The Role of Next Generation Sequencing in Diagnosis of Patients with Rare Syndromic Short Stature

Nadir Sendromik Boy Kısalığı Olan Olguların Tanısında Yeni Nesil Dizilemenin Rolü

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ABSTRACT

Objective: To examine the genetic causes of short stature, algorithms are applied to make the diagnosis in a stepwise manner by applying different genetic technique options at different stages of diagnosis, depending on the evaluation of the clinicians. In this study, we aimed to evaluate genetic diagnosis in Turkish patients with short stature of unknown etiology using next negeration sequencing (NGS).
Methods: In cases with an unknown etiology of short stature, NGS was applied. For this aim, 19 cases from 17 families were included. The clinically relevant variants detected in the cases were confirmed by the Sanger method.
Results: For 11 of 19 patients (57.9%) with short stature, a genetic diagnosis was obtained by NGS. In 11 patients, 9 different genetic variants were identified. We detected 4 novel variants (*LTBP3*: c.2919C>G, *TRPS1*: c.2894G>T, *CNOT1*: c.4967dup, *ANKRD11*: c.5273C>T). These novel variants were associated with Dental Anomalies and Short Stature syndrome (DASS), Trichorhinophalangeal syndrome (TRPS), Vissers-Bodmer syndrome (VIBOS) and KBG syndrome (KGBS).
Conclusion: These results show that NGS significantly increases the diagnostic yield of patients with short stature. NGS provides great benefits for both patients and clinicians in clinical use, and also enables the detection of novel rare variants.

Keywords: Short stature, Next generation sequencing, *LTBP3, TRPS1, CNOT1, ANKRD11*

Received: 04.18.2022 **Accepted:** 05.09.2022

ÖZET

Amaç: Boy kısalığının genetik nedenlerini incelemek için, klinisyenlerin değerlendirmesi doğrultusunda, tanının farklı aşamalarında farklı genetik teknik seçenekleri uygulanarak adım adım tanı koymak için algoritmalar uygulanır. Bu çalışmada etiyolojisi bilinmeyen boy kısalığı olan Türk hastalarda yeni nesil dizileme (NGS) kullanarak genetik tanıyı değerlendirmeyi amaçladık.
Yöntem: Nedeni bilinmeyen boy kısalığı olan olgularda, NGS yöntemi uygulandı. Bu amaçla 17 aileden 19 olgu çalışmaya dahil edildi. Vakalarda tespit edilen klinik olarak anlamlı varyantlar Sanger yöntemi ile doğrulandı.
Bulgular: Çalışmaya dahil edilen 19 hastanın 11'inde (%57.9) NGS ile genetik tanı konuldu. Ayrıca, 11 hastada 9 farklı genetik varyant tespit edildi. Bu varyantlardan 4'ünü yeni varyant olarak tespit ettik (LTBP3: c.2919C>G, TRPS1: c.2894G>T, CNOT1: c.4967dup, ANKRD11: c.5273C>T). Bu yeni varyantlar Diş Anomalileri ve Kısa Boy sendromu (DASS), Trikorhinofalangeal Sendrom (TRPS), Vissers-Bodmer sendromu (VIBOS) ve KBG sendromu (KGBS) sendromları ile ilişkilendirildi.
Sonuç: Bu sonuçlar, NGS'nin boy kısalığı olan hastalarda tanıyı önemli ölçüde desteklediğini göstermektedir. NGS, klinik kullanımda hem hastalar hem de klinisyenler için büyük faydalar sağlar ve aynı zamanda yeni nadir varyantların saptanmasını sağlar.

Anahtar Sözcükler: Boy kısalığı, Yeni nesil dizileme, *LTBP3, TRPS1, CNOT1, ANKRD11*

Geliş Tarihi: 18.04.2022 **Kabul Tarihi:**09.05.2022

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doi:http://dx.doi.org/10.12996/gmj.2022.87

INTRODUCTION

Short stature, is defined as a height below the 2 standard deviations (SDS) for age and sex (1). The underlying causes of short stature can be familial, structural, chronic diseases and genetic diseases (2). It is stated that approximately 80% of the stature diversity in the population is under genetic control (3). When short stature is a part of a more complex condition (syndromic), investigating underlying genetic causes is especially important.

