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Comprehensive Evaluation of the Molecular Role of PTPN11 Gene Analysis in the Prenatal Diagnosis and Early Detection of Noonan Syndrome

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Abstract

Rasopathies are a clinical group of disorders caused by dominant pathogenic variants in 29 genes within the RAS-MAPK pathway, which regulates cell growth, differentiation, aging, and the cell cycle. The most common Rasopathy is Noonan syndrome (NS), with PTPN11 variants detected in approximately 50% of cases, with 90% of these variants occurring in regions encoding the N-terminal SH2 and C-terminal catalytic domains. Prenatal findings include increased nuchal translucency (NT), lymphatic anomalies (such as cystic hygroma, pleural effusion, and ascites), cardiac anomalies, polyhydramnios, limb shortening, and macrocephaly. PTPN11 variants are found in 2-3% of fetuses with NT abnormalities (without chromosomal anomalies) and in >10% of cases with additional NS-related features. This study retrospectively analyzed PTPN11 gene results in 246 prenatal cases with NS-related ultrasound (USG) findings, excluding chromosomal anomalies. In 200 cases, target exons (3,4,7,8,13,14) were examined, while in 46 cases, the entire gene was analyzed using Sanger sequencing.

Five PTPN11 variants were detected in five cases (2%), including two novel variants (p.P107S and p.M504T). Two cases had isolated NT, while three had multiple USG findings. In six cases where no PTPN11 variant was found, SOS1 pathogenic variants were identified in two cases, and RAF1 pathogenic variants in one terminated pregnancy. The detection rate for NS-related pathogenic variants was 2.3% in both the isolated NT and multiple USG findings groups. Since 90% of pathogenic PTPN11 variants are located in targeted exons, prioritizing exon analysis as the initial step, followed by whole-gene and Rasopathy-related gene analysis when necessary.

Keywords: PTPN11 gene, Noonan syndrome, Nuchal Translucency, RAS-MAPK

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التقييم الشامل لفعالية تحليل جين PTPN11 في التشخيص السابق للولادة والاكتشاف المبكر لمتلازمة نونان

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المستخلص

الأمراض المرتبطة بمسار RAS-MAPK (Rasopathies) هي مجموعة سريرية ناتجة عن متغيرات جينية مرضية سائدة في 29 جينًا ضمن هذا المسار، الذي ينظم نمو الخلايا، تمايزها، الشيخوخة، وتنظيم الدورة الخلوية. تُعد متلازمة نونان (NS) الأكثر شيوعًا بين هذه الأمراض، حيث تم اكتشاف متغيرات PTPN11 في 50% من الحالات، و90% منها تحدث في المناطق المشفرة لمجالي N-terminal SH2 وC-terminal catalytic. تشمل العلامات قبل الولادة زيادة السماكة القفوية (NT)، تشوهات الجهاز اللمفاوي (مثل الورم اللمفاوي الكيسي، الانصباب الجنبي، والاستسقاء)، عيوب القلب، تعدد السائل الأمنيوسي، تقاصر الأطراف، وتضخم الرأس. تم العثور على متغيرات PTPN11 في 2-3% من الأجنة ذات NT غير الطبيعي (مع استبعاد الشذوذات الكروموسومية) وفي أكثر من 10% من الحالات التي تظهر عليها ميزات إضافية لمتلازمة نونان. تمت مراجعة نتائج تحليل جين PTPN11 بشكل رجعي في 246

