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Neurodegeneration with Brain Iron Accumulation

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Recent advances in magnetic resonance imaging and identification of causative genes led to the recognition of a new group of disorders named neurodegeneration with brain iron accumulation (NBIA). NBIA is a group of inherited disorders characterized by abnormal iron deposition in the brain, usually in the basal ganglia. The disorder shares the clinical features of movement disorders and is accompanied by varying degrees of neuropsychiatric abnormalities. In this review, the causative genes, clinical presentations, neuroimaging features, and pathological findings are summarized.

Key words: Neurodegeneration, Iron, NBIA

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INTRODUCTION

Neurodegeneration with brain iron accumulation (NBIA) is a group of inherited disorders with hallmark features that include abnormal iron accumulation in the basal ganglia, mainly the globus pallidus and substantia nigra, and progressive movement disorders that include dystonia, spasticity, parkinsonism, and varying degrees of intellectual disability, as well as other neuropsychiatric abnormalities [1]. Prevalence data is incomplete, but all forms of NBIA are rare, between one and three per million individuals in the general population [1,2].

Ten genes associated with different NBIA subtypes have been identified: *PANK2*, *PLA2G6*, *C19orf12*, *WDR45*, *COASY*, *FA2H*, *ATP13A2*, *FTL*, *CP*, and *DCAF17* [3,4]. The four most common NBIA disorders include pantothenate kinase-associated neurodegeneration (PKAN; NBIA1, MIM#234200), phospholipase A2-associated neurodegeneration (PLAN; NBIA2B, MIM#610217), mitochondrial membrane protein-associated neurodegeneration (MPAN; NBIA4, MIM#614298), and beta-propeller protein-associated neurodegeneration (BPAN; NBIA5, MIM#300894) [5]. Inheritance pattern of most NBIA disorders is autosomal recessive [1]. Neuroferritinopathy (NBIA3, MIM#606159) is the only autosomal-dominant subtype. BPAN is inherited in a dominant X-linked manner. Recently, a number of new NBIA subtypes are being recognized as a result of next-generation sequencing (NGS) [5]. *SCP2*, *GTPBP2*, *AP4M1*, *REPS1*, and *CRAT* can be considered NBIA genes [2,6].

Here, we review the known NBIA genes and their proposed disease mechanisms, and summarize the clinical, magnetic resonance imaging (MRI), and pathological features of NBIA disorders.

MOLECULAR MECHANISMS

Only two genes, *FTL* in NBIA3 and *CP* in aceruloplasminemia (MIM#604290), are directly associated with iron homeostasis [3,4]. The other forms are not directly involved in iron metabolism; instead, they are involved in diverse cellular pathways: *PANK2* in PKAN) and *COASY* in Coenzyme A synthase protein-associated neurodegeneration (CoPAN; NBIA6, MIM# 615643) code for enzymes responsible for coenzyme A (CoA) synthesis; *PLA2G6* in PLAN), *FA2H* in fatty acid hydrolase-associated neurodegeneration (FAHN; SPG35, MIM#612319), and *C19orf12* in MPAN appear to be related to lipid metabolism, membrane integrity, and mitochondrial function; *WDR45* in BPAN and *ATP13A2* in Kufor-Rakeb syndrome (MIM#606693) are implicated in autophagosome and lysosomal activity, while the *DCAF17* in Woodhouse-Sakati syndrome (MIM#241080) encodes a nucleolar protein of unknown function [2-4].

Although the presence of excessive iron accumulation enables these diseases to be included in the NBIA disorders, pathogenic processes leading to iron dyshomeostasis are still not fully elucidated [2,3]. The contribution of iron to disease pathophysiology also remains unclear owing to the lack of experimental models that fully recapitulate the human phenotype [2].

CLINICAL FINDINGS

Patients with NBIA commonly exhibit mixed dystonia, parkinsonism, and spasticity [1]. However, the clinical features of NBIA range from global neurodevelopmental delay in infancy to mild parkinsonism in adulthood, with wide variation between and within the specific NBIA subtype, making the diagnosis of these rare diseases challenging [1,5]. The late-onset forms of NBIA may also mimic the clinical presentations of other neurodegenerative diseases [7].

