS* should be conducted. Furthermore, genetic testing of *GH1, GHRHR, HESX1, SOX3, PROP1, POU1F1*, and *LHX3* should be considered if patients with isolated growth hormone deficiency have short stature below -3 standard deviation score, barely detectable serum growth hormone concentration, and other deficiencies of anterior pituitary hormone. In short patients with height SDS <-3 and high growth hormone levels, genetic testing should be considered to identify *GHR* mutations. Lastly, when severe short patients (height z score <-3) exhibit high levels of prolactin and recurrent pulmonary infection, genetic testing should be conducted to identify *STAT5B* mutations.

Key words: Short stature, Small for gestational age, Growth hormone deficiency, Insulin like growth factor, Growth hormone insensitivity

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INTRODUCTION

Short stature (<3rd percentile compared with the same sex and age) is one of the common reasons for visiting pediatric endocrinology. Its organic causes include renal failure, hypothyroidism, celiac disease, acid-base disorders, inflammatory bowel diseases, and impairment of thesomatotropin axis. However, in a previous study, the incidence of pathogenic causes of short stature was found in only 1.3% of 235 patients with short stature patients by the standard evaluation for identifying the above-mentioned underlying disease [1].

Also in a previous study, various monogenic causes of short stature were observed in a patient with idiopathic short stature, including short-stature homeobox-containing gene, natriuretic peptide receptor 2 gene, natriuretic peptide precursor C gene, fibroblast growth factor receptor 3 gene, and aggrecan gene [2]. Early identification of monogenic short stature prevents further diagnostic workup in patients and facilitates the evaluation of other medical comorbidities as well as the provision of genetic counseling to the family [3]. This article briefly reviews clinical and laboratory findings associated with monogenic short stature, including both the pre- and postnatal onsets of short stature.

CONSIDERATION OF GENETIC TESTING IN A PATIENT WITH PRENATAL ONSET OF SHORT STATURE

Factors known to inhibit intrauterine growth include congenital infections, smoking, placenta previa, and poor nutrition. However, a specific etiology is not identified in most infants that are small for gestational age (SGA) [3]. In a previous study, 80%–85% of idiopathic SGA infants exhibited rapid growth [4]. There are many underlying genetic conditions that result in pre- and postnatal growth restriction. Silver–Russell syndrome (SRS) is one of the common etiologies of SGA. SRS is mainly diagnosed based on clinical findings. According to the Netchine–Harbison scoring system, the presence of four of the following six criteria suggests SRS: low birth weight (≤-2 standard deviation score for gestational age), postnatal growth restriction, relative macrocephaly at birth, prominent forehead, asymmetric body, feeding difficulties, and/or low body mass index [4]. Although molecular testing has a diagnostic yield of only around 60% [5] and a lack of positive molecular results cannot rule out SRS, it confirms the presence of the disease, particularly in children aged <2 years, adolescents, and adult patients with less prominent signs and symptoms [6]. The most prevalent genetic cause is a hypomethylated imprinting region, including *IGF2* and *H19* genes, on the paternal allele of chromosome 11p15.5 [5], followed by uniparental disomy of chromosome 7. Methylation patterns could differ among leucocytes, buccal swabs, and skin fibroblasts [7,8]. The other candidate genes of SRS on chromosome 7 are *MEST* (7q32) and *GRB10* (7p12.1) [9,10]. Additional genetic testing includes a *CDKN1C* gain-of-function mutation and an *IGF2* loss-of-function mutation [11,12].

Another disorder with clinical features similar to SRS is 3-M syndrome. Patients with 3-M syndrome exhibit pre- and postnatal growth restriction, triangular faces, and normal head circumference [13]. Other features include normal intelligence, fleshy nasal tip, frontal bossing, and pointed chin, which become less pronounced as patient age [14]. The associated genes include *CUL7*, *OBSL1*, and *CCDC8*, which are inherited as a recessive condition [13]. Other disorders represented by severely restricted prenatal and postnatal growth with microcephaly are microcephalic osteodysplastic primordial dwarfism types I and II, Meier–Gorlin syndrome, and Seckel syndrome [15].

CONSIDERATION OF GENETIC TESTING IN A PATIENT WITH ISOLATED GROWTH HORMONE DEFICIENCY

Growth hormone (GH) conducts a critical role in human growth by itself or through the regulation of Insulin-like growth factor-1 (IGF-1) production. Isolated growth hormone deficiency (GHD) has an idiopathic cause [3]. In previous studies, genetic conditions associated with isolated GHD have been identified in 11%–34% of patients with isolated GHD. The candidate genes were *GH1*, *GHRHR*, *HESX1*, and *SOX3* [16,17].

Homozygous deletion, nonsense mutation, and compound heterozygous frameshift mutations of *GH1* induce GHD type 1A, the most severe form of isolated GHD. GHD type 1A is represented by severe postnatal growth deterioration, unverifiable serum GH, development of anti-GH antibodies, and autosomal recessive transmission. Conversely, GHD type 1B (less severe form) is induced by missense, nonsense, splice site, or frameshift mutations of *GH1* or *GHRHR*. It is represented by low but detectable serum GH concentrations and absence of anti-GH antibodies. Mutations within the first six nucleotides of intron 3 of *GH1* produce a shortened form of GH and induce type II GHD. The shortened GH has a dominant negative effect on GH receptor, which leads to reduced GH secretion and is possibly associated with multiple pituitary hormone deficiency (MPHD), including TSH, LH, and prolactin [3].

One of the important clinical features of children with isolated GHD with identified genetic causes was lower height SDS (-4.7±1.6 vs. -3.4±1.7) than those with unidentified genetic causes. However, no significant difference was observed in age at diagnosis, IGF-1 SDS, peak GH concentration after simulation test, and prevalence of anterior pituitary gland hypoplasia [17]. Further research on the criteria for conducting genetic testing in a patient with isolated GHD is warranted.

CONSIDERATION OF GENETIC TESTING IN A PATIENT WITH MPHD

PROP1

PROP1 antecedes and provokes the expression of *POU1F1*. It is also selectively expressed in the embryonic pituitary gland [18]. *PROP1* mutation is associated with MPHD, including GH, TSH, prolactin, LH, FSH, and ACTH deficiencies. However, physicians need to be cautious because panhypopituitarism may not be present from the beginning. The time of appearance and degree of hormone deficiencies vary significantly, particu-

larly for LH, FSH, and ACTH [19]. Thus, laboratory evaluation including FSH, LH, PRL, and ACTH levels in a patient with isolated GHD can help identify the patient at risk of developing panhypopituitarism. Another feature of MPHD is a small or normal-sized adenohypophysis. However, magnetic resonance imaging revealed pituitary hyperplasia in 33% of patients with multiple pituitary hormone deficiency. The pituitary enlargement exhibited a non-enhancing mass lesion located between the adenohypophysis and neurohypophysis [20].

POU1F1

POU1F1 is expressed in the developing pituitary gland and associated with the differentiation of cells responsible for the secretion of somatotropin, prolactin, and thyrotropin [21]. Patients with a *POU1F1* mutation exhibit complete GH and prolactin deficiency during the first years of life. However, the extent and onset of TSH deficiency vary significantly. Furthermore, central hypothyroidism is common but does not occur in all patients with a *POU1F1* mutation [22]. Radiologic findings of the pituitary gland include hypoplasia of adenohypophysis, normal infundibular stalk, and normally positioned neurohypophysis. However, normal-sized anterior lobes have been reported in rare cases [23]. Thus, genetic testing may be required in patients with GH and prolactin deficiencies, as well as TSH deficiency and structural abnormalities of the pituitary gland.

LHX3

LIM-homeobox 3 (MIM 600577) is expressed in the developing anterior pituitary gland and intermediate pituitary gland. It is important to determine all pituitary cell lineages, except corticotropes [24]. Patients with homozygous *LHX3* loss-of-function mutations harbor GH, TSH, LH, FSH, and prolactin deficiencies. Other clinical features of associated abnormalities in patients with *LHX3* mutations include a short neck with limited rotation as well as vertebral and skeletal problems.

GH insensitivity

Laron et al. reported GH insensitivity (GHI) earliest with the description of children with extremely short stature, the phenotype of hypopituitarism, and high level of serum GH [25]. GH derived from the pituitary gland stimulates IGF-I expression in both the hepatocytes and non-hepatic tissues [26]. In addition to the direct effect of GH on chondrocyte, GH also prompts longitudinal bone growth via the IGF-1-dependent mechanism. Binding of GH to cell surface homodimeric growth hormone receptor (GHR) activates cytosolic Janus kinase 2 (JAK2). JAK2 phosphorylates the signal transducer and activator of transcription (STAT)-5b. Subsequently, tyrosyl-phosphorylated-STAT5b produces a homodimer and translocates to the nucleus, producing IGF-1, IGFBP3, and acid-labile subunit (ALS).

GHR mutations include missense, nonsense, and splice mutations [27,28]. The majority of patients with *GHR* mutations have homozygous or compound heterozygous mutations as well as autosomal recessive inheritance [29]. In a previous study, IGF-1 disruption reduced fetal growth in mice significantly [30]. Heterozygous *GHR* mutations cause a mild phenotype by dominant negative effect [31]. One of the characteristic findings suggesting GHI was severe short stature. The height SDS of a patient with GHI ranged from -3 to -10 SDS [32]. Even in Japanese sibling patients with a mild phenotype due to compound heterozygous mutations of *GHR*, the height SDSs were -3.0 and -3.5, respectively. Another clinical characteristic finding was elevated GH levels. In previous reports, the basal levels of GH were between 1.0 and 44 ng/mL. Furthermore, the peak GH concentrations after the stimulation test were between 23.8 and 48.1 ng/mL [33]. Thereafter, in patients with height SDS <-3 and high GH levels, genetic testing should be considered to identify *GHR* mutations.

STAT5B

Human *STAT5B* mutations cause severe postnatal growth restriction as *STAT5b* is critical for GH-induced production of IGF-I [34]. Clinical and laboratory characteristics are generally normal birth weight; severely restricted postnatal growth; unresponsiveness to GH replacement; elevated and stimulated basal GH concentrations, 0.13–17.7 ng/mL and 12.5–53.8 ng/mL, respectively; low IGF-I levels, absent to 38 ng/mL; and high prolactin levels, 13–169 μg/L [35-37]. Another important feature is immune dysfunction resulting in recurrent pulmonary infections and autoimmune disease [35]. Immune dysfunction includes lymphopenia and low regulatory T cells [38]. However, brain development is normal. The genetic mutations reported to date were autosomal recessive and homozygous, indicating that haploinsufficiency had a negligible effect on IGF-I expression [26]. Therefore, in patients with height SDS <-3, the presence of high prolactin levels and recurrent pulmonary infection indicate the need for genetic testing to identify *STAT5B* mutations.

IGF1

The clinical phenotypes of patients with homozygous *IGF1* mutations include sensorineural deafness, severe deficit of in-

tellectual development, microcephaly, intrauterine growth retardation, and severe postnatal growth [39,40]. *In utero*, the development of the central nervous system appears to be associated with IGF-1 [24]. In previous reports, birth weight ranged from -5.4 to -1.2 SDS. IGF-1 levels were found to be very low [39,41,42], whereas IGFBP-3 and ALS levels were normal [41]. Heterozygous *IGF1* mutations may cause short stature through a dominant negative effect [42].

IGFALS

IGFALS mutations are characterized by GHI and IGF-1 and ALS deficiencies. *IGFALS* is located on chromosome 16p13.3 and encodes ALS by stimulating GH [43]. The clinical characteristics of patients with a homozygous IGFALS mutation included severe IGF-1, IGFBP3, and ALS deficiencies. However, relative mild growth failure and even a normal height were reported in some patients [44]. The known IGFALS mutations include missense and nonsense mutations, deletions, duplications, and insertions [45]. Patients with heterozygous IGFALS mutations exhibited approximately 1 SDS height loss compared with those with wild-type mutations [46].

