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Case Report

Unraveling the Genetic Puzzle: Could MAP3K7 Be a Candidate Gene for RASopathies? Case Presentation

Kizilcan Cetin S et al. A Candidate Gene for Noonan Syndrome

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What is already known about this topic?
While *MAP3K7* variants have been associated with disorders like Cardiospondylocarpofacial syndrome (CSCFS) and Frontometaphyseal dysplasia 2 (FMD2). The incidence of NS is approximately 1:1000, and while genetic confirmation is ideal, it may not be possible for all cases. Negative genetic test results do not necessarily exclude NS, emphasizing the importance of clinical evaluation.

What this study adds to the literature?
The patient's clinical features, such as short stature, valvular heart disease, and facial dysmorphisms, are compatible with NS characteristics despite the *MAP3K7* variant being classified as a variant of uncertain significance. This case raises the question of whether *MAP3K7* could be a candidate gene for Noonan Syndrome (NS).

Abstract
Noonan Syndrome (NS) diagnosis is challenging due to diverse clinical manifestations. Here, our case report highlights *MAP3K7*'s novel role in NS. A 10.4-year-old female patient presented with short stature and suggestive clinical findings of RASopathy. Despite atypical facial features, the patient met two major diagnostic criteria of Van der Burgt.Initial genetic testing for known NS-associated genes did not find any variants. Later, whole exome sequencing (WES) discovered a unique de novo heterozygous variant (c.65C>A, p.(P22H)) in the *MAP3K7*. This variant, categorized as a variant of uncertain significance (VUS) by the American College of Medical Genetics and Genomics (ACMG) criteria, raised questions about its potential role in NS. The patient's clinical presentation deviated from classical manifestations of *MAP3K7*-associated syndromes, underscoring the genetic and molecular mechanisms' complexity. Notably, this is the first case reported to associate *MAP3K7* variants with NS, advancing knowledge of the condition's genetic causes. Despite challenges in NS diagnosis, proper management, including recombinant growth hormone therapy, is crucial for optimizing growth potential. The case underscores *MAP3K7* as a potential candidate gene for NS, and more functional genetic investigations are required to clarify the delicate interaction between genetic abnormalities, the RAS/MAPK pathway, and clinical manifestations observed in NS cases.
Keywords: Noonan Syndrome, short stature, *MAP3K7*

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Introduction
A group of diseases known as rasopathies is caused by pathogenic gene changes that encode parts of the intracellular signaling pathway Rat-sarcoma-Mitogen-activated-protein-kinase (RAS/MAPK). These diseases are characterized by common clinical traits like distinctive facial features, short stature, and congenital heart disease(1). Noonan Syndrome (NS) is the group's most predominant disorder. Its prevalence ranges from 1 in 1,000 to 1 in 2,500 live births (2, 3). The diagnosis of NS presents a challenge due to the wide range of clinical manifestations. However, since 1994, Van der Burgt et al. ‘s scoring method has significantly assisted in diagnosing NS accurately (4, 5). A diagnosis of NS is confirmed if there is either a typical facial appearance coupled with one major / two minor clinical characteristic findings or if facial features indicative of NS are present along with two major / three minor clinical features (4, 5). This system considers major criteria such as typical facial dysmorphology, cardiac anomalies (such as pulmonary valve stenosis and characteristic ECG findings), short stature (below the 3rd percentile), chest wall deformities (such as pectus carinatum, pectus excavatum), and additional features (intellectual disability, cryptorchidism, or lymphatic dysplasia). Minor criteria involve suggestive facial dysmorphology, non-major heart defects, short stature (below the 10th percentile), broad chest, and suggestive features in first-degree relatives.
Nearly 20 genes (including *PTPN11, SOS1, SOS2, KRAS, NRAS, RIT1, RRAS, RASA1, RASA2, MRAS, RAF1, BRAF, MAP2K1, MAP3K8, SHOC2, PPP1CB, SPRY1, LZTR1, MYST4, A2ML1, CBL*), which are involved in the Ras/MAPK pathway and NS manifestation, have been discovered thus far (6, 7). As a result, around 85% of cases can now be explained by known genetic factors. However, negative tests do not necessarily rule out the disorder. Thus, clinical diagnosis remains vital(7, 8). The etiology of the unidentified cases where the pathogenesis remains unidentified holds the potential for uncovering new genes and mechanisms in the future, emphasizing the necessity of recognizing NS clinical features for precise diagnosis.
In this case report, we present a patient with a de novo heterozygous variant in *MAP3K7*,c.65C>A, p.(P22H), exhibiting NS suggestive facial features and meeting two major Van der Burgt criteria, thereby fulfilling NS diagnostic criteria. Our presentation highlights the novel role of *MAP3K7* in NS, prompting contemplation on the intricate interplay between genetic anomalies, the RAS/MAPK pathway, and the diverse clinical manifestations observed in NS cases.