PKAN may present in age-dependent phenotypes: the classic form with early onset and the atypical variant with later onset [8,9]. The classic form is characterized by pyramidal and extrapyramidal features with prominent dystonia and rapid progression [8]. Gait and postural difficulties are often the presenting features developing in the first decade of life. Retinopathy is often present. The atypical variant presents in the second or third decade of life with less severe and slow-progressive extrapyramidal and pyramidal signs [8]. Speech difficulties and psychiatric symptoms with cognitive decline are often observed.

Similar age-dependent presentations have also been recognized in PLAN; early-childhood-onset form results in infantile neuroaxonal dystrophy with severe progression, whereas later-onset PLAN may be milder and present commonly with dystonia-parkinsonism without cerebellar or sensory abnormalities [1,7,9]. These examples highlight the marked variability in clinical phenotype and suggest that NBIA should be considered as a possible diagnosis in patients of any age and irrespective of family history [7].

MPAN typically occurs in the first decade of life to early adulthood. The phenotype is characterized by spastic gait with upper motor neuron findings, followed by lower motor neuron signs; electrophysiologically, it is characterized as a motor neuronopathy/axonopathy, dystonia, and parkinsonism, commonly accompanied by optic atrophy and neuropsychiatric features [1,9,10].

BPAN is a biphasic disease: global developmental delay in childhood and further regression in early adulthood with progressive dystonia, parkinsonism, and dementia [1,9,11]. BPAN typically presents with seizures, spasticity, and Rett syndrome-like features [11].

Clinical features of FAHN comprise childhood-onset spasticity, ataxia, and dystonia [9]. FAHN is a disorder allelic to hereditary spastic paraplegia type 35 (SPG35). Kufor-Rakeb syndrome, also known as PARK9, is caused by mutations in the *APT13A2* and is characterized by a syndrome of juvenile-onset parkinsonism, spasticity, and cognitive decline [9]. Woodhouse-Sakati syndrome presents with hypogonadism, diabetes mellitus, alopecia, extrapyramidal movement disorders, intellectual disability, and sensorineural hearing loss [1].

Similar to PKAN, CoPAN due to mutations in the *COASY* presents with early-onset gait difficulty and learning disabilities, followed by progressive spasticity and extrapyramidal features [1,6].

Neuroferritinopathy and aceruloplasminemia typically present in mid-life with the average age of onset much later than other NBIA subtypes [1,9]. Neuroferritinopathy, often thought of as one of the Huntington-like disorders [9], is a dominantly-inherited syndrome of chorea, dystonia, parkinsonism, cognitive decline, and low serum ferritin. Aceruloplasminemia is characterized by diabetes, retinal disease, and a movement disorder consisting of facial dystonia, chorea, tremor, parkinsonism, ataxia, cognitive decline, and low or absent serum ceruloplasmin. Systemic iron accumulation results in peripheral retinal degeneration and liver iron storage [9].

MRI FEATURES

The diagnosis of NBIA is typically suspected when compati-

ble MRI features are identified along with representative clinical features [12]. Iron-sensitive sequences such as T2* and susceptibility-weighted imaging are more sensitive for demonstration of brain iron accumulation [13]. The established hallmark MRI features include hypointense lesions in the globus pallidus and substantia nigra on T2-weighted images [7,14].

In PKAN, iron-related MRI signal abnormalities are restricted to the globus pallidus and substantia nigra, and almost invariably exhibit the "eye of the tiger sign," in which a central hyperintense region in the globus pallidus is surrounded by a hypointense region [13,15]. In MPAN cases, pallidal hypointensity can be seen with hyperintense streaking in the region of the medial medullary lamina [1,16]. In BPAN, the hypointensity is most pronounced in the substantia nigra where it appears as a discrete linear streak. This same area on T1 sequences is surrounded by a hyperintense "halo" extending to the cerebral peduncles [1,18]. Cavitation involving the globus pallidus and putamen is unique to neuroferritinopathy [15]. However, the morphological patterns of these lesions can vary according to the patient's age [13,17]. Typical findings do not appear until adolescence or early adulthood.