To examine the genetic causes of short stature, algorithms are applied to make the diagnosis in a stepwise manner by applying different genetic technique options at different stages of diagnosis, depending on the evaluation of the clinicians. However, the rate of genetic diagnosis is low, especially due to genetic heterogeneity. With the technological developments in the field of genetics and the more affordable costs, the possibility of using genetic tests in clinical practice is increasing. Next-generation sequencing (NGS), that is a method provides to sequence enlarged regions of DNA, has increased the genetic diagnosis rates (4). It is faster than other molecular genetic techniques and replacing the previous tecniques by its advantages (4).

A significant number of studies reported mono-genic etiological causes detected by whole-exome sequencing (WES) or clinical exome sequencing (CES) (5–7). As demonstrated in these studies, exome or panel testing is likely to find a monogenic cause for syndromic short stature. A recent study investigating genetic etiology of short stature found a genetic cause in 50% of the patients, mostly using exome or panel testing (5).

The definition of genetic etiology would provide appropriate management and treatment of the disease and appropriate genetic counseling for the entire family members. In this study, we aimed to evaluate genetic diagnosis in Turkish patients with short stature of unknown etiology using a CES or WES.

METHODS

Patients

The cases who applied to the Department of Medical Genetics in Balıkesir Atatürk City Hospital between June 2018 and October 2021 due to short stature (height SD < −2 for age and sex) with accompanying dysmorphologic features or severe short stature (height SD < −3) were included in this study. Exclusion criteria in the study were as follows: 1) Cases with chromosomal abnormality as a result of karyotype analysis, 2) Cases with a diagnosis of achondroplasia/hypochondroplasia with a molecular diagnosis, 3) Cases with Noonan syndrome whose mutation in the PTPN11 gene was detected by target gene analysis, 4) Cases with a diagnosis of neurofibromatosis. Before the genetic testing, evaluation of phenotypic characteristics and previous genetic results of all cases were performed. In cases with an unknown etiology of short stature, next generation sequencing (CES or WES) was applied. For this aim, 19 cases from 17 families were included. The clinically relevant variants detected in the cases were confirmed by the Sanger method. In case of more than one patient from the same family, the genetic variant detected in the proband was screened for target mutations in family members with similar clinics.

Data collection and DNA extraction

Informed consent was provided from all patients before collection of blood sample. DNA extraction was performed from these blood samples. The obtained blood sample was 2 ml of venous blood collected in an EDTA tube. We extracted DNA from 200 µl peripheral blood samples using QIAamp DNA Blood Mini Kit (Qiagen Inc.).

Clinical exome sequencing (CES)/ whole-exome sequencing (WES)

The obtained genomic DNA was enriched two capture kits were used for CES: Twist Human Core Exome Kit (Twist Bioscience, San Francisco, CA, USA) and KAPA HyperCap DS CES Target Enrichment Probes (Roche, Germany). These were sequenced with the MGI-DNBSEQ-G400 (China). Additionally WES was performed using TruSeq Exome Enrichment kit from Illumina following the manufacturer's protocol. It was sequenced using HiSeq2500 Sequencer (Illumina)

Data analysis

The online SEQ Platform, a cloud based genomics software, was used and all samples were analyzed with this platform (Genomize Inc., Turkey**).** Exon-intron junctions (±10 bp) were included in the analysis and mapped to reference human genome (hg19). Variants with a frequency higher than 0.5% were filtered out. Human Phenotype Ontology was used for phenotypic filter and Online Mendelian Inheritance in Man (OMIM) was used for genesets. Mutations in all patients were classified according to ACMG criteria into three categories: pathogenic, likely pathogenic, and uncertain significance. Likely benign and benign variants according to ACMG have not been reported. Clinically relevant pathogenic variants were filtered by following steps, respectively: 1) all missense, nonsense, frameshift, frame and synonymous variants, 2) variants with a minor allele frequency of 1.0% in population studies [1000 Genomes (1000G) and Genome Aggregation Database (gnomAD)]. Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) and Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM and the criteria of the American College of Medical Genetics and Genomics (ACMG) were used to classifying all variants (8). Novel mutations and variants of uncertain significance (VOUS) were interpreted using in-silico variant prediction programs [SIFT (http://sift.jcvi.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.mutationtaster.org/) and CADD (Combined Annotation Dependent Depletion)].