Case Report
A 10.4-year-old female patient was admitted to our outpatient clinic due to short stature. Her medical history revealed that she was born to non-consanguineous parents at 38 weeks of gestation, with a birth weight of 2500 g. She had undergone a number of clinical assessments.

Her diagnosis remained uncertain for a very long time. She had pulmonary valve stenosis, pectus excavatum, and failure to thrive in early infancy, so she was suspected of NS. Chromosome analysis was 46,XX (100 metaphases). Targeted gene panel sequencing of *PTPN11, SOS1,* and *RAF1* showed no variants. The microarray analysis was normal. On follow-up, her height was 110.8 cm (-3.3 SD). Treatment with recombinant growth hormone (rhGH) was initiated at 0.2 mg/kg/week at the age of 8.5 years. Linear growth during rhGH treatment was as follows: 5 cm/year in the first year and 8 cm/year in the second year of treatment. Whole exome sequencing (WES) revealed a novel heterozygous c.65C>A, p.(Pro22His) variant in MAP3K7 (NM_145331.3) (Figure 1).According to The American College of Medical Genetics and Genomics (ACMG) criteria, this variant is classified as a variant of uncertain significance (VUS). The parents were informed by their doctor that all genetic tests were normal.

On admission, the patient's height was 124.5 cm (-2.57 SD), and her BMI was 14.58 kg/m2(-1.38 SD). The target height was 153.5 cm (-1.23 SD). Physical examination revealed the phenotypic features such as a synophrys, prominent supraorbital ridges, mild ptosis, low set ears, full cheeks, broad nasal tip, deep elongated philtrum, thin upper vermillion, low anterior hairline, triangular face, short webbed neck, superior pectus carinatum and inferior pectus excavatum, short metacarpals and metatarsals, clinodactyly, syndactyly, intellectual disability, dyslexia (Figure 2A).

Laboratory testing revealed normal hemogram and biochemical markers, such as blood glucose, thyroid function, tissue transglutaminase autoantibody IgA, serum total IgA, and liver and kidney function. Her urine test results were normal. GH stimulation tests with clonidine excluded GH deficiency (peak GH of 9,63 ng/ml). The bone age was seven years and ten months, and the bone survey was normal with incidental accessory bone, os tibiale externum. According to pituitary magnetic resonance imaging (MRI), the hypophysis length was 2.5 mm (normal range: 4.5±0.6 mm) and cranial MRI was normal. Before our admission, the patient had not been followed up properly. Her diagnosis was not certain, and the rhGH treatment was experimental. Her growth velocity slowed by 1 cm/six months on our follow-up, so rhGH was stopped. IGF-1 levels were within the acceptable range for the pubertal stage, gender, and age. There was no pathology on laboratory tests. The radiographic bone survey was normal (Figure 3). There was no finding suggestive of skeletal dysplasia. The patient underwent regular monitoring through electrocardiography, echocardiography, and abdominal and pelvic ultrasonography, which revealed no abnormal ultrasonographic findings. Subsequent echocardiograms showed a minimal atrial septal defect (ASD) with no evidence of hypertrophic cardiomyopathy.

The MAP3K7 c.65C>A variant was not identified in either parent through family segregation analysis, indicating a de novo occurrence .Clinically, the patient was diagnosed with NS based on Van der Burgt scoring (characterized by NS-like facial appearance, major short stature, cardiac symptoms, and pectus excavatum). Puberty (Tanner stage 2) began at 10.6 years of age.