Widespread brain iron accumulation involving the basal ganglia nuclei, thalami, dentate nuclei, and cerebral and cerebellar cortices may develop in aceruloplasminemia and neuroferritinopathy [15]. However, cases of genetically proven PLAN and Kufor-Rakeb syndrome with no evidence of iron deposition on MRI have also been described [7].

In addition to excessive iron, non-iron MRI abnormalities are helpful in diagnosis [14,16]. Cerebellar atrophy is a hallmark feature of PLAN [16,18]. Hypertrophy of the clava has been proposed as an important early feature of PLAN and may precede cerebellar atrophy [19, 20]. T2 hyperintense white matter abnormalities are prominent in FAHN, Woodhouse-Sakati syndrome, and aceruloplasminemia [16]. Thinning of the corpus callosum is seen in FAHN, PLAN, and BPAN [11,16].

NEUROPATHOLOGY

On a pathological level, iron accumulation may be accompanied by protein aggregates (e.g., Lewy bodies, hyperphosphorylated tau) and axonal swellings, depending on NBIA subtype [4]. For example, widespread alpha-synuclein-positive Lewy body pathology has been described in patients with PLAN [21]. In addition, neuropathological examination of a patient with the MPAN has shown the presence of Lewy bodies, spheroids, and tau-positive tangle pathology [10]. Tau-positive neurofibrillary tangles are common in the brains of patients with BPAN [4,11].

CONCLUSIONS

NBIA is a phenotypically and genetically heterogeneous group of disorders. There have been significant advances in identifying new genes/mutations involved in NBIA as a result of the development of technology based on NGS [5,6], which has also facilitated early detection of patients with nonspecific phenotype. Due to their clinical and pathophysiologic overlap with common neurodegenerative diseases, such as Parkinson's and Alzheimer's disease, NBIA disorders may stand at the forefront of understanding the common pathways in neurodegeneration [22].

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CONFLICTS OF INTEREST

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Laboratory Misdiagnosis of von Willebrand Disease Caused by Preanalytical Issues: Sample Collection, Transportation, and Processing

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von Willebrand disease (VWD) is a genetic bleeding disorders caused by a deficiency of von Willebrand factor (VWF). Diagnosis or exclusion of VWD is not an easy task for most clinicians. These difficulties in diagnosis or exclusion of VWD may be due to preanalytic, analytical and postanalytic laboratory issues. Analytical systems to diagnose VWD may produce misleading results because of limitations in their dynamic range of measurement and low sensitivity. However, preanalytical issues such as sample collection, processing, and transportation affect the diagnosis of VWD profoundly. We will review here the common preanlytical issues that may impact the laboratory diagnosis of VWD.

Key words: Preanalytical, Variables, von Willebrand Disease (VWD), von Willebrand Factor (VWF)

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INTRODUCTION

Von Willebrand disease (VWD) is a congenital bleeding disorder caused by a deficiency of von Willebrand factor (VWF) and typically characterized by mild mucosal bleeding [1]. VWD may be caused by either a quantitative or a qualitative defect in VWF. The quantitative VWF defects are divided clinically into mild-moderate defects, known as type 1 VWD, and severe defects with undetectable VWF, known as type 3 VWD. The qualitative variants are known as type 2 VWD and divided further based upon the specific defect in VWF present. The current classification of the type 2 variants separates these into four groups [2].

The laboratory workup of VWD involves determining the level of VWF by both functional and antigenic methods. Per recommendations from the National Heart Lung and Blood Institute (NHLBI) that were developed by a working group in 2008, initial testing for VWD should include 1) VWF Ristocetin Cofactor assay, 2) VWF antigen assay, and 3) coagulation FVIII activity assay [3]. Second-tier specialized testing should include multimeric analysis, VWF collagen binding assay, ristocetin induced platelet aggregation (RIPA), platelet VWF assessments, genetic sequencing, FVIII binding assessment, and the VWF propeptide assay.