Ethical Publication Statement

This study was approved in Balikesir University Medical Faculty Health Sciences Ethics Committee dated 24.11.2021 and numbered 2021/261. The study was evaluated as a research file and it was decided that it was scientifically and ethically appropriate.

RESULTS

The mean SDS for height of the 19 patients were −3.42±1.39. The median age for all cases was 14 years. The female and male ratio was 1.38 (11/8). For 11 of 19 patients (57.9%) with suspected syndromic short stature, a genetic diagnosis was obtained by CES or WES. Most patients displayed distinct facial features (9/11, 81.8%). Neurodevelopmental disorders such as autism spectrum disorder or intellectual disability were present in 5 of the 11 cases with a rate of 45.5% (Table 1).

In 11 patients, 9 different genetic variants were identified. We detected 4 novel variants (*LTBP3*: c.2919C>G, *TRPS1*:c.2894G>T, *CNOT1*: c.4967dup, *ANKRD11*: c.5273C>T). These novel variants were associated with Dental Anomalies and Short Stature syndrome (DASS), Trichorhinophalangeal syndrome (TRPS), Vissers-Bodmer syndrome (VIBOS) and KBG syndrome (KGBS). Following the family member testing and segregation studies, 3 more affected family members carrying the same genetic variants were detected. We determined 4 variants as pathogenic, 3 variants as likely pathogenic, and 2 variants as variants of uncertain significance (Table 1). These 9 different genetic variants were associated with rare skeletal disorders and connective tissue diseases [DASS, Spondyloepiphyseal dysplasia congenita (SEDC), TRPS, Laron Syndrome, Noonan syndrome type 10 (NS-10), VIBOS, KBGS, Noonan syndrome type 4 (NS-4), Weill-marchesani syndrome 2 (WMS2)].