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معلومات البحث
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حالة ما قبل الولادة مع ثبوت نتائج موجات فوق صوتية (USG) مرتبطة بمتلازمة نونان، بعد استبعاد الشذوذات الكروموسومية في 200 حالة، تم تحليل إكسونات مستهدفة (3، 4، 7، 8، 13، 14)، بينما خضعت 46 حالة لتحليل الجين بالكامل باستخدام طريقة Sanger sequencing.
تم اكتشاف خمس متغيرات في PTPN11 في خمس حالات (2%)، من بينها متغيران جديدان (p.P107S وp.M504T). في حالتين، لوحظ NT معزول، بينما كانت هناك تشوهات موجات فوق صوتية متعددة في ثلاث حالات. في ست حالات لم يتم العثور فيها على متغير PTPN11، تم تحديد متغيرات مرضية في SOS1 في حالتين ومتغير RAF1 في حالة واحدة، والتي انتهت بإنهاء الحمل. بلغت نسبة اكتشاف المتغيرات المسببة لمتلازمة نونان 2.3% في كل من مجموعة NT المعزول والمجموعة ذات النتائج المتعددة في الموجات فوق الصوتية. نظرًا لأن 90% من المتغيرات الممرضة في PTPN11 تقع في الإكسونات المستهدفة، فإن تحليل هذه الإكسونات كخطوة أولى، يليه تحليل الجين بالكامل والجينات المرتبطة بـ Rasopathy عند الضرورة.

الكلمات المفتاحية: جين PTPN11، متلازمة نونان، السماكة القفوية، الأمراض المرتبطة بمسار RAS-MAPK

Introduction

Genes involved in the Ras-mitogen-activated protein kinase (RAS-MAPK) pathway play a crucial role in regulating the cell cycle, cell differentiation, ageing, and all key stages of normal development [1]. Changes in these genes, which occur dominantly, lead to genetic and clinical heterogeneity in manifestations. As a result, conditions such as Noonan syndrome (NS), Lentiginous Noonan syndrome (LEOPARD-NSML), Costello syndrome (CS) [2], Cardio-Facio-Cutaneous syndrome (CFCS), and Legius syndrome (LS) are defined [3]. RAS-MAPK pathway disorders are among the most common diseases worldwide (1:1000–2500) and are referred to as "Rasopathies" or "Noonan spectrum disorders."[4] Among these Rasopathies, NS is the most commonly observed, with mutations in the PTPN11 gene found in 50% of cases [5].

Phenotypic findings observed in Noonan syndrome (NS) include a wide forehead, full lips, epicanthal folds, relative macrocephaly, downward slanting palpebral fissures, pulmonary valve stenosis [6], hypertrophic cardiomyopathy, growth and developmental delays, cancer susceptibility, chest deformities, short stature, hair anomalies, skin abnormalities, swallowing difficulties, and coagulation disorders (prolonged bleeding time) [7]. Additional findings in NS and Cardio-Facio-Cutaneous (CFC) syndrome include a deep philtrum, high-set ears, a high-arched palate, hypertelorism, ptosis, cardiac septal defects, vision impairments, and lymphatic system anomalies [8]. Among these findings, lymphatic anomalies leading to increased nuchal fluid (nuchal translucency, NT), cystic hygroma, oedema, pleural effusion, shortness of long bones (<5p) [9], polyhydramnios, cardiac anomalies, macrosomia, macrocephaly (>90 percentile), and renal anomalies can be detected through fetal ultrasound examinations at various stages of pregnancy [10]. Invasive procedures are recommended for genetic diagnosis, including karyotype analysis and molecular cytogenetic tests (microarray, aCGH) [11]. In cases where no anomalies are detected in these tests, it is necessary to investigate Rasopathy spectrum disorders [12]. Among the clinical findings, increased nuchal translucency (NT) is one of the most important parameters in the 11–14-week screening test, and its measurement is recommended for every pregnancy [13]. In 1% of pregnancies, NT measurements exceed 3.5 mm, and invasive procedures for fetal chromosomal analysis are performed [14]. Noonan Syndrome (NS) is considered in the differential diagnosis in