Despite these testing guidelines, several variables can affect the accuracy of VWF testing in general and this, in turn, may impact result interpretation and VWD diagnosis. Difficulties in achieving a correct diagnosis or exclusion of VWD might be due to analytical issues. Sometimes assays may generate a wrong result (i.e., due to analytical errors) or may have limitations in their dynamic range of measurement and/or their level of low analytical sensitivity, for example preventing

the correct identification of severe cases of VWD, which are characterized by very low factor levels. Less well recognized is the influence of preanalytical issues on the diagnosis of VWD [4]. During the pre-analytical phase, in case of hemostasis testing for patients with a positive history of hemorrhagic diathesis, certain steps regarding the preparation of patients and the execution of sampling (specimen collection, transportation, sample preparation, and storage) are of special importance. Therefore, this narrative review aims to provide an overview of some important preanalytical aspects such as sample collection, processing, and transportation that may affect the laboratory diagnosis of VWD.

SAMPLE COLLECTION

VWD studies tend to use blood anticoagulated with 3.2% sodium citrate, although 3.8% citrate concentrations are still in use in some centers. Most hemostasis researchers try to collect with minimal stasis or tourniquet use (to avoid hemoconcentration) and large-bore needles, or for platelet function to avoid platelet activation. Smaller one [23G] may be employed for pediatric use or difficult collections), as mentioned in several guidance publications [5-7]. There are no data to support the need for a discard tube for specialized hemostasis assays. However, due to a lack of sufficient evidence, the practice of drawing a discard tube should still be recommended [7]. Stress should be avoided under all circumstances (e.g., restless child) because stress increases acute phase proteins of which VWF, factor VIII, and fibrinogen are most important in particular in the workup for VWD. We also recognize the importance of proper tube fill volumes, with potential concern for both under-and over-filled tubes. Citrate tubes should contain 10% by volume of citrate anticoagulant, meaning 9 parts blood is added to 1 part anticoagulant. Under-filling tends to be most problematic, causing both dilution and over-citration ("over-anticoagulation") of plasma, leading to prolonged results for routine tests (e.g., PT and APTT), and false lowering of specific analytes (e.g., FVIII, FIX, VWF). This has the potential to cause misdiagnosis of VWD if analyte levels are close to cut-off or decision points (i.e., lower limit of the normal reference range, differentiating "normal" vs. "low"; typically around 50 U/dL, or "50% of normal"). Perhaps less well known/frequent, are effects of over-filling evacuated blood tubes (especially as available volumes facilitating over-filling are minimal for most blood tubes). The most likely effect here is to compromise adequate mixing of blood, potentially leading to sample clotting—either complete or partial [8]. Complete sample clotting leads to serum, with the potential loss of VWF through entrapment in the clot [9]. Partial sample clotting often leads to sample activation ("priming of coagulation"), which may shorten APTTs and artificially raise laboratory detected factor levels. Under-filled tubes may also lead to a false diagnosis of VWD or may exaggerate the perceived disorder. There are many situations where non-citrate anticoagulated tubes are collected for patients investigated for VWD. The best example is the EDTA tube for CBC, perhaps focusing on platelet counts as an alternative/complementary explanation of the bleeding disorder under investigation. EDTA samples are also used for VWF Multimer analysis and genetic testing [10].

Entrapment of VWF in clots may also lead to loss of VWF, preferentially high molecular weight (HMW) VWF, and thus misdiagnosis of VWD, especially type 2 [9]. Although workers in this field know such sample types to be inappropriate for standard VWD investigations, rejecting such samples when primary tubes were provided and thus visually inspected, problems may arise when testing laboratories are geographically distant from collection centers, with separation of "plasma" from centrifuged primary tubes, with (often frozen) material sent to test laboratories [11]. Once received frozen, the originating matrix is unclear, but can be determined if investigated. However, this adds cost and complexity and may not normally be executed.