Table 1. Genetic results and clinical findings in patients with short stature

Gene Ref. Seq. ID	Case	Age	Height	Gender	Zygosity	Location	Nucleotide variation	Amino acid variation	Inheritance	ACMG Clasification	Dysmorphologic features	Additional Clinical Findings	Associated phenotype
LTBP3 NM_001130144.2 CES Twist	1	10 months	61 cm (-4, 44 SDS)	Male	Hom	Exon 21	**c.2919C>G***	**p.Asp973Glu***	mat/pat	VOUS	Short stature	-	DASS
COL2A1 NM_001844.5 CES Twist	2	19 months	75 cm (-2.25 SDS)	Female	Het	Exon 23	c.1510G>A	p.Gly504Ser	de-novo	Pathogenic	Short stature dwarfism, short-trunk, short neck, frontal bossing, depressed nasal root, short neck	IUGR, oligohidramnios	SEDC
TRPS1 *NM_014112.5* *WES*	3	34 years	156,7 cm (-3,16 SDS)	Male	Het	Exon7	**c.2894G>T***	**p.Arg965Leu***	NA	Likely Pathogenic	Short stature, protruding ears, laterally sparse eyebrows, thin upper lip, high palate, crowded teeth, cone-shaped epiphyses, brachydactyly, Sparse hair	-	TRPS
GHR NM_000163.5 WES	4	31 years	123,8 cm (-6.69 SDS)	Female	Hom	Intron 2	c.70+1G>A	-	NA	Likely Pathogenic	Short stature, short limbs, small face, blue sclerae, acrohypoplasia, clinodactyly, delayed menarche, high-pitched voice	Target resistance to the action of GH	Laron Syndrome
	5 (cousin of case 4)	21 years	140,7 cm (-5.76 SDS)	Male					NA		Short stature, short limbs, small face, delayed bone age, acrohypoplasia	Target resistance to the action of GH	
LZTR1 NM_006767.4	6	29 years	151 cm (-2.06 SDS)	Female	Het	Exon 10	c.1018C>T	p.Arg340Ter	NA	Pathogenic	Short stature, hypertelorism, strabismus short neck	ID	NS-10
CNOT1 NM_016284.5 WES	7	1 year 11 months	79 cm (-2,22 SDS)	Male	Het	Exon 35	**c.4967dup***	**p.Ala1657SerfsTer21***	de-novo	Likely Pathogenic	Short stature, low set ears, hypertelorism,upslanted palpebral fissure, epicanthal folds, low nasal bridge, short neck, pes planus	ASD	VIBOS
ANKRD11 NM_001256183.2 CES roche	8	12 years 4 months	138 cm (-2,63 SDS)	Female	Het	Exon 9	**c.5273C>T***	**p.Pro1758Leu***	pat	VOUS	Short stature, large prominent ears, high palate, pes planus	ID	KBGS
	9 (father of case 8)	37 years	155 cm (-3.44 SDS)	Male					NA		Short stature	ID	
SOS1 NM_001382395.1 CES Roche	10	13 months	69 cm (-2,77 SDS)	Male	Het	Exon 10	c.1655G>C	p.Arg552Thr	de-novo	Pathogenic	Short stature, short neck, macrocephaly, hypertelorism, cryptorchidism, high anterior hairline, ptosis	-	NS- 4
FBN1 *NM_000138.5* CES Twist	11	22 years	150 cm (-2,23 SDS)	Female	Het	Exon 9	c.863A>G	p.Asp288Gly	NA	Pathogenic	Short stature, proportionate, scoliosis, increased lumbar lordosis	ID	WMS2

*Novel variant

SDS: Standart deviation, VOUS: Variant of uncertain significance, ID: Intellectual disability, DASS: Dental anomalies and short stature, SEDC: Spondyloepiphyseal dysplasia congenita, TRPS: Trichorhinophalangeal syndrome, NS-10: Noonan syndrome type 10, VIBOS: Vissers-Bodmer syndrome, KBGS: KBG syndrome, NS-4: Noonan syndrome type 4, WMS2: Weill-marchesani syndrome 2

DISCUSSION

Nineteen cases with short stature (height SD < −2 for age and sex) with accompanying dysmorphologic features or severe short stature (height SD < −3) were evaluated to determine genetic etiology. We detected 4 novel variants in *CNOT1* (c.4967dup), *TRPS1* (c.2894G>T), *LTBP3* (c.2919C>G) and *ANKRD11* (c.5273C>T) genes. Two of these variants were found in more than one affected case in the same family (*TRPS1 and ANKRD11).*

In 2 studies published in 2019, authors reported a new syndrome including pancreatic agenesis and holoprosencephaly-12 associated with a recurrent de novo missense variant in the *CNOT1* gene (9,10). Then, in 2020, another study was published through international collaborations involving 37 families carrying a pathogenic *CNOT1* variant (11). In this study, another *CNOT1* gene-related syndrome named Vissers-Bodmer syndrome (VIBOS) was defined by demonstrating the role of *CNOT1* in neurodevelopmental disorders. The most common phenotypic features associated with VIBOS syndrome were described in the study of Vissers et al. (11) as neurodevelopmental disorders, facial abnormalities, problems with growth and development, and skeletal abnormalities. As seen in these studies, genotype-phenotype correlations associated with *CNOT1* gene variants have been established recently and have been shown in very few studies. In the same line with the study of Vissers et al. (11), we reported a case carrying a novel *CNOT1* variant (c.4967dup) presenting short stature, low set ears, hypertelorism, upslanted palpebral fissure, epicanthal folds, low nasal bridge, short neck, pes planus and autism spectrum disorder. The variant in our study, which occurs as a result of the addition of a nucleotide (adenine) at position 4967 of the CNOT1 gene DNA sequence, causes a frameshift mutation. The location of this variant was near a highly conserved position among the species. This variant is categorized as likely pathogenic (PVS1, PM2) according to the ACMG criteria.