Throughout follow-up, insufficient growth at 1 cm/6 months was noted. We reinitiated rhGH treatment at 0.35 mg/kg/week, which was the recommended dosage for NS. Adjustments were made to the dose based on IGF-1 levels, eventually reducing it to 0.20 mg/kg/week (Figure 2).

At the latest evaluation, at 13 years of age, the patient was in Tanner stage 3 with a height of 138 cm (-3.2 SD) and a BMI of 15.23 kg/m2 (SDS: -2.18). During the approximately 3-year clinical follow-up, we observed a more pronounced chin-pointed appearance (Figure 2B). Yearly follow-ups included abdominal and pelvic ultrasonography and echocardiographic assessments, all revealing no pathological findings.

Methods

Genomic DNA was isolated from the patient's peripheral blood samples using established protocols. Genetic analyses were conducted through next-generation sequencing (Miseq, Illumina, San Diego, CA, USA) in accordance with the manufacturer's guidelines.

Results

The identified variant in the patient is exceptionally rare (gnomAD Allele Frequency = 6.207e-7) and results in a change within a highly conserved gene across species. Computational tools predict it to be 'disease causing,' supported by MutationTaster, and deleterious with a CADD score of 23.0. Notably, this variant has not been documented in major databases such as ClinVar or dbSNP.

Discussion

Diagnosing NS in patients who lack the phenotypic characteristics might be challenging. Atypical clinical anomalies could lead to a misdiagnosis. The diagnostic difficulty may cause delays in appropriate management and interventions. Although genetic testing has advanced, it's noteworthy that a substantial percentage (about 15%) of people with a clinical diagnosis of NS lack a definitive genetic diagnosis(7). Therefore, the clinical assessment remains highly significant even now. An accurate understanding of WES is crucial for identifying variants related to the syndrome. Effective collaboration between clinicians and geneticists with expertise in dysmorphology and disease-specific features is significant. The guidelines established by ACMG provide a framework for analyzing VUS, highlighting the need for collaboration between doctors and genetics professionals in determining these cases. The evolution of VUS classifications from uncertain significance to likely pathogenic or pathogenic underscores the dynamic nature of genetic research and the potential for reclassification as our understanding deepens(9).

The *MAP3K7* (MIM*602614) is a 17-exon gene located on chromosome 6q15. Mitogen-activated protein Kinases (MAPKs), also known as Extracellular Signal-Regulated Kinases (ERKs), are activated by a wide range of stimuli and serve as a convergence point for signaling pathways(1). Notably, with its autosomal inheritance pattern, NS often exhibits de novo variants(7). Segregation analysis, which demonstrates that neither of the patient's parents had the detected *MAP3K7* variant, supports the idea that this variant was pathogenic. However, it's important to consider the incomplete penetrance and variable expressivity, particularly in dominantly inherited conditions like those observed in RASopathy spectrum disorders. The parents might have also carried the variant with milder or asymptomatic presentations, highlighting the complexity of genetic inheritance and phenotypic expression in RASopathy spectrum disorders.

Pathogenic variants in *MAP3K7* have recently been linked to two disorders: cardiospondylocarpofacial syndrome (CSCFS) and frontometaphyseal dysplasia 2 (FMD2)(10-15). Interestingly, her presentation did not align with the typical features associated with either CSCFS or FMD2, particularly the absence of spinal and bone fusions in CSCFS(10-12) and the incongruence with flexion contractures of the elbow seen in FMD2(13-15). The patient's unique clinical manifestation, combined with the discovery of a novel likely pathogenic variant (c.125_127del, p.(Val42del)) in *MAP3K7* in another case, underscores the need for comprehensive functional studies to clarify the precise mechanisms linking these genetic variants to the observed phenotypic traits (16). However, this patient displayed spinal and bone fusions in the hands and feet, which were associated with CSCFS (10-12), and elbow flexion contractures, a characteristic of FMD2(13-15), though not CSCFS; the patient exhibited apparent "opposite" features. The two *MAP3K7*-associated syndromes that overlap might indicate the presence of a single disorder. This patient had followed up with NS diagnosis for years according to Var der Burgt criteria(16). The complexity of this case extends beyond the phenotypic range of CSCFS and FMD2, raising concerns about the underlying genetic and molecular mechanisms. The situation in this case resembled the underlying mechanism of Neurofibromatosis-Noonan syndrome (NFNS). NF1 and NS can exhibit similar features in some patients, leading to NFNS. The genetic basis of NFNS is not fully understood, and there is ongoing debate about whether it represents a variable manifestation of NF1 or NS or a distinct clinical entity. Some NFNS patients have variants in both *PTPN1*1 and *NF1*, but the majority only have *NF1* variants. As a result, most authors attribute NFNS syndrome to *NF1* variants(17, 18). In another case report, an Asian male with CSCFS presented a novel missense variant in MAP3K7 (NM_145331.3: c.467A > T: p.Asp156Val) and exhibited a mixed phenotype resembling Ehlers-Danlos syndrome andNS. This overlap in phenotypes suggested potential diagnostic