The effect of blood hematocrit is another issue commonly discussed in sample collections and especially as related to pathology testing. The Clinical and Laboratory Standards Institute guideline on blood collection recommends undertaking citrate adjustments when hematocrit values are >0.55 [7].

Finally, all collected blood tubes should be labeled in front of the patient, using not less than two identifiers to avoid patient mix-ups, and attributing (analytically accurate) test results to the wrong patient.

SAMPLE TRANSPORTATION AND PROCESSING

Blood samples are usually need processed and transported to testing laboratories after blood collection. When possible, samples should be drawn directly in a laboratory. Immediately after drawing, whole blood should remain capped for transport both for safety reasons and to minimize loss of CO_2, which causes pH to increase, leading to prolongation of PT and/or aPTT [12]. Which comes first depends on geographical distances involved. For short distances, samples may be transported

whole, then centrifuged, then tested immediately or separated/frozen for subsequent testing. For long distances, samples may be centrifuged and then separated plasma transported (sometimes frozen) to testing laboratories [11]. Whole blood should be transported preferably upright in tube racks to avoid excessive agitation [7,9] and at "normal ambient" temperature (i.e., between 16 and 24°C). Extremes of temperature (low/refrigerated or high/"back of a van") should be avoided. Transport at high temperatures or even delayed transport at ambient temperature will lead to loss of labile factors and thus potentially to a false diagnosis of disease. Cold storage of citrated whole blood induces drastic time-dependent losses in VWF that contribute potential for misdiagnosis of VWD [13]. Refrigerated transport of whole blood should cause absorption of VWF (especially HMW VWF), and activation of platelets and FVII, thus leading to possible misidentification of VWD (especially as type 2); if transported this way, misidentification may occur in a normal sample, or from a type 1 VWD [14].

Whenever a delay in transport is expected, it might be advisable to perform local centrifugation and separation. Plasma is generally prepared by centrifugation of a whole blood sample. A temperature-controlled centrifuge is required for processing routine coagulation assays. Centrifugation should take place at room temperature (15–25°C) [7]. It is recommended to centrifuge the primary tube for coagulation testing at 1,500 g for no less than 15 min with a centrifuge brake set off. The centrifuge should be validated before use, every 6 months or after modifications, to assure that platelet-poor plasma (PPP) is achieved. Using relative centrifugal forces greater than 1,500 g is not recommended as this may induce platelet activation, hemolysis or other unwanted effects [7]. Alternate centrifugation, double centrifugation, protocols may be acceptable, providing residual platelet count is minimized and platelets are not activated by excessive centrifugal force. This low residual platelet count is perceived most important for lupus anticoagulant (LA) investigations, where double centrifugation protocols are commonly employed [15]. Depletion of platelets is especially important, should plasma be frozen/thawed, because residual platelets will be damaged and generate lysates quenching LA, thus potentially generating false-negative test results. For centrifugation, a swing-out bucket is recommended to minimize the remixing of plasma with platelets/erythrocytes, with resultant cell lysates on freeze/thawing. Filtration of plasma may cause loss of adhesive proteins (e.g., fibrinogen, VWF), leading to misdiagnosis of VWD [16], and is no longer encouraged, with double centrifugation now standard [15].