Our case carrying a novel variant of the *TRPS1* gene (c.2894G>T) presented with short stature accompanying facial dysmorphological features, brachydactyly and sparse hair and was diagnosed with Tricho-rhino-phalangeal syndrome (TRPS). We found the same variant in the 11-year-old son of this case. Different from his father who carried the same variant, he had facial dysmorphological features and brachydactyly without short stature. The variant in our study causes premature stop codon by resulting one nucleotide alterations in exon 7 of the TRPS1 gene. The variant introduces a more hydrophobic residue at this position. This can result in loss of hydrogen bonds and/or disturb correct folding. This variant is classified as likely pathogenic (PM2, PM5, PM1, PP3) in silico variant analysis databases according to the ACMG. TRPS is characterized by craniofacial and skeletal abnormalities including sparse hair, facial anomalies, cone-shaped epiphyses, short stature and shortening of metacarpals and metatarsals (12), and are distinguished TRPS I, TRPS II and TRPS III. An international working group focusing on TRPS investigated phenotypic variability and genotype-phenotype correlation in 85 individuals carrying *TRPS1* variants (13). Maas et al. (13)reported short stature (height at birth < -2SD) in 8 of 34 cases carrying variants of the *TRPS1* gene. Also, the rate of short stature (height < -2SD) was higher in adult males (15/22) and adult females (22/44) than the rate of short stature at birth. Although *TRPS1* gene variants are not commonly determined in cases primarily applying with short stature, cone-shaped epiphyses are hallmarks in radiological findings. Also, osteopenia and decreased linear growth are common. Variabilities in phenotypic characteristics were reported, even in the same families. All these reasons may explain why we did not detect short stature in an 11-year old-case carrying a variant of the *TRPS1* gene. Detection of short stature is more common in adult cases, it may be possible to decrease the height postnatally and should be followed up carefully. Careful monitoring of height is important because the therapeutic role of growth hormone was implied in a case diagnosed with TRPS (14).

Pathogenic variants of the *LTBP3* gene are known genetic cause of DASS syndrome. This syndrome affects two organs as the skeleton and teeth (15,16). We detected a novel *LTBP3* variant (c.2919C>G) in a case of 10 months presenting only short stature. This variant of the *LTBP3* gene has not been previously reported in any database in the homozygous and/or heterozygous state. It is classified as VOUS (PM2, BP4) according to ACMG criteria. We evaluated that the patient who presented with severe short stature was compatible with this syndrome, which was associated with isolated short stature and dental anomalies in the literature. To reveal the clinical relationship of the variant, it will be appropriate to follow up for dental anomalies in the syndrome. DASS syndrome should be kept in mind before dental findings occur in young children with isolated short stature and in the presence of consanguineous marriage.

KBG syndrome is a rare disorder caused by pathogenic variants of the *ANKRD11* gene and characterized by intellectual disability, short stature and facial features (17). We determined a novel VOUS variant of the *ANKRD11* gene (c.5273C>T). Although this variant of *ANKRD11* gene was classified as VOUS (PM2, PP3), this variant was detected in two affected cases from same family. In the same line with literature, our 2 cases had short stature and intellectual disability. We interpret that this VOUS variant was related to phenotypic features of our patients, since the clinical features of the cases were consistent with the KBG syndrome in the literature and it was found in 2 affected individuals from the same family.

As a conclusion, we obtained genetic diagnosis in 57.9% of the patients with short stature of unknown etiology using a CES or WES. A recent study investigating genetic etiology of short stature found a genetic cause in 50% of the patients, mostly using exome or panel testing (5). These results show that NGS significantly increases the diagnostic yield of patients with short stature. NGS provides great benefits for both patients and clinicians in clinical use, and also enables the detection of new rare variants, as in our study.