implications for identifying CSCFS. In contrast, our case differs because the patient did not exhibit clinical features typical of CSCFS or FMD2(19). This distinction underscores the variability in presentations associated with *MAP3K7* variants, contributing to the complexity of clinical diagnosis and genetic characterization. As of our current knowledge, *MAP3K7* has not been previously associated with NS. The patient's comprehensive bone X-ray examination revealed no findings of skeletal dysplasia, which ruled out both types. The intriguing association between *MAP3K7* and NS provides new insight into the underlying genetics of this condition. This finding encourages us to further investigate the interplay between genetic variants, the RAS/MAPK pathway, and the variable clinical manifestations. To further explore the potential impact of this variant, according to the KEGG pathway database, *MAP3K7* is a key component of the MAPK signaling pathway (hsa04010), which interacts with several other genes known to cause RASopathies, such as *KRAS, BRAF*, *RAF1 SOS1* and *NF1*. The involvement of MAP3K7 in this pathway underscores its potential role in the pathogenesis of RASopathies.In our case, the absence of skeletal dysplasia features may lead to consider *MAP3K7* as the direct cause of the NS clinical presentation. Whether this gene is directly related to the NS clinical condition in our patient or if its interaction with a different gene in the Rasopathy pathway is a speculative question. The answer to this will likely be provided by future functional analysis studies. This point is the limitation of the case report. Remarkably, our patient is the second documented case in literature featuring a *MAP3K7* variant that doesn't exclusively result in the two recognized types of skeletal dysplasia and, more importantly, the first case with thorough evidence of its relationship with NS, as far as current information allows. This case significantly highlights *MAP3K7* as a key candidate gene for NS. While this association is encouraging, more functional genetic research focusing on the precise pathways to which *MAP3K7* variants contribute is necessary to establish a definitive connection. The identified variant in the patient is exceptionally rare (gnomAD Allele Frequency = 6.207e-7). Functional validation through protein structure modeling or in vitro studies is crucial to elucidate its impact on protein function and disease pathogenesis.

Without functional analysis, it is challenging to definitively establish the pathogenicity of this variant and its role in the phenotypic presentation of NS. Future functional studies are crucial to validate our findings and elucidate the molecular mechanisms involved. Moreover, the delayed diagnosis of NS has a domino effect on initiating appropriate medical interventions. In NS, rGH is effective, and the contribution of the final height is essential (20, 21). Most adults' heights remain below the 3rd percentile without rGH treatment (21). Other pathologies that would cause low BMI did not exist during clinical or laboratory evaluations. In NS patients, the growth response is more favorable when rGH treatment is initiated earlier and maintained longer. The duration of rGH usage before puberty and the height at the onset of puberty also impact near-final height (21). Delayed diagnosis may postpone growth hormone therapy, limiting optimal growth potential. This delay carries enduring implications that influence our case's achievable final adult height. The relation within the RAS/MAPK pathway can vary based on genotypic variation. Additionally, differences in growth characteristics may depend on whether other pathways related to RAS/MAPK (such as PI3K/AKT, JAK2/STATb5) are influenced or unaffected (21). It is reported that growth differences can occur depending on the nature of the genetic variant. It is well-established that the RAS/MAPK pathway is implicated in various malignancies (20). Therefore, it is crucial to investigate the incidence of cancers in disorders associated with *MAP3K7* mutations. Accordingly, detailed information was provided to the family, and rGH therapy was initiated with their consent. Furthermore, it is essential to assess the safety of GH treatment in these conditions. The patient was monitored with three-month-follow-ups, including close IGF-1 level monitoring and abdominal-pelvic imaging studies.