Whole blood assays should be performed within 4 hr after blood sampling and centrifugation should ideally be taken within 1 hr. Cold storage of citrated whole blood before centrifugation, by placing samples either in an ice bath or in refrigerated (2–8°C) storage, is no longer recommended. Improper storage of whole blood at cold temperature may cause VWF and factor VIII values to fall below normal reference threshold levels, which may potentially lead to a false suspicion of VWD due to inappropriate pre-analytical handling of blood [13,17]. When testing is not performed immediately, plasma can be frozen for later testing. Normal domestic freezers can be used for short-term storage (< 1 week), except if they are subject to cyclic freeze/thaw events (i.e. frost-free freezers). Otherwise, low-temperature freezers (≤ 70°C) should be used for long-term storage. Please refer to expert guidance on storage conditions [7,18]. Inappropriate thawing and mixing of plasma is another important issue. Different procedures may be used (i.e., gentle end-over-end inversions, blood tube rockers, vortex mixers), generating modest differences in clotting assays, including VWF [5,19-21]. Worse, no mixing leads to the clear potential for VWD misdiagnosis [20]. Therefore, laboratories should select one mixing procedure and then use standard operating procedures containing clear indications of the approved technique that should then always be followed for mixing thawed plasma. Mixing applies not only to test laboratories about to perform tests after samples are frozen/thawed but also to sample processing laboratories, which may thaw plasma post freezing to aliquot and dispatch to a distant laboratory.

CONCLUSIONS

The misleading results due to preanalytical issues can be minimized by the cooperation of laboratorians and clinicians. Clinicians should provide all relevant clinical information sufficiently for the laboratorians to adopt the proper techniques, and laboratorians should provide thorough post-analytical guidance on the test results including limitations and potential drawbacks and as well as for subsequent follow-up strategy.

Preanalytical issues arise uniquely for the specific reagents and analysis systems. These considerations imply that various preanalytical events may affect different test results, leading to complex test-panel patterns, and difficulty in interpretation, with subsequent potential for misdiagnosis of VWD. Standardizing efforts are needed to reduce the overall chances of the preanalytical phase, to generate high-quality test results and to

guarantee patient safety [5,22]. International external quality assessment schemes are also now starting to provide tools that cover the preanalytical issues of hemostasis testing.

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Coffin-Lowry Syndrome – The First Genetically Confirmed Case in Korea Diagnosed by Whole Exome Sequencing

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Coffin-Lowry syndrome (CLS) is a genetic disorder characterized by intellectual disability, typical facial features, and skeletal abnormalities. But this syndrome shows highly variable clinical manifestations, and can't be diagnosed with conventional chromosome analysis or comparative genomic hybridization, leading to delayed diagnosis. Here we report an 18-year-old boy with CLS diagnosed by whole exome sequencing. Our patient initially presented with developmental delay, facial dysmorphism at the age of 1. At the age of 18, he developed orthopnea due to mitral regurgitation. At the 22 years of age, he was diagnosed as CLS diagnosed by whole exome sequencing. Our case implies that clinical suspicion is important for early diagnosis, and advanced diagnostic tools such as WES should be considered in suspected cases.

Key words: Coffin-Lowry syndrome, Diagnosis, Whole exome sequencing

BRIEF REPORT

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INTRODUCTION

Coffin-Lowry syndrome (CLS) is a rare X-linked genetic syndrome causing severe intellectual disability. Other slow physical growth, distinctive facial appearance, large soft hands, and minor skeletal changes with general muscular hypotonia [1]. Early diagnosis of CLS is essential for proper management, including survey of some specific complications, and for genetic counselling. Establishing the diagnosis in very young children is often much more difficult than in older patients since clinical manifestations are milder or not specific [2]. We describe here a case of CLS diagnosed by whole exome sequencing (WES).

PATIENT REPORT

An 18-year-old boy was referred to our genetic, metabolic disease clinic for genetic evaluation due to intellectual disability and facial dysmorphism.

He was born full term with normal birth weight of 3 kg. His growth was normal, but his development was delayed. When he was about 1 year old, he visited other hospital due to developmental delay. But no definite diagnosis was obtained, and he received physical therapy with no more investigation.

At the age of 18, he developed orthopnea and visited pediatric cardiology clinic. On Echocardiography mitral regurgitation, grade III was observed, and enalapril, furosemide, spironolactone and digooxin were prescribed. And he was referred to genetic, metabolic disease clinic for evaluation for genetic disease.

On physical examination, his height and weight were normal range with 172 cm,

62 kg. He had malar hypoplasia, hypertelorism, thick lips, irregular teeth, long face, and low set ear. He had severe intellectual disability and mild hearing difficulty.