IGF1R

IGF1R belongs to the insulin receptor family and is a transmembrane receptor; it binds IGF-1 and IGF-2. The *IGF1R* gene is located on chromosome 15q26.3 [26]. The clinical features of *IGF1R* mutations are impaired pre- and postnatal growth, small head circumference, developmental delay, micrognathia, and relatively high IGF-1 levels [47]. The signs and symptoms that suggest *IGF1R* mutation include small birth weight or length, postnatal growth failure, small head circumference, developmental delay and micrognathia, and relatively high IGF-1 levels [47]. The IGF-1 levels reported in previous literatures ranged from -2.78 to 2.9 SDS. The IGFBP3 levels were between -1.33 and +1.24 SDS [47-49]. A heterozygous mutation could result in a reduced amount of wild-type IGF1R protein and impaired postreceptor IGF-1 signaling [50].

CONCLUSION

Growth is a complex process involving various genetic factors both pre- and postnatally. Recent advancements in genetics have improved the molecular diagnosis for patients with short statures. Clinicians should be aware of the clinical characteristics of patients to consider genetic testing and use a broad-based genetic approach in children with short stature.

REFERENCES

1. Sisley S, Trujillo MV, Khoury J, Backeljauw P. Low incidence of pathology detection and high cost of screening in the evaluation of asymptomatic short children. J Pediatr 2013;163:1045-51. doi: 10.1016/j.jpeds.2013.04.002.
2. Kang MJ. Novel genetic cause of idiopathic short stature. Ann Pediatr Endocrinol Metab 2017;22:153-7. doi: 10.6065/apem.2017.22.3.153.
3. Dauber A, Rosenfeld RG, Hirschhorn JN. Genetic evaluation of short stature. J Clin Endocrinol Metab 2014;99:3080-92. doi: 10.1210/jc.2014-1506.
4. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? Pediatr Res 1995;38:267-71. doi: 10.1203/00006450-199508000-00022.
5. Azzi S, Salem J, Thibaud N, Chantot-Bastaraud S, Lieber E, Netchine I, et al. A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. J Med Genet 2015;52:446-53. doi: 10.1136/jmedgenet-2014-102979.
6. Netchine I, Rossignol S, Dufourg MN, Azzi S, Rousseau A, Perin L, et al. 11p15 imprinting center region 1 loss of methylation is a common and specific cause of typical Russell-Silver syndrome: clinical scoring system and epigenetic-phenotypic correlations. J Clin Endocrinol Metab 2007;92:3148-54. doi: 10.1210/jc.2007-0354.
7. Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol 2017;13:105-24. doi: 10.1038/nrendo.2016.138.
8. Azzi S, Blaise A, Steunou V, Harbison MD, Salem J, Brioude F, et al. Complex tissue-specific epigenotypes in Russell-Silver Syndrome associated with 11p15 ICR1 hypomethylation. Hum Mutat 2014;35:1211-20. doi: 10.1002/humu.22623.
9. Begemann M, Spengler S, Kanber D, Haake A, Baudis M, Leisten I, et al. Silver-Russell patients showing a broad range of ICR1 and ICR2 hypomethylation in different tissues. Clin Genet 2011;80:83-8. doi: 10.1111/j.1399-0004.2010.01514.x.
10. Eggermann T, Spengler S, Begemann M, Binder G, Buiting K, Albrecht B, et al. Deletion of the paternal allele of the imprinted MEST/PEG1 region in a patient with Silver-Russell syndrome features. Clin Genet 2012;81:298-300. doi: 10.1111/j.1399-0004.2011.01719.x.
11. Monk D, Wakeling EL, Proud V, Hitchins M, Abu-Amero SN, Stanier P, et al. Duplication of 7p11.2-p13, including GRB10, in Silver-Russell syndrome. Am J Hum Genet 2000;66:36-46. doi: 10.1086/302717.
12. Brioude F, Oliver-Petit I, Blaise A, Praz F, Rossignol S, Le Jule M, et al. CDKN1C mutation affecting the PCNA-binding domain as a cause of familial Russell Silver syndrome. J Med Genet 2013;50:823-30. doi: 10.1136/jmedgenet-2013-101691.
13. Begemann M, Zirn B, Santen G, Wirthgen E, Soellner L, Büttel HM, et al. Paternally Inherited IGF2 Mutation and Growth Re-

striction. N Engl J Med 2015;373:349-56. doi: 10.1056/NEJMoa1415227.
14. Clayton PE, Hanson D, Magee L, Murray PG, Saunders E, Abu-Amero SN, et al. Exploring the spectrum of 3-M syndrome, a primordial short stature disorder of disrupted ubiquitination. Clin Endocrinol (Oxf) 2012;77:335-42. doi: 10.1111/j.1365-2265.2012.04428.x.
15. Maksimova N, Hara K, Miyashia A, Nikolaeva I, Shiga A, Nogovicina A, et al. Clinical, molecular and histopathological features of short stature syndrome with novel CUL7 mutation in Yakuts: new population isolate in Asia. J Med Genet 2007;44:772-8. doi: 10.1136/jmg.2007.051979.
16. Klingseisen A, Jackson AP. Mechanisms and pathways of growth failure in primordial dwarfism. Genes Dev 2011;25:2011-24. doi: 10.1101/gad.169037.
17. Mullis PE. Genetic control of growth. Eur J Endocrinol 2005;152:11-31. doi: 10.1530/eje.1.01797.
18. Alatzoglou KS, Turton JP, Kelberman D, Clayton PE, Mehta A, Buchanan C, et al. Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. J Clin Endocrinol Metab 2009;94:3191-9. doi: 10.1210/jc.2008-2783.
19. Sornson MW, Wu W, Dasen JS, Flynn SE, Norman DJ, O'Connell SM, et al. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. Nature 1996;384:327-33. doi: 10.1038/384327a0.
20. Böttner A, Keller E, Kratzsch J, Stobbe H, Weigel JF, Keller A, et al. PROP1 mutations cause progressive deterioration of anterior pituitary function including adrenal insufficiency: a longitudinal analysis. J Clin Endocrinol Metab 2004;89:5256-65. doi: 10.1210/jc.2004-0661.
21. Voutetakis A, Argyropoulou M, Sertedaki A, Livadas S, Xekouki P, Maniati-Christidi M, et al. Pituitary magnetic resonance imaging in 15 patients with Prop1 gene mutations: pituitary enlargement may originate from the intermediate lobe. J Clin Endocrinol Metab 2004;89:2200-6. doi: 10.1210/jc.2003-031765.
22. Simmons DM, Voss JW, Ingraham HA, Holloway JM, Broide RS, Rosenfeld MG, et al. Pituitary cell phenotypes involve cell-specific Pit-1 mRNA translation and synergistic interactions with other classes of transcription factors. Genes Dev 1990;4:695-711. doi: 10.1101/gad.4.5.695.
23. Pfäffle R, Klammt J. Pituitary transcription factors in the aetiology of combined pituitary hormone deficiency. Best Pract Res Clin Endocrinol Metab 2011;25:43-60. doi: 10.1016/j.beem.2010.10.014.
24. Turton JP, Reynaud R, Mehta A, Torpiano J, Saveanu A, Woods KS, et al. Novel mutations within the POU1F1 gene associated with variable combined pituitary hormone deficiency. J Clin Endocrinol Metab 2005;90:4762-70. doi: 10.1210/jc.2005-0570.
25. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, et al. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med 2003;349:2211-22. doi: 10.1056/NEJMoa010107.
26. Laron Z, Pertzelan A, Mannheimer S. Genetic pituitary dwarfism with high serum concentation of growth hormone--a new inborn error of metabolism? Isr J Med Sci 1966;2:152-5.
27. David A, Hwa V, Metherell LA, Netchine I, Camacho-Hübner C, Clark AJ, et al. Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev 2011;32:472-97. doi: 10.1210/er.2010-0023.
28. Baumann G. Genetic characterization of growth hormone deficiency and resistance: implications for treatment with recombinant growth hormone. Am J Pharmacogenomics 2002;2:93-111. doi: 10.2165/00129785-200202020-00003.
29. Savage MO, Attie KM, David A, Metherell LA, Clark AJ, Camacho-Hübner C. Endocrine assessment, molecular characterization and treatment of growth hormone insensitivity disorders. Nat Clin Pract Endocrinol Metab 2006;2:395-407. doi: 10.1038/ncpendmet0195.
30. Diniz ET, Jorge AA, Arnhold IJ, Rosenbloom AL, Bandeira F. [Novel nonsense mutation (p.Y113X) in the human growth hormone receptor gene in a Brazilian patient with Laron syndrome]. Arq Bras Endocrinol Metabol 2008;52:1264-71. doi: 10.1590/s0004-27302008000800010.
31. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Dev Biol 2001;229:141-62. doi: 10.1006/dbio.2000.9975.
32. Ayling RM, Ross R, Towner P, Von Laue S, Finidori J, Moutoussamy S, et al. A dominant-negative mutation of the growth hormone receptor causes familial short stature. Nat Genet 1997;16:13-4. doi: 10.1038/ng0597-13.
33. Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958-2003. J Clin Endocrinol Metab 2004;89:1031-44. doi: 10.1210/jc.2003-031033.
34. Iida K, Takahashi Y, Kaji H, Nose O, Okimura Y, Abe H, et al. Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. J Clin Endocrinol Metab 1998;83:531-7. doi: 10.1210/jcem.83.2.4601.
35. Rosenfeld RG, Belgorosky A, Camacho-Hubner C, Savage MO, Wit JM, Hwa V. Defects in growth hormone receptor signaling. Trends Endocrinol Metab 2007;18:134-41. doi: 10.1016/j.tem.2007.03.004.
36. Vidarsdottir S, Walenkamp MJ, Pereira AM, Karperien M, van Doorn J, van Duyvenvoorde HA, et al. Clinical and biochemical characteristics of a male patient with a novel homozygous STAT5b mutation. J Clin Endocrinol Metab 2006;91:3482-5. doi: 10.1210/jc.2006-0368.
37. Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, et al. Growth hormone insensitivity associated with a STAT5b mutation. N Engl J Med 2003;349:1139-47. doi: 10.1056/NEJMoa022926.
38. Pugliese-Pires PN, Tonelli CA, Dora JM, Silva PC, Czepielewski M, Simoni G, et al. A novel STAT5B mutation causing GH insensitivity syndrome associated with hyperprolactinemia and immune dysfunction in two male siblings. Eur J Endocrinol 2010;

163:349-55. doi: 10.1530/eje-10-0272.
39. Bernasconi A, Marino R, Ribas A, Rossi J, Ciaccio M, Oleastro M, et al. Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. Pediatrics 2006;118:e1584-92. doi: 10.1542/peds.2005-2882.
40. Netchine I, Azzi S, Houang M, Seurin D, Perin L, Ricort JM, et al. Partial primary deficiency of insulin-like growth factor (IGF)-I activity associated with IGF1 mutation demonstrates its critical role in growth and brain development. J Clin Endocrinol Metab 2009;94:3913-21. doi: 10.1210/jc.2009-0452.
41. Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, et al. Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab 2005;90:2855-64. doi: 10.1210/jc.2004-1254.
42. Camacho-Hübner C, Woods KA, Miraki-Moud F, Clark A, Savage MO. Insulin-like growth factor-I deficiency caused by a partial deletion of the IGF-I gene: effects of rhIGF-I therapy. Growth Horm IGF Res 1999;9 Suppl B:47-51; discussion -2. doi: 10.1016/s1096-6374(99)80081-1.
43. van Duyvenvoorde HA, van Setten PA, Walenkamp MJ, van Doorn J, Koenig J, Gauguin L, et al. Short stature associated with a novel heterozygous mutation in the insulin-like growth factor 1 gene. J Clin Endocrinol Metab 2010;95:E363-7. doi: 10.1210/jc.2010-0511.
44. Ooi GT, Cohen FJ, Tseng LY, Rechler MM, Boisclair YR. Growth hormone stimulates transcription of the gene encoding the acid-labile subunit (ALS) of the circulating insulin-like growth factor-binding protein complex and ALS promoter activity in rat liver. Mol Endocrinol 1997;11:997-1007. doi: 10.1210/mend.11.7.9942.
45. Domené HM, Scaglia PA, Lteif A, Mahmud FH, Kirmani S, Frystyk J, et al. Phenotypic effects of null and haploinsufficiency of acid-labile subunit in a family with two novel IGFALS gene mutations. J Clin Endocrinol Metab 2007;92:4444-50. doi: 10.1210/jc.2007-1152.
46. Domené HM, Hwa V, Argente J, Wit JM, Camacho-Hübner C, Jasper HG, et al. Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. Horm Res 2009;72:129-41. doi: 10.1159/000232486.
47. Fofanova-Gambetti OV, Hwa V, Wit JM, Domene HM, Argente J, Bang P, et al. Impact of heterozygosity for acid-labile subunit (IGFALS) gene mutations on stature: results from the international acid-labile subunit consortium. J Clin Endocrinol Metab 2010;95:4184-91. doi: 10.1210/jc.2010-0489.
48. Ester WA, van Duyvenvoorde HA, de Wit CC, Broekman AJ, Ruivenkamp CA, Govaerts LC, et al. Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. J Clin Endocrinol Metab 2009;94:4717-27. doi: 10.1210/jc.2008-1502.
49. Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruithof MF, et al. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. J Clin Endocrinol Metab 2006;91:3062-70. doi: 10.1210/jc.2005-1597.
50. Kawashima Y, Kanzaki S, Yang F, Kinoshita T, Hanaki K, Nagaishi J, et al. Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. J Clin Endocrinol Metab 2005;90:4679-87. doi: 10.1210/jc.2004-1947.
51. Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, et al. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. J Clin Endocrinol Metab 2007;92:1542-8. doi: 10.1210/jc.2006-2354.