Our study include several limitations. We had negative cases with unsolved genetic diagnosis. WES or CES would not resolve every case. Further studies should include further genetic investigation. Segregation study could not be performed in some families and that copy number variations could not be analyzed. Also, increased sample sizes and more cases would help improve our understanding and validate these rare novel variants. It could improve the molecular diagnosis of short stature.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Ranke MB. Towards a consensus on the definition of idiopathic short stature. Hormone research in paediatrics. 1996;45(Suppl. 2):64–6.

2. Baron J, Sävendahl L, de Luca F, Dauber A, Phillip M, Wit JM, et al. Short and tall stature: a new paradigm emerges. Nature Reviews Endocrinology. 2015;11(12):735–46.

3. McEvoy BP, Visscher PM. Genetics of human height. Economics & Human Biology. 2009;7(3):294–306.

4. Bahsi T, Ergun S, ERGÜN M, Percin F. Comparison of the Diagnostic Accuracy of Next Generation Sequencing and Microarray Resequencing Methods for Detection of BRCA1 and BRCA2 Gene Mutations. Gazi Medical Journal. 2018;29(2).

5. Kamil G, Yoon JY, Yoo S, Cheon CK. Clinical relevance of targeted exome sequencing in patients with rare syndromic short stature. Orphanet J Rare Dis. 2021;16(1):1–19.

6. Toydemir RM, Bamshad MJ. Sheldon-hall syndrome. Orphanet J Rare Dis. 2009;4(1):1–5.

7. Guo MH, Shen Y, Walvoord EC, Miller TC, Moon JE, Hirschhorn JN, et al. Whole exome sequencing to identify genetic causes of short stature. Hormone research in paediatrics. 2014;82(1):44–52.

8. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine. 2015;17(5):405–23.

9. de Franco E, Watson RA, Weninger WJ, Wong CC, Flanagan SE, Caswell R, et al. A specific CNOT1 mutation results in a novel syndrome of pancreatic agenesis and holoprosencephaly through impaired pancreatic and neurological development. The American Journal of Human Genetics. 2019;104(5):985–9.

10. Kruszka P, Berger SI, Weiss K, Everson JL, Martinez AF, Hong S, et al. A CCR4-NOT transcription complex, subunit 1, CNOT1, variant associated with holoprosencephaly. The American Journal of Human Genetics. 2019;104(5):990–3.

11. Vissers LELM, Kalvakuri S, de Boer E, Geuer S, Oud M, van Outersterp I, et al. De novo variants in CNOT1, a central component of the CCR4-NOT complex involved in gene expression and RNA and protein stability, cause neurodevelopmental delay. The American Journal of Human Genetics. 2020;107(1):164–72.
12. Giedion A, Burdea M, Fruchter Z, Meloni T, Trosc V. Autosomal-dominant transmission of the tricho-rhino-phalangeal syndrome. Report of 4 unrelated families, review of 60 cases. Helv Paediatr Acta. 1973;28(3):249–59.
13. Maas SM, Shaw AC, Bikker H, Lüdecke HJ, van der Tuin K, Badura-Stronka M, et al. Phenotype and genotype in 103 patients with tricho-rhino-phalangeal syndrome. Eur J Med Genet. 2015;58(5):279–92.
14. Merjaneh L, Parks JS, Muir AB, Fadoju D. A novel TRPS1 gene mutation causing trichorhinophalangeal syndrome with growth hormone responsive short stature: a case report and review of the literature. International Journal of Pediatric Endocrinology. 2014;2014(1):1–7.
15. Huckert M, Stoetzel C, Morkmued S, Laugel-Haushalter V, Geoffroy V, Muller J, et al. Mutations in the latent TGF-beta binding protein 3 (LTBP3) gene cause brachyolmia with amelogenesis imperfecta. Hum Mol Genet. 2015;24(11):3038–49.
16. Noor A, Windpassinger C, Vitcu I, Orlic M, Rafiq MA, Khalid M, et al. Oligodontia is caused by mutation in LTBP3, the gene encoding latent TGF-β binding protein 3. The American Journal of Human Genetics. 2009;84(4):519–23.
17. Brancati F, Sarkozy A, Dallapiccola B. KBG syndrome. Orphanet Journal of Rare Diseases. 2006;1(1):1–6.