Laboratory findings showed increased uric acid level (9.4 mg/dL), and other tests including thyroid function test, liver function test, glucose and lipid were normal. Chromosome study was normal. Further evaluation such as array comparative genomic hybridization (CGH) was recommended, but he was lost to follow up.

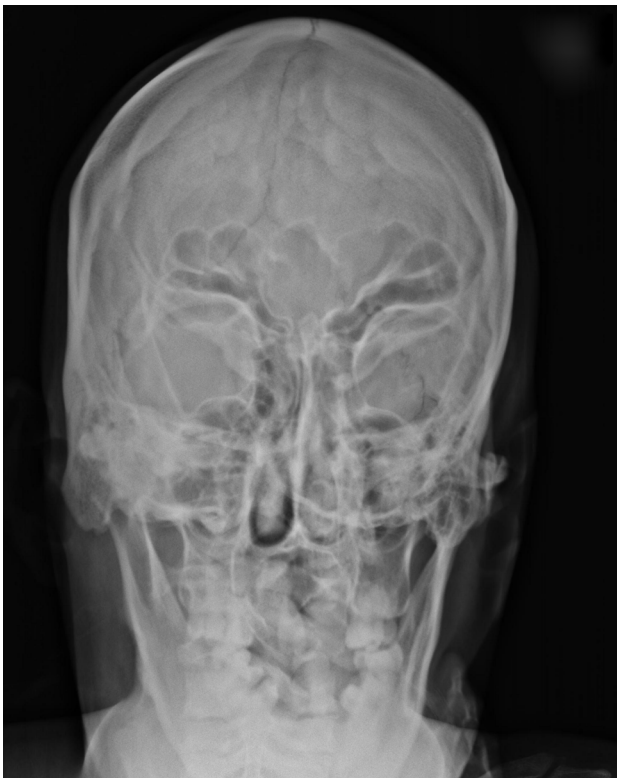

Fig. 1. Skull X-ray showed diffuse thickened cranial vault, enlarged frontal sinus, and protruded mandible.

After 4 years, symptoms of heart failure aggravated, and he received operation (open heart annuloplasty of mitral valve, tricuspid annuloplasty). At that time, he was again referred to our clinic. Laboratory findings showed increased uric acid level (8.0 mg/dL). His liver function test (AST 115 IU/L, ALT 25 IU/L) and lipid profile (total cholesterol 244 mg/dL, triglyceride 219 mg/dL, HDL 40 mg/dL, LDL 119 mg/dL) were also abnormal.

On X-ray exam, diffuse thickened cranial vault, enlarged frontal sinus, and protruded mandible were observed (Fig. 1). Liver ultrasonography findings suggested mild fatty liver. WES was performed to identify underlying genetic cause in the patient. Informed consent was obtained from patients and their family. The average coverage for WES was 100X. Variants in the dbSNP135 and TIARA databases for Koreans and the variants with minor allele frequencies >0.5% of the 1000 Genomes database were excluded. We selected only the functional variants. WES revealed a novel mutation in *RPS6KA3* gene (likely pathogenic, PM2+PM4+PP3+PP4), which was confirmed by direct sequencing, resulting in hemizygous frameshift mutation (c.889_890delAG, p.Leu298Phefs*21) (Fig. 2). Trio testing showed that this mutation was derived from his mother. He had one sister, and his parents and sister had no symptoms associated with CLS.

He is now on regular follow up with anti-hypertensive agents (cancesartan, amlodipine), diuretics (furosemide), and gout medication (colchicine, febuxostat).