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Clinical Application of Chromosomal Microarray for Germline Disorders

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Chromosomal microarray (CMA) is primarily recommended for detecting clinically significant copy number variants (CNVs) in the genetic diagnosis of developmental delay, intellectual disability, autism, and congenital malformations. Prenatal CMA is recommended when a fetus has major congenital malformations. The main principles of CMA can be divided into array comparative genomic hybridization and single-nucleotide polymorphism arrays. In the current CMA platforms, these two principles are combined, and detection of genetic abnormalities including CNVs and absence of heterozygosity is facilitated. In this review, I described practical assessment of CMA testing regarding to laboratory management of CMA, interpretation of CNVs, and special considerations for comprehensive genetic counseling.

Key words: DNA microarray, Hereditary disease, Genetic testing

REVIEW ARTICLE

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INTRODUCTION

Karyotyping has limitations in detecting copy number variants (CNVs) less than 5–10 Mb in size, and may not identify the origin of CNVs corresponding to marker chromosomes or unbalanced translocations. Fluorescence in situ hybridization (FISH) can only identify a few CNVs in specific genomic regions because of the limited application of FISH probes. Chromosomal microarray (CMA) analysis overcomes the limitations of karyotyping and FISH (Table 1) [1].

Array comparative genomic hybridization (aCGH) is a method used for detecting CNVs in a patient by attaching fluorescent probes to the patient and control samples, hybridizing the two samples to a DNA array, and measuring the relative fluorescence to call the CNVs. A single nucleotide polymorphism (SNP) array is used to measure the genotype signals in SNPs using allele-specific oligonucleotide probes complementary to a wild-type sequence or a SNP, in which genotype information such as B-allele frequency (a normalized allelic intensity ratio) can be obtained. CNV as well as the absence of heterozygosity (AOH) can be detected using SNP arrays [2]. Currently, a platform combining array CGH and SNP array is widely used in clinical laboratories [3], and also CNV detection has become possible through a relative comparison between patient data and control data from hundreds of healthy subjects eliminating the need for a control sample which was necessary in conventional aCGH [4,5].

Clinical indications for postnatal CMA testing include developmental delay, intellectual disability, autism, and multiple congenital malformations [6]. Prenatal CMA is recommended when one or more major fetal malformations are observed on prenatal ultrasound [7]. The diagnostic yield of postnatal CMA is 15%–20% and in prenatal CMA, 6% of the cases with abnormal prenatal ultrasonography

Table 1. Comparison of abnormality detection by cytogenetic tests

Abnormality	Karyotyping	FISH	CMA
Aneuploidy	+	+	+
Balanced rearrangement	+	+	-
Copy number variation (>10 Mb)	+	+	+
Copy number variation (<5–10 Mb)	-	+	+
Mosaicism	>20%	>5%	>20%
Marker chromosomes	+	+	+
Target	Whole genome	Limited	Whole genome

FISH, fluorescence in situ hybridization; CMA, chromosomal microarray.

and normal karyotype results show clinically significant CNVs [6,8].

VALIDATION, VERIFICATION AND QUALITY CONTROL OF CMA

According to the standards and guidelines for constitutional CMA presented by the American College of Medical Genetics (ACMG), using appropriate positive samples, evaluation of accuracy, precision, analytical sensitivity/specificity, and reportable ranges is recommended for validation, and evaluating accuracy, precision, and reportable range is recommended for verification with FDA-approved CMA platforms [9].

In terms of quality control, the values of quality control metrics (QC metrics) presented by manufacturers need to be managed. In the wet bench process, DNA concentration, OD 260/280, and the size of PCR and fragmentation products are important. Further, in the bioinformatics process, statistical values such as MAPD (median absolute pairwise difference) and SNPQC (SNP QC metric) need to be within the allowable ranges and managing data by batch is necessary. Retesting can be considered if QC metric values are outside the allowable range. However, in some cases, retesting may not be necessary if no significant deviation from routine results is determined after checking the raw data, which is both applicable to prenatal and postnatal CMA according to the policy of the laboratory [10]. Notably, a retest may be necessary even if the QC metrics are within the allowable range, if the noise is severe or if there are many false CNV calls [10].

INTERPRETATION OF CMA RESULTS

When CMA testing is completed and QC metrics are within the allowable range, it is important to check whether the detected CNV and AOH values are true values using the analysis software provided by the manufacturer. For true CNV and AOH values identified in this manner, the clinical significance of variants is assessed using existing laboratory data (in-house data), CNVs reported in open databases, and literature review; the CNV classification is then determined as P (pathogenic), LP (likely pathogenic), VUS (variant of uncertain significance), LB (likely benign), and B (benign) according to the CNV interpretation guidelines presented by the ACMG [11,12]. In the recently proposed CNV interpretation guidelines, CNV classification is facilitated by scoring the evidence with digitization; in particular, quantification according to the number of genes in the CNV is possible, and there are differences between copy number loss and gain as follows.

Loss: 0–24 genes (0 points)/25–34 genes (0.45 points)/35 or more genes (0.9 points)

Gain: 0–34 genes (0 points)/35–49 genes (0.45 points)/50 or more genes (0.9 points)

Further, the CNV guidelines state that uncoupling between patient clinical information and CNV classification is important, which means that the same CNV should not be classified differently for each patient [12].

When recommending a CNV public database or useful website for CNV interpretation, firstly checking the ClinGen Dosage Sensitivity map information is important; genes or regions of haploinsufficiency or triplosensitivity are reviewed by dosage sensitivity curation working group in ClinGen. Then additional checking of the information in OMIM or ClinVar may be required for the genes which were not curated in ClinGen. The public databases recommended for CNV interpretation are as follows [13,14].

ClinGen Dosage Sensitivity map: https://dosage.clinicalgenome.org/

Database of Genomic Variants (DGV): http://dgv.tcag.ca/dgv/app/home

Decipher: https://decipher.sanger.ac.uk/

UCSC Genome Browser: https://genome.ucsc.edu/

Orphanet: https://www.orpha.net/

ClinGen Dosage Sensitivity map has curated information of genes or genomic regions in which haploinsufficiency or triplosensitivity is associated with genetic diseases. DGV shows structural variants in healthy control samples. Decipher has cases with pathogenic CNVs and phenotype information. In UCSC Genome Browser, comprehensive analysis of CNV using combined other databases including DGV, ClinVar, OMIM, ClinGen, and Decipher is possible. Orphanet provides general

information according to cytogenetic abnormalities such as microdeletion/duplication syndromes.

SPECIAL CONSIDERATIONS

Secondary findings

Among the genes included in a CNV, it is taken into consideration when genes that are not related to the current phenotype or those associated with late onset disease are included. A laboratory policy should be established on whether to report the detection of CNV associated with late-onset diseases such as PMP22 duplication [12]. Additionally, referring to the list of 81 genes reported in the ACMG secondary findings to confirming dosage sensitivity is necessary [15].

Carrier status

In general, heterozygous deletions of genes associated with autosomal recessive genetic disorders are not considered for reporting. However, CNVs that have genes associated with recessive genetic diseases may be considered for reporting, if the allele frequency is high and the loss of function is a well-known mechanism for pathogenesis, or if the CNVs appear to be closely related to patient clinical information. In particular, if dosage-sensitive genes are included in CNVs on the X chromosome, reporting of female carriers may be considered [12].

Duplication of part of a gene

In most cases, duplications that contain only a part of the gene (not intragenic) (>80%) are direct-oriented tandem duplications, and no effect on gene function is expected. However, the possibility of other duplication types such as inverted duplications or insertions cannot be ruled out [16]. Therefore, in rare cases of duplications involving only a subset of genes whose haploinsufficiency is associated with disease pathogenesis, VUS reporting should be considered.

Consanguinity and uniparental disomy

If consanguinity is suspected because of an increased AOH ratio throughout autosomal chromosomes, the laboratory should establish its own policy regarding reporting methods. Additionally, AOH observed at a uniparental disomy (UPD) disorder-related site can be reported [3]. To confirm heterodisomy, a trio analysis of the CMA test can be performed, and the genotype signal patterns can be compared and confirmed. However, the CMA cannot cover all UPD disorders. In Prader-Willi syndrome, up to 90% of the cases can be diagnosed by CMA, but the rest can only be diagnosed by performing methylation analysis [17].

Prenatal CMA

The American College of Obstetricians and Gynecologists recommends CMA testing when one or more major structural abnormalities are observed in the fetus on prenatal ultrasound and an invasive prenatal examination is performed [7]. In prenatal CMA, selecting an appropriate platform is important considering the resolution and cost, and caution is required when reporting the VUS. CNVs often have a low genotype-phenotype correlation [18]. In the case of prenatal CMA, CNVs showing a low correlation with malformation are often detected; therefore, patients may suffer from toxic knowledge when reporting unnecessary VUS. The Society of Obstetricians and Gynecologists of the Canadian College of Medical Geneticists states that VUS with deletions of 500 kb or less, or duplications of 1 Mb or less, should not be reported. These can only be reported when there is sufficient evidence for the pathogenic possibility of the relevant part. Further, comprehensive genetic counseling considering various possible situations, such as secondary findings and the possibility of consanguinity is essential [19].

The possibility of maternal cell contamination is also considered. Contamination of maternal cells in prenatal specimens can affect the results of cytogenetic analysis. In general, the ACMG guidelines recommend that prenatal genetic testing should include a check for maternal cell contamination [9]. However, in practice, for prenatal CMA, the need for checking maternal cell contamination should be considered carefully because the test price is increased when an additional STR analysis is performed. Further, the usefulness of information obtained from the confirmation of maternal cell contamination in interpreting the test results should be considered. In general, slight contamination by maternal cells in the prenatal CMA test (<20%), does not significantly affect the test results. Further, increased maternal cell contamination can be checked by CMA itself because QC metric values such as SNPQC fall outside the allowable ranges. Thus, whether to implement a confirmation test for maternal cell contamination considering various factors and test characteristics needs to be determined [20].

Internal database management

Establishing and managing a laboratory database of detected CNV information is necessary to provide a reference for the frequency and content of previous reports on detected CNVs. Currently, a database needs to be built by considering the range

of coverage and overlapping percentage for each CNV (an appropriate algorithm design is required) [21].

CONCLUSION

CMA is a state-of-the-art molecular cytogenetic test that can easily detect CNVs among structural variants in the genome and check for information on AOH, compensating for the shortcomings of conventional karyotyping and FISH tests. Its clinical utility has been proven sufficient as a primary test for congenital disorders after childbirth; notably, it can supplement the limitations of conventional tests even in prenatal testing. In clinical laboratories, management of QC metrics is important for quality control, and comprehensive confirmation of clinical information, open databases, and literature reviews are required for accurate interpretation of results. An improved understanding of the characteristics of CMA testing can help diagnose various genetic diseases and increase its clinical value when used in conjunction with other genetic tests.

CONFLICTS OF INTEREST

I declare that I do not have any conflicts of interests.