Our case had a low BMI. There is no difference in macronutrient intake in NS patients compared to healthy children, which might be attributed to increased energy expenditure(22). A distinctive chest deformity with pectus carinatum in the upper section and pectus excavatum in the lower part of the chest is a remarkable anomaly at any age our patient had (23, 24). The dysmorphic qualities of NS show variations depending on age, with more pronounced characteristics in infancy, while facial features may not be readily noticeable during adolescence and adulthood. During the adolescent and young adult periods, the face takes on a more triangular contour (25). Throughout approximately three years of follow-up, there was notable, a remarkable enhancement in the patient's jawline. All clinical findings were consistent with NS.

In conclusion, the identification of a novel de novo heterozygous variant (c.65C>A, p.(P22H)) in the *MAP3K7* raises intriguing questions about its role in NS development. Notably, this is the first documented case associating *MAP3K7* variants with NS, expanding our understanding of genetic factors implicated in the condition. The patient's unique clinical presentation, distinct from classical manifestations of *MAP3K7*-related syndromes, highlights the intricate nature of genetic and molecular mechanisms. This finding raises more questions about how the RAS/MAPK system, clinical symptoms seen in NS patients, and *MAP3K7* variants interact.

Statements

Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

Medical Practices:SC, ZA, ZS, EO,SC, MB , Concept:SC, ZS, MB, Design: SC, EO, ZS, ZA MB, Data collection: SC, ZA, ZS, EO,SC Analysis: SC, ZA, ZS, EO,SC Literature Search: SC, ZS, EO,SC, MB, Writing: SC, ZS, EO, MB

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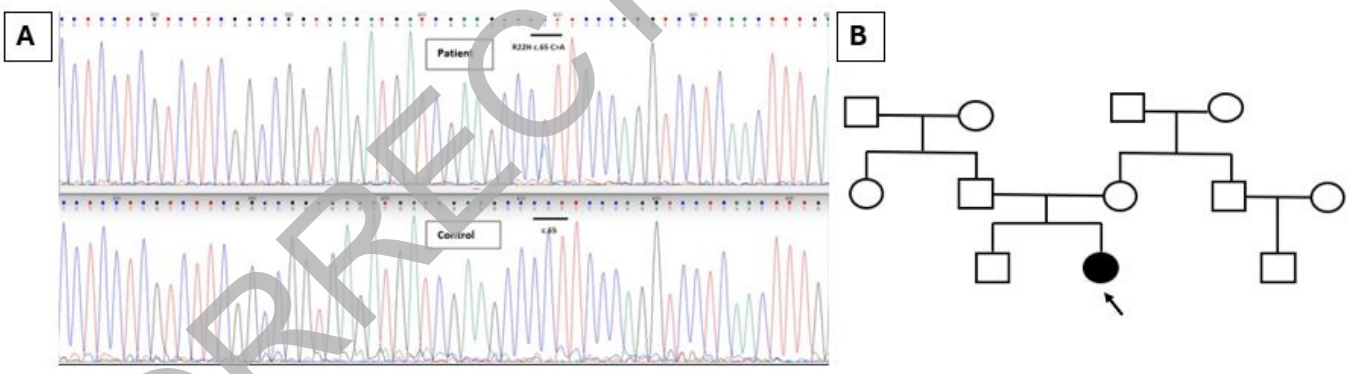

Fig.1. Electropherograms of the Sanger sequencing and the family tree of the patient.