samples with a normal karyotype, and specific molecular analyses are applied [15]. This approach is also followed in the presence of other ultrasound findings (such as ascites, pleural effusion, cystic hygroma, etc.) that may be associated with the NS spectrum [16]. Today, 29 genes associated with Noonan Syndrome (NS) have been identified, including A2ML1, BRAF, CBL, CDC42, HRAS, KAT6B, KRAS, LZTR1, MAP2K1, MAP2K2, MAP3K8, MRAS, NF1, NRAS, NSUN2, PPP1CB, PTPN11, RAF1, RALA, RASA2, RIT1, RRAS, RRAS2, SHOC2, SOS1, SOS2, SPRY1, and ZNF526 [17]. Ongoing genetic research indicates that the number of known genes related to the NS spectrum is likely to increase, and there may also be undiscovered genes, making the prenatal diagnostic process more challenging [18]. However, since NS is the most frequently observed syndrome within the spectrum, with half of its mutations found in the PTPN11 gene [19], and these mutations are particularly clustered in certain exons, this has allowed for the inclusion of quick and cost-effective steps in the molecular testing algorithm [20]. The PTPN11 (protein tyrosine phosphatase, non-receptor type 11) gene is localized on 12q24.13 and consists of 15 exons that encode 593 amino acids [21]. According to the Human Mutation Database 2020.1 (HGMD®), the distribution of various mutations identified in the PTPN11 gene across the exons is as follows: 35% in exon 3, 19.6% in exon 13, 11.9% in exon 7, 8.4% in exon 8, 2.2% in exons 4, 12, and 14, 2.1% in exons 2, 6, 10, and 11, 1.4% in exon 5, and 0.7% in exons 1 and 9, with no mutations reported in exon 15. According to the Human Mutation Database 2020.1 (HGMD®), the distribution of various mutations identified in the PTPN11 gene across the exons is as follows: 35% in exon 3, 19.6% in exon 13, 11.9% in exon 7, 8.4% in exon 8, 2.2% in exons 4, 12 [22]. According to OMIM and literature data, pathogenic variants show a distribution in specific exons (exons 2, 3, 4, 7, 8, 11, 12, 13, 14), with 35-73% of all mutations occurring in exon 3, 20-40% in exon 8, and 10-13% in exon 13. This information indicates that examining only exons 3, 7, 8, and 13 in postnatal cases can lead to a mutation detection rate of 85-90% [23]. In prenatal diagnosis, it is believed that applying a similar algorithm would be a rational approach for cost-effective testing and obtaining rapid results. The application of next-generation sequencing (NGS) technologies allows for examining all known associated genes in this group of diseases as a panel [24]. However, detecting numerous density-related or incidental variants that are unknown to be associated, derived from large datasets, can create significant challenges in analysis and interpretation [25]. In our study, 246 cases with excluded chromosomal anomalies, where ultrasound findings/indications placed Noonan Syndrome (NS) in the differential diagnosis, were investigated using different molecular diagnostic approaches: 1) Sanger sequencing of PTPN11 gene target exons, 2) Sanger sequencing of all exons of PTPN11 gene, 3) In cases where no mutation was detected in PTPN11, Sanger sequencing of target exons in four other genes (RAF1, KRAS, SOS1, SHOC2). Additionally, in three post-mortem cases terminated due to ultrasound findings, panel testing including 16 Rasopathy-related genes was conducted. This study explored the contribution of various molecular genetic testing approaches to the diagnosis.

Items of Research (Materials and methods)

The study included 246 cases referred between 2020 and 2024 to the High Institute for Infertility

Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq, and Kadhimiya Teaching Hospital, Department of Obstetrics and Gynecology. These cases had a first-trimester nuchal translucency (NT) measurement of 3.1 mm or more or ultrasound findings associated with syndromes (such as lymphatic system anomalies and heart anomalies), normal karyotype analysis, and informed consent was obtained.

Genomic DNA was isolated from the acquired tissues (amniotic fluid, fetal cord blood, chorionic villus) of all prenatal cases, and skin biopsy samples were taken from three post-mortem cases using the standard column purification method (Qiagen). Specific PCR primers were designed for Sanger sequencing. Based on literature and database reviews, genes that should be included in the Rasopathy panel were identified, and multiplex primer designs for these genes were carried out using the Ion Ampliseq system. Sanger sequencing was performed using the ABI capillary electrophoresis system, and next-generation sequencing (NGS) was conducted on the Ion Torrent S5XL sequencing platform. The results of the three different approaches were evaluated.