REFERENCES

1. Zneimer SM. Cytogenetic abnormalities: Chromosomal, FISH and Microarray-Based Clinical Reporting. 1st ed. ChiChester, UK: Wiley-Blackwell, 2014:3.
2. Szuhai K. Array-CGH and SNP-Arrays, the New Karyotype. In: Jordan B, ed. Microarrays in Diagnostics and Biomarker Development: Current and Future Applications. Berlin, Heidelberg: Springer, 2012:39-52.
3. Gonzales PR, Andersen EF, Brown TR, Horner VL, Horwitz J, Rehder CW, et al. Interpretation and reporting of large regions of homozygosity and suspected consanguinity/uniparental disomy, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2022;24:255-61. doi: 10.1016/j.gim.2021.10.004.
4. Uddin M, Thiruvahindrapuram B, Walker S, Wang Z, Hu P, Lamoureux S, et al. A high-resolution copy-number variation resource for clinical and population genetics. Genet Med 2015;17:747-52. doi: 10.1038/gim.2014.178.
5. Zahir FR, Marra MA. Use of Affymetrix Arrays in the Diagnosis of Gene Copy-Number Variation. Curr Protoc Hum Genet 2015;85:8.13.1-8.13.13. doi: 10.1002/0471142905.hg0813s85.
6. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010;86:749-64. doi: 10.1016/j.ajhg.2010.04.006.
7. Committee on Genetics and the Society for Maternal-Fetal Medicine. Committee Opinion No.682: microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology. Obstet Gynecol 2016;128:e262-8. doi: 10.1097/AOG.0000000000001817.
8. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012;367:2175-84. doi: 10.1056/NEJMoa1203382.
9. Shao L, Akkari Y, Cooley LD, Miller DT, Seifert BA, Wolff DJ, et al. ACMG Laboratory Quality Assurance Committee. Chromosomal microarray analysis, including constitutional and neoplastic disease applications, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2021;23:1818-29. doi: 10.1038/s41436-021-01214-w.
10. Seol CA, Ha JS, Won D, Kim IS. Practical guidelines for chromosomal microarray analysis for constitutional abnormalities: part I, general and prenatal. Lab Med Online 2023;13:141-53. doi.org/10.47429/lmo.2023.13.3.141.
11. Won D, Seol CA, Ha JS, Kim IS. Practical guidelines for chromosomal microarray analysis for constitutional abnormalities: part II, reporting and interpretation. Lab Med Online 2023;13:154-64. doi.org/10.47429/lmo.2023.13.3.154.
12. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med 2020;22:245-57. doi: 10.1038/s41436-019-0686-8.
13. Thaxton C, Good ME, DiStefano MT, Luo X, Andersen EF, Thorland E, et al.; ClinGen Gene Curation Working Group; ClinGen Dosage Sensitivity Working Group. Utilizing ClinGen gene-disease validity and dosage sensitivity curations to inform variant classification. Hum Mutat 2022;43:1031-40. doi: 10.1002/humu.24291.
14. Mikhail FM, Biegel JA, Cooley LD, Dubuc AM, Hirsch B, Horner VL, et al. Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). Genet Med 2019;21:1903-16. doi: 10.1038/s41436-019-0545-7.
15. Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, et al.; ACMG Secondary Findings Working Group. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2023;25:100866. doi: 10.1016/j.gim.2023.100866.
16. Newman S, Hermetz KE, Weckselblatt B, Rudd MK. Next-generation sequencing of duplication CNVs reveals that most are tandem and some create fusion genes at breakpoints. Am J Hum

Genet 2015;96:208-20. doi: 10.1016/j.ajhg.2014.12.017.
17. Smith A, Hung D. The dilemma of diagnostic testing for Prader-Willi syndrome. Transl Pediatr 2017;6:46-56. doi: 10.21037/tp.2016.07.04.
18. Maya I, Sharony R, Yacobson S, Kahana S, Yeshaya J, Tenne T, et al. When genotype is not predictive of phenotype: implications for genetic counseling based on 21,594 chromosomal microarray analysis examinations. Genet Med 2018;20:128-31. doi: 10.1038/gim.2017.89.
19. Armour CM, Dougan SD, Brock JA, Chari R, Chodirker BN, DeBie I, et al.; On-Behalf-Of the Canadian College of Medical Geneticists. Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada. J Med Genet 2018;55:215-21. doi: 10.1136/jmedgenet-2017-105013.
20. Seol CA. Clinical application of prenatal chromosomal microarray. J Genet Med 2022;19:43-8. doi.org/10.5734/JGM.2022.19.2.43.
21. Yang X, Song Z, Wu C, Wang W, Li G, Zhang W, et al. Constructing a database for the relations between CNV and human genetic diseases via systematic text mining. BMC Bioinformatics 2018;19(Suppl 19):528. doi: 10.1186/s12859-018-2526-2.

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Pseudohypoparathyroidism: Clinical Review of Diagnosis and Genetic Etiology

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Pseudohypoparathyroidism (PHP) is very rare and shows heterogeneity with impaired genetic components. PHP is characterized by parathyroid hormone resistance to target organ, related with a *GNAS* (guanine nucleotide-binding protein α-subunit) mutation and epimutation. PHP receptor is coupled with the stimulatory G protein which activates cyclic adenosine monophosphate formation. PHP type 1A is caused by inactivating mutations on the maternal allele of the *GNAS* whereas paternal allele mutations cause pseudopseudohypoparathyroidism. PHP type 1B is caused by abnormal patterns of methylation in differentially methylated region which can be divided into partial or complete. This disease has some difficulties to diagnose according to these different molecular alterations caused by complex genetic and epigenetic defects. According to this different molecular alterations, genetic confirmation must be done to discriminate their etiology.

Key words: GNAS, Hypocalcemia, Parathyroid hormone, Pseudohypoparathyroidism

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INTRODUCTION

Pseudohypoparathyroidism (PHP) is very rare and shows heterogeneity with impaired genetic components. Its exact prevalence is unknown because of rarity, the estimated prevalence as 0.34 in 100,000 and 1.1 in 100,000 in Japan [1] and Denmark [2] respectively. PHP is characterized by parathyroid (PTH) resistance to target organ, related with a *GNAS* (guanine nucleotide-binding protein α-subunit) mutation and epimutation. PHP comprises OMIM #103580 for PHP type 1A (PHP-1A), #603233 for PHP type 1B (PHP-1B), and #6212462 type 1C (PHP-1C), which share common features of hypoparathyroidism [3].

To understand PHP pathophysiology, it is necessary to identify the PHP receptor and its signaling pathway. PHP receptor is coupled with the stimulatory G protein (Gs) which activates cAMP formation. Some cases show the resistance of other hormones (such as thyroid stimulating hormone (TSH), gonadotropin, calcitonin and growth hormone-releasing hormone) which have receptors coupled to Gs [4,5].

Albright hereditary osteodystrophy (AHO) comprises heterogeneous clinical manifestations such as ectopic ossification, round face, central obesity, short stature, brachydactyly and mental retardation. AHO was described with PHP in 1942, PHP type 1 is differentiated to the presence (PHP-1A and PHP-1C) and absence (PHP-1B) of AHO [6,7].

CLINICAL PRESENTATIONS AND GENETIC CAUSES

PHP-1A and PHP-1C

PHP-1A has multiple hormone resistances as well as PTH and TSH, gonadotro-

pin, and growth hormone releasing hormone (GHRH) which act via Gs-coupled receptor. The PTH resistance to renal proximal tubule leads to hypocalcemia, hyperphosphatemia, and elevated circulating PTH, generally preceding hypocalcemia usually over 1st years of life. PHP-1A shows clinical features of AHO, but these clinical features are very vague, it is diagnosed based on severe hypocalcemic symptoms such as seizure, tetany, paresthesia during childhood, requiring more calcium [3, 8]. Along with PHP resistance, almost PHP-1A patients have TSH resistance which is generally mild. But some patients are misdiagnosed only as congenital hypothyroidism by newborn screening test [9]. PHP-1A patients, especially females, have hypogonadism which is diagnosed as delay puberty, amenorrhea, or infertility. Almost all PHP-1A have an autosomal dominant pattern and caused by heterozygous loss-of function mutation in the *GNAS* gene [10,11]. Interestingly, patients inheriting from maternal mutation present phenotype of AHO as well as multiple hormone deficiency. However, patients inheriting from paternal mutation of this gene, show only AHO features without multiple hormone resistance (pseudo-pseudohypoparathyroidism, PPHP) [12].

GNAS is an imprinting gene which encodes Gsα coupled to PTH receptor, and locus is located on chromosome 20q13.22. This Gsα have a predominant maternal expression in specific maternal expression in some endocrine tissues, therefore is known as tissue-specific paternal imprinting of gene. This parent-of-origin-specific difference, *i.e.*, tissue-specific hormone responsive is expected in renal proximal tubule, thyroid, and ovary. The *GNAS* gene contains 13 exons which can be affected by loss-of function alterations [13]. Altered translation initiation or aberrant mRNA splicing by small insertion/deletions or amino acids substitutions have been reported. PPHP patients usually coexist, those patients have no hormone resistance but a partial deficiency (50%) of Gsα activity in various cell (fibroblast, erythrocytes) and show only the AHO. The diagnosis of PPHP with only phenotype is very difficult. The phenotype of PHP-1C is like PHP-1A, which is the AHO and multiple hormone resistance, but there is no deficiency of Gsα activity in various cell which PHP-1A showed. Few inactivating mutations is reported about PHP-1C.

PHP-1B

PHP-1B is characterized with resistance to PTH and normal Gsα activity without AHO features. Recently mild TSH elevations have been reported. These defects are often sporadic but caused by familial with an autosomal dominant pattern. Interestingly, like PHP-1A, PTH resistance develops with maternal inheritance, whereas paternal inheritance is not related with hormone resistance. There are no clinical differences between sporadic and familial mutations. PHP-1B is caused by methylation defects of the imprinted *GNAS* cluster. *GNAS* locus comprises several transcripts like the Gsα extra-large variant (XLαs), the neuroendocrine protein (NESP 55), untranslated exon A/B, and an additional antisense transcript. The familial form of PHP-1B is associated with loss of methylation at GNAS exon A/B or microdeletions on the cis-acting control elements on *STX16* gene in maternally inheritance with autosomal dominant pattern [14,15].

GNAS GENE AND IMPRINTING

The human *GNAS* gene comprises 13 exons and maps to chromosome 20q13. The mutations, loss-of-function, are developed in any coding region. Insertions, deletions, amino acids substitutions, nonsense, point mutations can alter translation initiation and aberrant mRNA splicing. PHP subtypes are caused by autosomal dominantly inheritance (mainly) or de novo mutation or epigenetic alterations (sporadic or familial). *GNAS* is imprinting gene, biallelic expression is shown in most cells but maternal expression is primarily observed in thyroid, renal proximal tubule, pituitary, and ovary cells. Therefore, this tissue specific monoallelic expression determines clinical outcome that depends on origin of paternal *GNAS* mutations.

PHP-1A is caused by inactivating mutations on the maternal allele of the *GNAS* whereas paternal allele mutations cause PPHP. Point mutation is easily detected by sequencing but genomic rearrangement needs analysis like multiple ligation-dependent probe amplification (MLPA) or comparative genomic hybridization [16,17].

PHP-1B is caused by abnormal patterns of methylation in the differentially methylated regions (DMR) which can be divided into partial or complete. *GNAS* has four different DMRs which are one paternally methylated DMR and three maternally methylated DMR. About 15%–20% are familial among the PHP-1B, and the methylation defect is generally limited at *GNAS* A/B:TSS-DMR. Methylation defects can be usually detected by methylation-sensitive MLPA [18].

CONCLUSION

Patients with PHP and related disorders develop a wide spectrum of clinical manifestations from early infancy to adulthood.

These symptoms include changed mineral metabolisms, which can cause seizures and other endocrine deficiencies. These highly heterogeneous features need early accurate genetic confirmation. This disease has some difficulties to diagnose according to these different molecular alterations caused by complex genetic and epigenetic defects. Therefore, patients sometimes need time-consuming to be established for a correct molecular diagnosis. This review might be helpful to diagnosis PHP and more genetic analysis for *GNAS* gene seems to be necessary.