Fig.2. Fig.2.A. Physical characteristics of the patient (synophrys, prominent supraorbital ridges, mild ptosis, low set ears, full cheeks, broad nasal tip, deep elongated philtrum, thin upper vermillion, low anterior hairline, triangular face, short webbed neck, superior pectus carinatum, and inferior pectus excavatum, short metacarpals and metatarsals, clinodactyly, syndactyly) Fig.2.B. Chin-pointed appearance of the patient

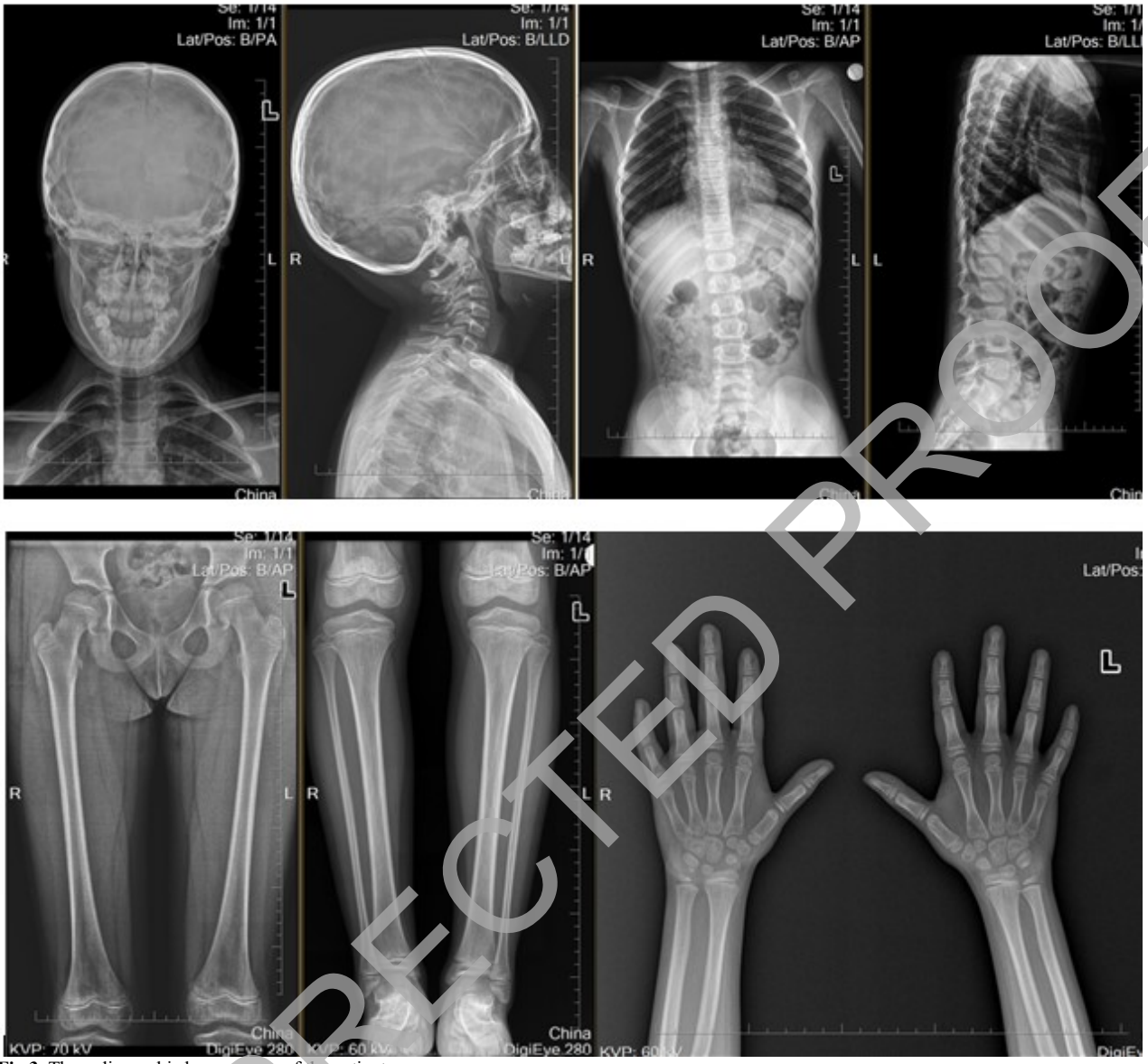

Fig.3. The radiographic bone survey of the patient