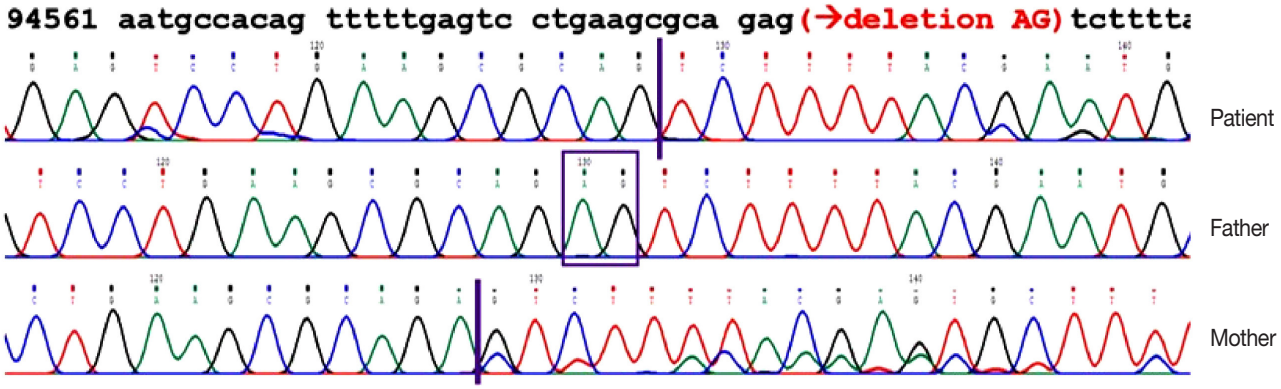

Fig. 2. Sanger sequencing from the patient and parents revealed a variant with 2 bp deletion in exon11 (NM_004586.2), c.889_890delAG (p.Leu298Phefs*21), which caused frameshifts starting from codon 298 with a premature stop codon.

DISCUSSION

CLS is a rare genetic disease, with a prevalence of 1 in 50,000 to 1 in 100,000 in males [3]. It is caused by loss-of-function mutations of the *RPS6KA3* gene, which encodes RSK2 (ribosomal S6 kinase 2) [4]. RSK2 is highly expressed in neocortex, the hippocampus, and Purkinje cells, which are important cognitive function and learning [5]. Over 140 inactivating mutations of *RPS6KA3* gene have been reported [3]. In Korea, clinically suspected CLS case was first reported in 2007 [6], and our case is the first report of Korean patients with genetically confirmed CLS. Newborn males often show hypotonia and hyperlaxity of joints, whereas growth parameters are usually within normal range. Facial abnormalities such as hypertelorism, frontal bossing are usually apparent by the second year of life and show progressive coarsening [3]. Common musculoskeletal findings include kyphoscoliosis, sclerosis of skull, and distal tufting of hands [7]. Variable enlargement or hypoplasia of the frontal sinuses also can be observed, like in our case. Most patients show severe cognitive deficiencies. Our patient showed abnormal facial, skeletal findings and intellectual disability. And interestingly, he developed dyslipidemia and fatty liver at young age, though he was not obese. The chronic health conditions in adult CLS patients including metabolic/endocrine disorders remains to be further investigated.

Patients with CLS can usually be diagnosed on the basis of clinical presentation and radiological findings. But patients with this syndrome do not show growth restriction or severe physical abnormal findings in prenatal or neonatal period. Also, clinical presentations are markedly variable. So significant portion of patients seem to remain undiagnosed or misdiagnosed for a long time, like our case. Usual screening test for developmental delay is conventional chromosome study and array CGH. But CLS cannot be diagnose with these tests, which is another reason of delayed diagnosis in our case. For diagnosis of CLS, suspicion with careful history taking and physical examination is most important. And evaluation with WES can be useful, like in our case.

Our study is the first report of genetically confirmed CLS in Korea. Clinical suspicion of physicians and advanced diagnostic tools such as WES will reduce undiagnosed or misdiagnosed cases. More patients with CLS are reaching adulthood due to improved medical care, and concerns for chronic medical conditions will be required to improve quality of life.

CONFLICTS OF INTEREST

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

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Nonessential tables and figures may accompany articles as

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Short communications of original research are published as case report. The purpose of the category is to permit publication of very important, high-quality mechanistic studies that can be concisely presented. These manuscripts should include a short nonstructured abstract (150 words maximum), Introduction, Methods, Results, and Discussion.

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