REFERENCES

1. Nakamura Y, Matsumoto T, Tamakoshi A, Kawamura T, Seino Y, Kasuga M, et al. Prevalence of idiopathic hypoparathyroidism and pseudohypoparathyroidism in Japan. J Epidemiol 2000;10:29-33. doi: 10.2188/jea.10.29.
2. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Pseudohypoparathyroidism - epidemiology, mortality and risk of complications. Clin Endocrinol (Oxf) 2016;84:904-11. doi: 10.1111/cen.12948.
3. Mantovani G, Bastepe M, Monk D, de Sanctis L, Thiele S, Usardi A, et al. Diagnosis and management of pseudohypoparathyroidism and related disorders: first international Consensus Statement. Nature Reviews Endocrinology 2018;14:476-500. doi: 10.1038/s41574-018-0042-0.
4. Cheloha RW, Gellman SH, Vilardaga JP, Gardella TJ. PTH receptor-1 signalling-mechanistic insights and therapeutic prospects. Nat Rev Endocrinol 2015;11:712-24. doi: 10.1038/nrendo.2015.139.
5. Mannstadt M, Jüppner H, Gardella TJ. Receptors for PTH and PTHrP: their biological importance and functional properties. Am J Physiol 1999;277:F665-75. doi: 10.1152/ajprenal.1999.277.5.F665.
6. Wilson LC, Hall CM. Albright's hereditary osteodystrophy and pseudohypoparathyroidism. Semin Musculoskelet Radiol 2002;6:273-83. doi: 10.1055/s-2002-36726.
7. Goswami M, Verma M, Singh A, Grewal H, Kumar G. Albright hereditary osteodystrophy: a rare case report. Journal of Indian Society of Pedodontics and Preventive Dentistry 2009;27:184-8. doi: 10.4103/0970-4388.57101.
8. Hwang SK, Shim YJ, Oh SH, Jang KM. Early diagnosis of pseudohypoparathyroidism before the development of hypocalcemia in a young infant. Children (Basel) 2022;9. doi: 10.3390/children9050723.
9. Germain-Lee EL. Management of pseudohypoparathyroidism. Curr Opin Pediatr 2019;31:537-49. doi: 10.1097/mop.0000000000000783.
10. Lu D, Dong A, Zhang J, Guo X. A novel GNAS mutation in pseudohypoparathyroidism type 1a in a Chinese man presented with recurrent seizure: a case report. BMC Endocrine Disorders 2021;21:12. doi: 10.1186/s12902-020-00651-z.
11. Bastepe M. The GNAS locus and pseudohypoparathyroidism. Adv Exp Med Biol 2008;626:27-40. doi: 10.1007/978-0-387-77576-0_3.
12. Simpson C, Grove E, Houston BA. Pseudopseudohypoparathyroidism. The Lancet 2015;385:1123. doi: 10.1016/S0140-6736(14)61640-8.
13. Aldred MA, Trembath RC. Activating and inactivating mutations in the human GNAS1 gene. Hum Mutat 2000;16(3):183-9. doi: 10.1002/1098-1004(200009)16:3<183::AID-HUMU1>3.0.CO;2-L.
14. Mantovani G. Pseudohypoparathyroidism: diagnosis and treatment. The Journal of Clinical Endocrinology & Metabolism 2011;96:3020-30. doi: 10.1210/jc.2011-1048.
15. Matta-Coelho C, Mesquita J, Souto SB. Pseudohypoparathyroidism Type 1B with Asymptomatic Hypocalcemia. AACE Clinical Case Reports 2018;4:249-51. doi: 10.4158/ACCR-2017-0064.
16. Shoemaker AH, Jüppner H. Nonclassic features of pseudohypoparathyroidism type 1A. Curr Opin Endocrinol Diabetes Obes 2017;24:33-8. doi: 10.1097/med.0000000000000306.
17. Bastepe M, Fröhlich LF, Hendy GN, Indridason OS, Josse RG, Koshiyama H, et al. Autosomal dominant pseudohypoparathyroidism type Ib is associated with a heterozygous microdeletion that likely disrupts a putative imprinting control element of GNAS. The Journal of Clinical Investigation 2003;112:1255-63. doi: 10.1172/JCI19159.
18. Poradosu S, Bravenboer B, Takatani R, Jüppner H. Pseudohypoparathyroidism type 1B caused by methylation changes at the GNAS complex locus. BMJ Case Rep 2016;2016. doi: 10.1136/bcr-2016-214673.

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Resistance to Thyroid Hormone Syndrome Mutation in *THRB* and *THRA*: A Review

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Resistance to thyroid hormone syndrome (RTH) is a genetic disease caused by the mutation of either the thyroid hormone receptor-β (*THRB*) gene or the thyroid hormone receptor-α (*THRA*) gene. RTH caused by *THRB* mutations (RTH-β) is characterized by the target tissue's response to thyroid hormone, high levels of triiodothyronine and/or thyroxine, and inappropriate secretion of thyroid-stimulating hormone (TSH). *THRA* mutation is characterized by hypothyroidism that affects gastrointestinal, neurological, skeletal, and myocardial functions. Most patients do not require treatment, and some patients may benefit from medication therapy. These syndromes are characterized by decreased tissue sensitivity to thyroid hormones, generating various clinical manifestations. Thus, clinical changes of resistance to thyroid hormones must be recognized and differentiated, and an approach to the practice of personalized medicine through an interdisciplinary approach is needed.

Key words: Thyroid hormone receptor-β, Thyroid hormone receptor-α, Thyroid hormone

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INTRODUCTION

The thyroid gland secretes hormones, namely, thyroxine (T4) and triiodothyronine (T3). Subsequently, 80% of T4 is converted to T3 in the target organ. Various biological actions of thyroid hormones (THs) are mediated by proteins and receptors located in the cell membrane that allows hormones to enter cells. Deiodines that convert T4 and T3 to metabolites with lower iodine content control the cytoplasmic metabolic action of THs. Finally, nuclear receptors bind more closely or exclusively to T3; thus, the genomic actions of THs are mediated by T3 [1]. T3 binds to nuclear receptors so that gene expression and isoforms of TH receptors (TRα1, TRα2, TRβ1, and TRβ2) are encoded by *THRA* and *THRB*, located in chromosomes 17q21.1 and 3p24.2 [2,3]. Resistance to TH syndromes (RHT) have a genetic origin and are characterized by decreased tissue sensitivity to THs. Most cases are of mutations in *THRB* that encodes for one of the two types of the T3 nuclear receptor, TRβ1 and TRβ2 [4].

RTH CAUSED BY *THRB* MUTATIONS (RTH-β)

RTH-β is a rare condition caused by *THRB* mutations [5]. RTH was first described in 1967 in a family with deaf congenital disease and pointed epiphysis. Disease prevalence is unclear because routine detection tests for congenital hypothyroidism are based solely on the determination of TSH, which is often normal under this condition [6]. Most RTH-β cases are caused by heterozygous variants in *THRB* [4]. *THRB* mutations have been associated with a T3 receptor binding alteration, retaining homo- and heterodimerization capability, or with an aberrant ability to dissociate from the correlator in the presence of T3. The series of

hydrophobic repeats are crucial for homodimer and heterodimer formation in TH receptors, which do not usually exhibit mutations. *THRB* mutations are predominantly grouped into three "hot" regions in the c-terminal region of *THRB*, involved with T3 hormone binding of protein [7]. A retrospective study of 14 patients with RTH and late manifestations of RTH syndrome identified that all had point mutations, located between exons 7–10 within the union domain to T3. Seven patients had widespread resistance to TH, and four showed euthyroidism. However, the other three patients experienced thyroid dysfunction before the age of 10 years, and all patients with early onset had a short stature and different degrees of learning disruption [8]. RTH-β is characterized by high levels of T4, T3, and rT3 accompanied by normal or high serum TSH levels [4]. The clinical symptoms of patients with RTH vary from manifestations of hyperthyroidism in organs primarily expressing TRα (heart and bone) to hypothyroidism in tissues expressing TRβ (hypophysis, brain, and thyroid gland). Hypercholesterolemia, delayed growth, and learning disorders (suggestive of hypothyroidism), can coexist with weight loss, heat intolerance, hyperactivity, and tachycardia [9].

The clinical manifestations of RTH are classified into two subgroups according to the absence or presence of symptoms of thyrotoxicosis, selective pituitary resistance, and generalized resistance of TH (GRTH). Patients with selective pituitary resistance show variable symptoms of hyperthyroidism; however, patients with GRTH exhibit "compensated hypothyroidism," which controls the effect of high TH levels. In several cohorts, the cardiac phenotypes of patients with different mutations, which included tachycardia and palpitations, were examined, although flutter or atrial fibrillation has been reported up to 20% of patients. No heart failure cases were reported, and no correlation was found between the genotype and the cardiac phenotype [10]. The main differential diagnosis of this condition is thyrotropoma, a TSH-producing pituitary tumor. TSH-producing pituitary tumors are characterized by a biochemical pattern of TH similar to RHT; however, unlike RTH, it presents an increase in α-subunits and sex hormone binding globulin. The genetic study is definitive for differentiation, and approximately 15% of cases have failed to identify the genetic cause [11].

The treatment mainly aims to maintain a balance between the overstimulation of the tissues expressing TRβ in patients with RTH [5]. Currently, no definitive therapy is available to correct the molecular defect that causes RTH syndrome, and most patients do not require treatment [5,9]. Patients with tachycardia and palpitations at rest may benefit from β-blocker therapy. If thyroid toxicity symptoms are present, triiodothyroacetic acid (TRIAC) or dextrothyroxine (DT4) can be administered to treat thyrotoxicosis. This compound, through a feedback mechanism, reduces TSH secretion and decreases circulating T4 levels. TRIAC given at a dose of 1.4–2.8 mg/day divided into two or three administrations is effective in both pediatric and adult patients with RTH [6]. The use of dopamine drugs and somatostatin analogs has limited success because TSH secretion quickly escapes the inhibitory effects of both drugs, as T4 reduction strongly stimulates TSH secretion (Table 1) [4,9].

Table 1. Manifestations of resistance to thyroid hormone syndrome

	RTH-β	RTH-α
Mutation receptor	TR-β1, TR-β2	TR-α1, TR-α2
Mutation gene	Thyroid hormone receptor-β (*THR-β*) gene	Thyroid hormone receptor-α (*THR-α*) gene
Clinical manifestations	Vary from hyperthyroidism to hypothyroidism or simple goiter	Anemia, Constipation, Growth retardation, Developmental delay, Motor and Cognitive development delay
Treatment	Most patients do not require treatment β-blocker (with tachycardia) TRIAC or DT4 (with thyrotoxicosis) Dopamine drugs Somatostatin analogs	Levothyroxine

RTH, Resistance to thyroid hormone syndrome; TR, thyroid hormone receptors; TRIAC, triiodothyroacetic acid; DT4, dextrothyroxine.

RTH CAUSED BY *THRA* MUTATIONS (RTH-α)

RTH-α is characterized by tissue-dependent hypothyroidism findings, and >40 *THRA* mutations have been recorded [12]. Most mutations selectively involve the c-terminal domain of TRα, generating severe loss of function and other mutation types associated with a variable-form function of TRα1 [13]. In these cases, the phenotype is characterized by manifestations of hypothyroidism that affects gastrointestinal, neurological, skeletal, and myocardial functions. Clinical characteristics associated with hypothyroidism (e.g., macrocephaly, hypertelorism, flattened nasal bridge, and a broad face) became more evident with time, together with poor linear growth, resulting in a dysmorphic appearance. Patients with RTH-α have normal hormonal response in the pituitary hypothalamic axis and liver. The severity of the clinical phenotype appears to be associated with the mutation location and type in *THRA*. When hetero-

zygous mutations in this gene are present, abnormalities include anemia, constriction, and delayed growth and development [9]. The A263V mutation of TRα1 showed partial loss of function, with higher T3 levels. TRα2 has no mutation-related phenotypes identified; however, some unusual clinical characteristics, such as micrognasia, clavicular agenesis, hyperparathyroidism, and chronic diarrhea, were reported in an adult woman with a different mutation (N359Y), common for both TRα1 and TRα2 [14]. In RTH-α, serum T3 levels can be high or normal, T4 and rT3 levels are in the normal range or low, and TSH levels are normal or slightly high [3].

Levothyroxine therapy generates the normalization of T4 levels, with additional increase in T3 and suppressed TSH levels, suggesting conserved negative feedback of TH over TSH secretion (Table 1) [3].

CONCLUSION

RTH is a group of genetic-related syndromes that result from different mutations, either in the genes they encode for receptors. These syndromes are characterized by decreased tissue sensitivity to THs, generating various clinical manifestations. Thus, clinical changes of resistance to TH must be recognized and differentiated, and an approach to the practice of personalized medicine through an interdisciplinary approach is needed.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Bernal J. Síndromes de resistencia a las hormonas tiroideas. Endocrinol Nutr 2011;58:185-96. doi: 10.1016/j.endonu.2011.02.001.
2. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest 2006;116:2571-9. doi: 10.1172/JCI29812.
3. Van Gucht AL, Moran C, Meima ME, Visser WE, Chatterjee K, Visser TJ, et al. Resistance to thyroid hormone due to heterozygous mutations in thyroid hormone receptor alpha. Curr Top Dev Biol 2017;125:337-55. doi: 10.1016/bs.ctdb.2017.02.001.
4. Groeneweg S, Peeters RP, Visser TJ, Visser WE. Therapeutic applications of thyroid hormone analogues in resistance to thyroid hormone (RTH) syndromes. Mol Cell Endocrinol 2017;458:82-90. doi: 10.1016/j.mce.2017.02.029.
5. Vela A, Pérez-Nanclares G, Ríos I, Rica I, Portillo N, Castaño L. Thyroid hormone resistance from newborns to adults: a Spanish experience. J Endocrinol Invest 2019;42:941-9. doi: 10.1007/s40618-019-1007-4.
6. Singh BK, Yen PM. A clinician's guide to understanding resistance to thyroid hormone due to receptor mutations in the TRα and TRβ isoforms. Clin Diabetes Endocrinol 2017;15:8. doi: 10.1186/s40842-017-0046-z.
7. Censi S, Barollo S, Watutantrige-Fernando S, Manso J, Ferrara AM, Mian C. A Novel thyroid hormone receptor beta mutation (G357R) in a family with resistance to thyroid hormone beta: extending the borders of the "hot" region in the THRB Gene. Thyroid 2019;29:449-51. doi: 10.1089/thy.2018.0201.
8. Han R, Ye L, Jiang X, Zhou X, Billon C, Guan W, et al. Characteristics of patients with late manifestation of resistance thyroid hormone syndrome: a single-center experience. Endocrine 2015;50:689-97. doi: 10.1007/s12020-015-0622-x.
9. Persani L, Campi I. Syndromes of resistance to thyroid hormone action. En: genetics of endocrine diseases and syndromes. Genetics of Endocrine Diseases and Syndromes 2019;55-84. doi: 10.1007/978-3-030-25905-1_5.
10. Illouz F, Briet C, Mirebeau-Prunier D, Bouhours-Nouet N, Coutant R, Sibilia P, et al. Cardiac complications of thyroid hormone resistance syndromes. Ann Endocrinol 2021;82:167-9. doi: 10.1016/j.ando.2020.03.008.
11. Sun H, Cao L, Zheng R, Xie S, Liu C. Update on resistance to thyroid hormone syndromeβ. Ital J Pediatr 2020;46:168. doi: 10.1186/s13052-020-00929-x.
12. National Library of Medicine (NIH). ClinVar [Internet]. https://www.ncbi.nlm.nih.gov/ clinvar/
13. Moran C, Agostini M, McGowan A, Schoenmakers E, Fairall L, Lyons G, et al. Contrasting phenotypes in resistance to thyroid hormone alpha correlate with divergent properties of thyroid hormone receptor α1 mutant proteins. Thyroid 2017;27:973-82. doi: 10.1089/thy.2017.0157.
14. Moran C, Chatterjee K. Resistance to thyroid hormone α-emerging definition of a disorder of thyroid hormone action. J Clin Endocrinol Metab 2016;101:2636-9. doi: 10.1210/jc.2016-2317.

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Effect of Synthetic CaM and NFAT Oligodeoxynucleotide on MPP$^+$-Stimulated Mesencephalic Neurons

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Background: Ca^{2+} signaling plays a vital role in neuronal signaling and altered Ca^{2+} homeostasis in Parkinson's disease (PD). Overexpression of αSYN significantly promote the Ca^{2+}-Calmodulin (CaM) activity and subsequent nuclear translocation of nuclear factor of activated T cells (NFAT) transcription factor in dopaminergic neurons of midbrain. However, the exact role of Ca^{2+}-CaM and NFAT in PD pathology is yet to be elucidated.
Methods: We designed the CaM-NFAT-oligodeoxynucleotide (ODN), a synthetic short DNA containing complementary sequence for NFAT transcription factor and CaM mRNA. Then, the effect of CaM-NFAT-ODN on 1-methyl-4-phenylpyridinium (MPP$^+$)-mediated neurotoxicity was investigated in mimic PD model *in vitro*.
Results: First, the expression of αSYN and CaM was strongly increased in substantia nigra (SN) of PD and the expression of tyrosine hydroxylase (TH) was strongly increased in control SN. Additionally, the expression of apoptosis marker proteins was strongly increased in SN of PD. Transfection of CaM-NFAT-ODN repressed CaM and pNFAT, the target genes of this ODN in rat embryo primary mesencephalic neurons. It also reduced ERK phosphorylation, a downstream target of these genes. These results demonstrated that CaM-NFAT-ODN operated successfully in rat embryo primary mesencephalic neurons. Transfection of CaM-NFAT-ODN repressed TH reduction, αSYN accumulation, and apoptosis by MPP$^+$-induced neurotoxicity response through Ca^{2+} signaling and mitogen-activated protein kinases (MAPK) signaling.
Conclusion: Synthetic CaM-NFAT-ODN has substantial therapeutic feasibility for the treatment of neurodegenerative diseases.

Key words: Dopaminergic neurons, Oligodeoxynucleotide, Tyrosine hydroxylase, Apoptosis, Calmodulin, Nuclear factor of activated T cells

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INTRODUCTION

Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders [1]. The pathology of the disease is the selective and progressive loss of the dopaminergic neurons, leading to the formation of Lewy bodies in substantia nigra (SN) pars compacta of midbrain [2]. Lewy bodies are formed by fibrils of alpha-synuclein (αSYN), and this cascade is an important step in the development of PD [3]. Aggregated αSYN regulates dopamine biosynthesis by triggering dopamine transporter (DAT) recruitment of dopamine and reducing the activity or the phosphorylation status of tyrosine hydroxylase (TH), thus leading to neuronal demise [4]. To date, no drugs have been developed to alleviate the loss of dopaminergic neurons in PD and the pathogenesis of PD remains controversial.

Ca^{2+} homeostasis plays a key role in the degeneration of dopaminergic neurons [5]. Additionally, the increase in cytosolic Ca^{2+} is dependent on Ca^{2+} channel activity and αSYN expression, and these two pathogenic factors were reported to act in PD [4]. The degeneration of dopaminergic neurons is a major contributor to the pathogenesis of midbrain disorders [6]. Ca^{2+} is important for many cellular processes, such as proliferation, growth, differentiation, development, and cell death [7]. Calcium signaling is essential for regulating dopamine concentration

levels in neurons [8]. Exposure to Ca^{2+} overload has been reported to be an important determinant, and pharmacological treatments that inhibits Ca^{2+} entry reduce neuronal damage in chemical models of PD [9]. Calmodulin (CaM), the best studied Ca^{2+}-binding protein, is abundantly expressed in the brain [10]. CaM contains globular domains at its C- and N-terminus respectively [11]. Each globular domain of CaM has a pair of Ca^{2+} binding sequences, and after binding Ca^{2+}, a hydrophobic surface is exposed [12]. The expression of CaM is up regulated in response to the increase in intracellular Ca^{2+} concentration and can thus lead to NFAT (nuclear factor of activated T-cells) dephosphorylation and nuclear translocation [9].

Recent reports have highlighted the important role of the NFAT transcription factor family in neurons involved in the regulation of synaptic plasticity, axonal growth and neuronal survival [13]. The NFAT regulatory domain is highly phosphorylated in the inactive state, covering the nuclear localization sequence and causing the NFAT protein to remain in the cytoplasm [14]. NFAT proteins directly regulate the expression of apoptosis-related genes and with the promotion of inflammatory process [15]. Both apoptosis and inflammation are processes widely involved in the development of PD [7,16].

The synthetic oligodeoxynucleotide (ODN) technique is a gene therapy strategy that DNA or RNA based molecular compounds that are utilized to disrupt gene transcription or translation [17]. Antisense ODN are used for selective impairment of protein synthesis based on the specific sequence of the target RNA [18,19]. Decoy ODN is employed to block transcription factor activity using synthetic double stranded ODN containing DNA binding sites, which works at the pre-transcription level [20,21]. Advances in gene therapy strategies have provided new technologies to inhibit dual-target gene expression based on short RNA and DNA strategies [22,23]. To improve a new therapeutic approach, in this study we used a combination of antisense ODN and decoy ODN to synthesize CaM-NFAT-ODN, which inhibits both CaM and NFAT. Although these ODN have proven beneficial in several disease models, it has not yet been demonstrated whether CaM-NFAT-ODN can attenuate the development of molecular mechanisms of neurotoxicity. Therefore, we investigated the effect of CaM-NFAT-ODN on neuronal cytotoxicity in an *in vitro* model of Parkinson's disease.

Table 1. Human postmortem tissues used for Figure 1A

KBBN ID	Age	Gender	Final diagnosis	Tissue
07004509	50–59	F	Control	SN
07004529	60–69	M	PD	SN

F, Female; M, Male; SN, Midbrain with substantia nigra; PD, Parkinson disease.

METHODS

Synthesis of oligodeoxynucleotides

Synthetic ODNs were commissioned by Macrogen (Seoul, Korea). To generate the ODN of CaM, it was selected through sequential overlapping simulations of the secondary structure using the S-Fold program. The sequence of CaM antisense ODN is 5′-GAA TTC GGT ATC AAG AAC TAG ATA TAC C-3′ and the sequence of NFAT decoy ODN is 5′-GAA TTC TGA CTT TCC TCA TGA AAA CAT GAG GAA AGT AC-3′. The underline is the target site as well as the agreed combining order. These ODNs were structured by annealing and each ODN was mixed with T4 ligase (Takara Bio, Otsu, Japan) to obtain a covalent ligation for ring-type ODN molecules.

Human Brain Samples and rat embryo primary mesencephalic neurons culture

A total of eight postmortem brain samples were obtained (Tables 1 and 2); with clinically and neuropathologically diagnosed PD and controls. Brain sections and tissues were provided by the Korea Brain Bank Network (KBBN) operated through the National Brain Bank Project funded by the Ministry of Science and ICT. The project was approved by the Daegu Catholic University Institutional Review Board, DCU IRB (IRB number: CR-20-149).

Mesencephalic neuron cultures were prepared from the ven-

Table 2. List of human postmortem tissues used for Figure 1B

Sample No.	KBBN ID	Age	Gender	Final diagnosis	Tissue
1	07004507	50–59	F	Control	SN
2	07004508				
3	06012591	60–69	M		
4	06012592				
5	06012596	50–59	M		
6	06012597				
1	07004517	70–79	M	PD	SN
2	07004518				
3	07004527	60–69	M		
4	07004528				
5	05007340	70–79	F		
6	05007344	80–89	F		
7	05007348	70–79	M		

F, Female; M, Male; SN, Midbrain with substantia nigra; PD, Parkinson disease.

tral mesencephalic tissues of embryonic day 13–14 rats, as described previously [7,24]. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Daegu Catholic University Medical Center (EXP-IRB number: DCIAFCR-191112-07-Y) in accordance with criteria outlined in the Institutional Guidelines for Animal Research. Briefly, dissociated cells were seeded on poly-d-lysine and laminin-coated plates. Cells were cultured in a Dulbecco's modified Eagle's medium/Ham's F-12 medium (Gibco, NY, USA) containing ITS premix (Sigma-Aldrich, St Louis, MO, USA) and 1% penicillin-streptomycin (Gibco).

Transfection and Morphology examination

Rat embryo primary mesencephalic neurons were transfected with synthetic ODN using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The transfection efficiency of synthetic ODN was observed using ODN labeled with fluorescein isothiocyanate (FITC) using the Label IT nucleic acid labeling kit (Mirus Bio, Madison, WI, USA). After transfection, the rat embryo primary mesencephalic neurons were cultured in 50 μM of MPP^+ for 24 hr.

Nissl bodies in human brain slides were detected by a previously described Nissl staining method [25]. Nissl Staining Solution (Cresyl violet stain solution; Abcam, Cambridge, MA, USA) manufacturer's instructions. Tissues sections were washed in cold phosphate-buffered saline (PBS) for 15 min and block with universal blocking solution (0.3% Triton™ X-100, 1% BSA, 0.05% Tween 20, 0.1% cold fish gelatin and 0.05% sodium azide in PBS) for 1 hr at room temperature.

The morphology of rat embryo primary mesencephalic neurons was observed using an inverted phase contrast microscope (Olympus CKX41SF, Tokyo, Japan, ×200 or ×400 magnification).

Immunoblot analysis

Protein samples were prepared from human brain tissue of the SN (Table 2) and rat embryo primary mesencephalic neuron with a protein extraction buffer (N-PER™, Thermo Fisher Scientific, Waltham, MA, USA) according to the instruction manual. The protein samples were separated on precast gradient polyacrylamide gels (Bolt™ 4%–12% Bis-Tris Plus Gels; Thermo Fisher Scientific) and transferred to nitrocellulose membranes (GE Healthcare, Madison, WI, USA) by using Bolt™ Mini Blot Module and Mini Gel Tank (Thermo Fisher Scientific), according to the manufacturer's recommendations. The membrane blocked with 5% bovine serum albumin was probed with a primary antibody and horseradish peroxidase-conjugated secondary antibody. Following a repeat of the wash step, the membrane was kept in enhanced chemiluminescence detection reagents (Thermo Fisher Scientific). Signal intensity was measured with an image analyzer (ChemiDoc™ XRS+; Bio-Rad Laboratories). The protein expression values were normalized to GAPDH and β-actin expression values. The primary antibodies used were anti-TH (Cat no: 13106, Cell Signaling Technology, Danvers, MA, USA), anti-αSYN (Cat no: 2628, Cell Signaling Technology), anti-PARP (Cat no: 9542, Cell Signaling Technology), anti-CaM (Cat no: sc-137079, Santa Cruz Biotechnology, TX, USA), anti-pNFATc3 (Cat no: sc-8405, Santa Cruz Biotechnology), anti-NFATc3 (Cat no: sc-365883, Santa Cruz Biotechnology), anti-pERK (Cat no: 4377, Cell Signaling Technology), anti-ERK (Cat no: 4695, Cell Signaling Technology) and anti-β-actin (Cat no: SAB3500350, Sigma-Aldrich), anti-GAPDH (Cat no: sc-47724, Santa Cruz Biotechnology).

Immunofluorescent staining

Human brain tissues were deparaffinized in xylene and subjected to citrate antigen retrieval prior to immunohistochemistry [25]. Cells were incubated with primary antibodies for 1 hr at room temperature and they were incubated with the Alexa Flour 488 (excitation/emission = 495/519 nm, green, Invitrogen, CA, USA) and Alexa Flour 594 (excitation/emission = 590/617 nm, red, Invitrogen) for 30 min at room temperature. Cells were counterstained with DAPI (excitation/emission = 330–380 nm/460 nm, ImmunoChemistry, MN, USA). Slides were mounted using ProLong® Gold antifade reagent (Molecular Probes® by Life Technologies™, CA, USA). Immunolabeling was examined using an Eclipse Ti-U and confocal microscope (Nikon, Tokyo, Japan).

RESULTS

Expression of TH, αSYN, PARP-1 and CaM in the SN of human Parkinson's disease brain

Increase in cytosolic Ca^{2+} is dependent on Ca^{2+} channel activity and αSYN expression [4]. To determine whether these findings are relevant to PD, we examined human postmortem SN from control versus PD patients. Nissl-stained positive neurons in the SN were significantly reduced in the PD brain compared to human control brain (Table 1 and Fig. 1A). αSYN and CaM expression was strongly increased in SN of PD, and expression of TH, a dopaminergic neuron marker, was strongly increased in control SN (Table 2 and Fig. 1B). Cleaved-PARP-1, an apoptosis marker protein, was also confirmed to be strongly increa-

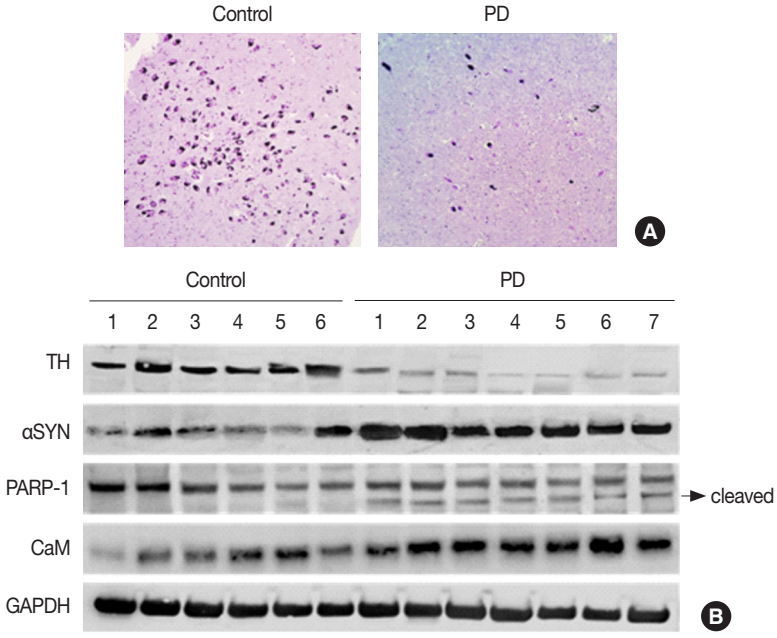


Fig. 1. Expression of TH, αSYN, PARP-1 and CaM in the substantia nigra of human Parkinson's disease brain. (A) Nissl staining, Magnification ×200, (B) Immunoblot analysis of TH, αSYN, PARP-1 and CaM in the SN of human control and Parkinson's disease brain. GAPDH was used to confirm equal sample loading.

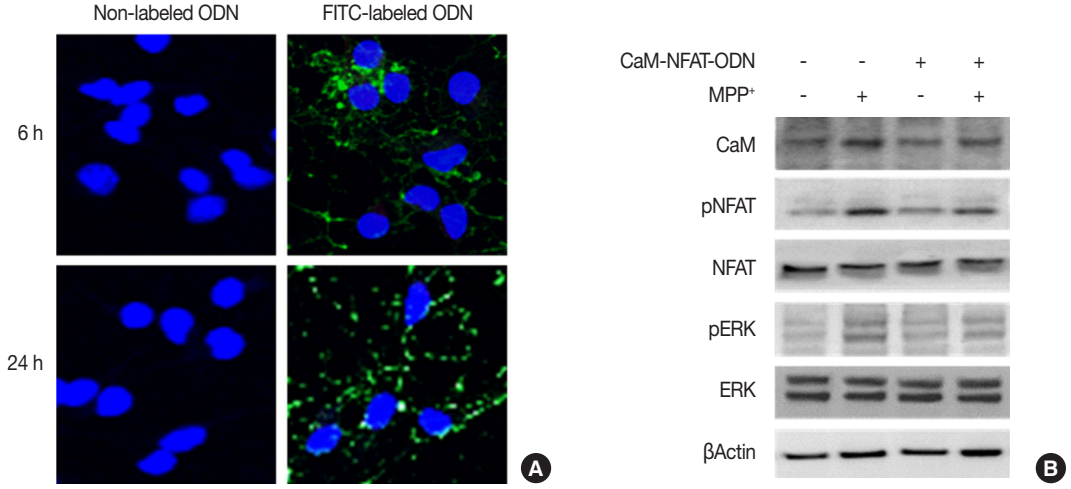


Fig. 2. Effect of CaM-NFAT-ODN in rat embryo primary mesencephalic neurons. (A) Representative immunofluorescence images showing the fluorescence activity in both the cytoplasm and nucleus detected via FITC-labeled ODN deposition (green). Cells were counterstained with DAPI (blue). Magnification ×1,600. (B) Immunoblot analysis of CaM, pNFAT, NFAT, pERK and ERK in rat embryo primary mesencephalic neurons. βActin was used to confirm equal sample loading.

sed in SN of PD (Fig. 1B). These results demonstrated that CaM is related to apoptosis of dopaminergic neurons and PD.

CaM-NFAT-ODN protects rat embryo primary mesencephalic neurons against MPP^+-Induced Neurotoxicity

To confirm the efficacy and distribution of the CaM-NFAT-ODN, we transfected rat embryo primary mesencephalic neurons with FITC-labeled ODN. As shown in Fig. 2A, FITC-CaM-NFAT-ODN was successfully transfected and delivered into the nucleus and cytoplasm of rat embryo primary mesencephalic neurons and observed for 24 hr. Moreover, immunoblot analysis showed that the CaM-NFAT-ODN decreased the protein levels of CaM, pNFAT, as well as the key downstream targets of

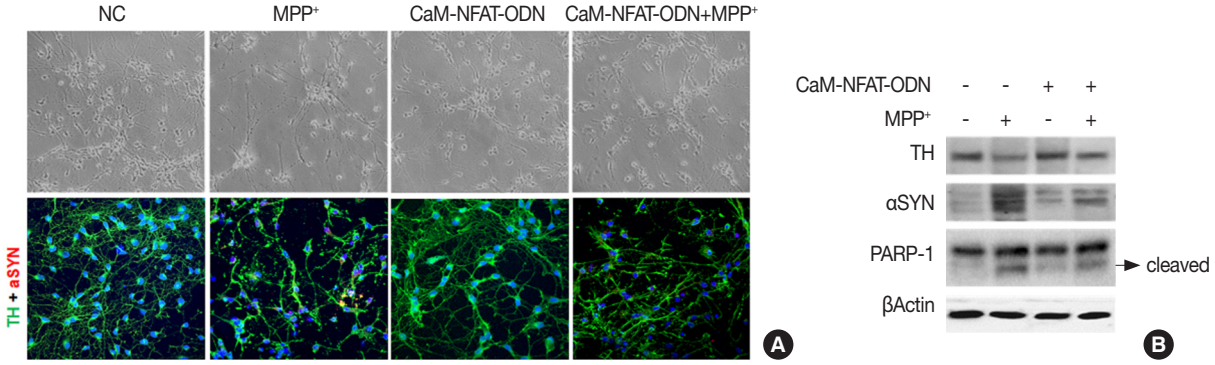


Fig. 3. The CaM-NFAT-ODN influenced the expression of TH, αSYN and PARP-1 in MPP^+ treated rat embryo primary mesencephalic neurons. (A) The morphological changes, TH and αSYN expression in dopaminergic neurons after exposure to MPP^+ in the presence or absence of APM. Immunofluorescence staining for TH (green) and αSYN (red) localization. Cells were counterstained with DAPI (blue). Magnification ×400. (B) CaM-NFAT-ODN strongly reduced expression of TH reduction, accumulation of αSYN and cleaved PARP-1 expression in rat embryo primary mesencephalic neurons, βActin was used to confirm equal sample loading.

ERK phosphorylation (Fig. 2B).

To determine whether Synthetic CaM-NFAT-ODN could regulate the expression of MPP^+-induced TH and αSYN in rat embryo primary mesencephalic neurons. The rat embryo primary mesencephalic neurons showed obvious neurites, and the neurons transfected with only CaM-NFAT-ODN were observed similar to normal neurons (Fig. 3A). When rat embryo primary mesencephalic neurons were exposed to the neurotoxin MPP^+, neurites were reduced, and neuronal debris increased. Interestingly, it was rescued by CaM-NFAT ODN transfection.

Rat embryo primary mesencephalic neurons were confirmed to be healthy TH-positive neurons with extensive neurites through immunofluorescence staining using a TH antibody. However, exposure to neurotoxin for 24 hr decreased the number of TH-positive neurons and induced accumulation of αSYN. When transfected with CaM-NFAT ODN, the number and morphology of TH-positive neurons were similar to normal neurons. Exposure of MPP^+ in CaM-NFAT-ODN transfected neurons appears to protect the cells from loss of TH-positive neurons and accumulation of αSYN. Consistent with immunofluorescence staining, CaM-NFAT-ODN attenuated TH reduction and accumulation of αSYN in rat embryo primary mesencephalic neurons (Fig. 3B). CaM-NFAT-ODN inhibited MPP^+-induced cleaved PARP-1, apoptosis marker proteins, in rat embryo primary mesencephalic neurons.

DISCUSSION

Appearance of PD symptoms is attributed to the loss of dopaminergic neurons in the striatum and substantia nigra [10]. Regarding PD, many CaM-regulated Ca^{2+} channels seem to be implicated in this pathology [26]. Neuronal cell death may be due to increased Ca^{2+}, which is commonly observed in neurons in various areas of PD brain [5,8]. Additionally, the increase in cytosolic Ca^{2+} is not due to MPP^+-induced oxidative stress but is dependent on Ca^{2+} channel activity and αSYN expression, and these two pathogenic factors have been reported to act in PD [27]. Our result showed that αSYN and CaM expression was strongly increased in Parkinson's disease SN, and expression of TH, a dopaminergic neuron marker, was strongly increased in control SN.

A Study has reported on potential neuroprotective agents through attenuation of MPP^+ and Ca^{2+}-overload-induced excitotoxicity in dopaminergic neurons [28]. Many reports suggest that Ca^{2+} is involved in the pathogenesis of PD and that regulation of Ca^{2+} may be a potential therapeutic target for neuroprotection in PD [29]. For an effective treatment that targets the causes of PD, it is necessary to systemically determine the therapeutic component based on the inhibition of initial molecule mechanism.

We have reported the anti-inflammatory and anti-fibrotic properties of synthetic targeted ODN in chronic diseases [17, 20,21,23]. Recently, we also reported the effects of a selective antagonist of the small-conductivity Ca^{2+}-activated K^+ channels antagonist on neuroinflammation and neurotoxicity [7,30]. However, the molecular mechanisms of MPP^+-induced neurotoxicity of ODN in PD models have not yet been elucidated.

In this study, we investigated the effects of CaM-NFAT-ODN, which simultaneously regulates the NFAT and CaM, on the neurotoxicity of rat embryo primary mesencephalic neurons.

In PC12 cells, MPP^+-induced loss of cell viability was medi-

ated by increased intracellular Ca^{2+} levels and CaM action [31]. Overexpression of αSYN activates the CaM-NFAT pathway in cell lines and dopaminergic neurons, whereas inhibition of this pathway protects dopaminergic neurons against αSYN-mediated toxicity [32]. The Ca^{2+}-CaM-ERK mitogen-activated protein kinase signaling pathway induces neuronal cell death in rat brain [33]. Our result showed that CaM-NFAT-ODN inhibited ERK phosphorylation, reduction of TH, αSYN expression and apoptosis in MPP^+-induced rat embryo primary mesencephalic neurons. Thus, gene therapy targeted to suppress mRNA level of CaM and transcription activity of NFAT simultaneously might provide a new therapeutic strategy to prevent various neurological disorders.

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CONFLICTS OF INTEREST

Not applicable.

AUTHOR CONTRIBUTIONS

Investigation: Park J and Jang KM. Writing original draft preparation: Park J and Jang KM. Writing review: Jang KM. Funding acquisition: Park J. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Zhao D, Feng PJ, Liu JH, Dong M, Shen XQ, Chen YX, et al. Electromagnetized-nanoparticle-modulated neural plasticity and recovery of degenerative dopaminergic neurons in the mid-brain. Advanced Materials 2020;32:2003800. doi: 10.1002/adma.202003800.
2. Wu J, Xu X, Zheng L, Mo J, Jin X, Bao Y. Nilotinib inhibits microglia-mediated neuroinflammation to protect against dopaminergic neuronal death in Parkinson's disease models. International Immunopharmacology 2021;99:108025. doi: 10.1016/j.intimp.2021.108025.
3. Wang X, Cao G, Ding D, Li F, Zhao X, Wang J, et al. Ferruginol prevents degeneration of dopaminergic neurons by enhancing clearance of α-synuclein in neuronal cells. Fitoterapia 2022;156:105066. doi: 10.1016/j.fitote.2021.105066.
4. Lieberman OJ, Choi SJ, Kanter E, Saverchenko A, Frier MD, Fiore GM, et al. α-Synuclein-dependent calcium entry underlies differential sensitivity of cultured sn and vta dopaminergic neurons to a parkinsonian neurotoxin. eNeuro 2017;4. doi: 10.1523/eneuro.0167-17.2017.
5. Sun Y, Zhang H, Selvaraj S, Sukumaran P, Lei S, Birnbaumer L, et al. Inhibition of L-type Ca2+ channels by TRPC1-STIM1 complex is essential for the protection of dopaminergic neurons. Journal of Neuroscience 2017;37:3364-77. doi: 10.1523/JNEUROSCI.3010-16.2017.
6. Chen L, Huang Y, Yu X, Lu J, Jia W, Song J, et al. Corynoxine protects dopaminergic neurons through inducing autophagy and diminishing neuroinflammation in rotenone-induced animal models of parkinson's disease. Front Pharmacol 2021;12:642900. doi: 10.3389/fphar.2021.642900.
7. Park J, Jang KM, Park KK. Effects of apamin on MPP(+)-induced calcium overload and neurotoxicity by targeting CaMKII/ERK/p65/STAT3 signaling pathways in dopaminergic neuronal cells. Int J Mol Sci 2022;23. doi: 10.3390/ijms232315255.
8. Pawar A, Pardasani KR. Computational model of calcium dynamics-dependent dopamine regulation and dysregulation in a dopaminergic neuron cell. The European Physical Journal Plus 2023;138:30. doi: 10.1140/epjp/s13360-023-03691-1.
9. Chen L, Song M, Yao C. Calcineurin in development and disease. Genes & Diseases 2022;9:915-27. doi: 10.1016/j.gendis.2021.03.002.
10. Bohush A, Lesniak W, Weis S, Filipek A. Calmodulin and its binding proteins in Parkinson's disease. Int J Mol Sci 2021;22. doi: 10.3390/ijms22063016.
11. Ren R, Guo J, Chen Y, Zhang Y, Chen L, Xiong W. The role of Ca2+/Calcineurin/NFAT signalling pathway in osteoblastogenesis. Cell Proliferation 2021;54:e13122.
12. An S. The emerging role of extracellular Ca(2+) in osteo/odontogenic differentiation and the involvement of intracellular Ca(2+) signaling: from osteoblastic cells to dental pulp cells and odontoblasts. J Cell Physiol 2019;234:2169-93. doi: 10.1002/jcp.27068.
13. Sant'Anna R, Robbs BK, Freitas JAd, Santos PPd, König A, Outeiro TF, et al. Alpha-synuclein oligomers activate NFAT proteins modulating synaptic homeostasis and apoptosis. bioRxiv 2023:2023.02.21.529374. doi: 10.1101/2023.02.21.529374.
14. Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev 2003;17:2205-32. doi: 10.1101/gad.1102703.
15. Mognol GP, Carneiro FRG, Robbs BK, Faget DV, Viola JPdB. Cell cycle and apoptosis regulation by NFAT transcription factors: new roles for an old player. Cell death & disease 2016;7:e2199. doi: 10.1038/cddis.2016.97.
16. Park J, Jang KM, Park KK. Apamin suppresses LPS-induced neuroinflammatory responses by regulating sk channels and TLR4-mediated signaling pathways. Int J Mol Sci 2020;21. doi: 10.3390/ijms21124319.
17. Gu H, An HJ, Gwon MG, Bae S, Zouboulis CC, Park KK. The effects of synthetic SREBP-1 and PPAR-gamma decoy oligodeoxynucleotide on acne-like disease in vivo and in vitro via lipogenic

regulation. Biomolecules 2022;12. doi: 10.3390/biom12121858.
18. Bennett CF, Swayze EE. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annual Review of Pharmacology and Toxicology 2010; 50:259-93. doi: 10.1146/annurev.pharmtox.010909.105654.
19. Mann MJ, Dzau VJ. Therapeutic applications of transcription factor decoy oligonucleotides. The Journal of Clinical Investigation 2000;106:1071-5. doi: 10.1172/JCI11459.
20. Lee SJ, Kim YA, Park KK. Anti-Fibrotic effect of synthetic noncoding decoy ODNs for TFEB in an animal model of chronic kidney disease. International Journal of Molecular Sciences 2022; 23:8138. doi: 10.3390/ijms23158138.
21. Gwon MG, Leem J, An HJ, Gu H, Bae S, Kim JH, et al. The decoy oligodeoxynucleotide against HIF-1α and STAT5 ameliorates atopic dermatitis-like mouse model. Molecular Therapy-Nucleic Acids 2023;34:102036. doi: 10.1016/j.omtn.2023.102036.
22. Kim KH, Park KK. Small RNA-and DNA-based gene therapy for the treatment of liver cirrhosis, where we are? World Journal of Gastroenterology: WJG 2014;20:14696-705. doi: 10.3748/wjg.v20.i40.14696.
23. Kim JY, An HJ, Kim WH, Gwon MG, Gu H, Park YY, et al. Antifibrotic effects of synthetic oligodeoxynucleotide for TGF-beta1 and smad in an animal model of liver cirrhosis. Mol Ther Nucleic Acids 2017;8:250-63. doi: 10.1016/j.omtn.2017.06.022.
24. Park JH, Seo YH, Jang JH, Jeong CH, Lee S, Park B. Asiatic acid attenuates methamphetamine-induced neuroinflammation and neurotoxicity through blocking of NF-kB/STAT3/ERK and mitochondria-mediated apoptosis pathway. J Neuroinflammation 2017;14:240. doi: 10.1186/s12974-017-1009-0.
25. Nam JH, Park ES, Won SY, Lee YA, Kim KI, Jeong JY, et al. TRPV1 on astrocytes rescues nigral dopamine neurons in Parkinson's disease via CNTF. Brain 2015;138(Pt 12):3610-22. doi: 10.1093/brain/awv297.
26. Bohush A, Leśniak W, Weis S, Filipek A. Calmodulin and its binding proteins in Parkinson's disease. International Journal of Molecular Sciences 2021;22:3016. doi: 10.3390/ijms22063016.
27. Chen TS, Koutsilieri E, Rausch WD. MPP+ selectively affects calcium homeostasis in mesencephalic cell cultures from embryonal C57/B16 mice. Journal of Neural Transmission/General Section JNT 1995;100:153-63. doi: 10.1007/BF01271538.
28. Egunlusi AO, Malan SF, Omoruyi SI, Ekpo OE, Palchykov VA, Joubert J. Open and rearranged norbornane derived polycyclic cage molecules as potential neuroprotective agents through attenuation of MPP(+)- and calcium overload-induced excitotoxicity in neuroblastoma SH-SY5Y cells. Eur J Med Chem 2020;204: 112617. doi: 10.1016/j.ejmech.2020.112617.
29. Swart T, Hurley MJ. Calcium channel antagonists as disease-modifying therapy for Parkinson's disease: therapeutic rationale and current status. Cns Drugs 2016;30:1127-35. doi: 10.1007/s40263-016-0393-9.
30. Park J, Jang KM, Park KK. Apamin suppresses LPS-induced neuroinflammatory responses by regulating SK channels and TLR4-mediated signaling pathways. International Journal of Molecular Sciences 2020;21:4319. doi: 10.3390/ijms21124319.
31. Lee DH, Han YS, Han ES, Bang H, Lee CS. Differential involvement of intracellular Ca 2+ in 1-methyl-4-phenylpyridinium-or 6-hydroxydopamine-induced cell viability loss in PC12 cells. Neurochemical Research 2006;31:851-60. doi: 10.1007/s11064-006-9088-9.
32. Sompol P, Furman JL, Pleiss MM, Kraner SD, Artiushin IA, Batten SR, et al. Calcineurin/NFAT signaling in activated astrocytes drives network hyperexcitability in Aβ-bearing mice. Journal of Neuroscience 2017;37:6132-48. doi: 10.1523/JNEUROSCI.0877-17.2017.
33. Sticozzi C, Belmonte G, Meini A, Carbotti P, Grasso G, Palmi M. IL-1β induces GFAP expression in vitro and in vivo and protects neurons from traumatic injury-associated apoptosis in rat brain striatum via NFκB/Ca2+–calmodulin/ERK mitogen-activated protein kinase signaling pathway. Neuroscience 2013;252:367-83. doi: 10.1016/j.neuroscience.2013.07.061.

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