1- Sanger sequencing of target exons (13, 14) of the PTPN11 gene (NM_002834.5; NP_002825.3) (n=100)
2- Sanger sequencing of all exons of the PTPN11 gene (n=50)
3- For cases with normal results in PTPN11 full gene sequence analysis, Sanger sequencing of target exons for 4 other genes (RAF1 (NM_002880), KRAS (NM_033360), SOS1 (NM_005633), SHOC2 (NM_007373)) (n=50)

DNA samples from three pregnancies with no pathogenic variants detected in the target exon analysis of the PTPN11 gene and terminated due to ultrasound findings were examined using next-generation sequencing (NGS) with a targeted panel approach for 16 genes (BRAF, CBL, HRAS, KAT6B, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, SHOC2, SOS1, RIT1, NF1, SPRED1, RRAS), which have a higher mutation detection rate compared to others. The ethical committee approved this study.

Results

The average maternal and paternal ages of the cases were 31 (18-45) and 34.5 (23-57), respectively. In the case group, 147 invasive procedures (59.8%) were performed at 10-14 weeks of gestation (WG), 86 (34.9%) at 15-24 WG, and 16 at 25 weeks and beyond (average 16.7 WG). Of the invasive procedures, 68 were amniocentesis, 130 were chorionic villus sampling, and 10 were cordocentesis. In the series, mutations were detected in two of the 200 cases analyzed by target exon analysis of the PTPN11 gene and in three of the 46 cases where all exons of the gene were analyzed. However, these mutations were also located in the target exons (Table 1). Thus, the mutation detection rate in the PTPN11 gene was determined to be 2.03%.

In the six cases where no mutation was found in the PTPN11 gene, mutations were detected in the SOS1 gene in two cases through target exon analysis of RAF1, KRAS, SOS1, and SHOC2 genes. Among the postmortem three cases that were analyzed with the next-generation sequencing (NGS) method for 16 genes, a mutation was found in the RAF1 gene in one of the cases where no pathogenic variant was detected in the target exons of the PTPN11 gene. as shows Table (1).

Table (1): List of variants associated with clinical and USG findings in cases where pathogenic variants were detected

Case no.	Pregnancy week	NT (mm)	USG Findings	Gene	Exon; Nucleotide; peptide
1	20	7.7	Increased NF	PTPN11	Ex 13; c.1529A>C; p.Q510P
2	11	3.7	Increased NT	PTPN11	Ex 13; c.1511T>C; p.M504T
3	12	15	Increased NT, cystic hygroma, generalized edema, hypoplastic left heart, pelvic renal dilatation, unilateral polydactyly (in the foot).	PTPN11	Ex 3; c.319C>T; p.P107S
4	28	2.1	PE, left hydrothorax, polyhydramnios.	PTPN11	Ex 4; c.417G>C; p.E139D
5	19	2.5	PE, short femur and ulna, strawberry-shaped head appearance, SUA	PTPN11	Ex 6; c.755T>C; p.I252T
6	15	4.7	Increased NT	SOS1	Ex 6; c.755T>C; p.I252T
7	20	16	Increased NT, cystic hygroma, generalized ascites, right aortic arch, pyelectasis.	SOS1	Ex 7; c.775T>C; p.S259P
8	25	7.7	Cystic hygroma	RAF1	Ex 4; c.417G>C; p.E139D

PE: Pleural Effusion, **USG**: Ultrasonography, **NF**: Nuchal fold, **NT**: Nuchal translucency, **SUA**: Single umbilical artery, **UNI**.: Unilateral, **EX**: Exon

Discussion

The high prevalence of Noonan spectrum disorders (1:1000-1:2500), the fact that changes in a single gene are responsible for approximately half of postnatal cases, and the ability to detect certain findings even in early fetal ultrasound examinations demonstrate the significant contribution of molecular analyses performed on materials obtained from invasive procedures in cases with normal chromosomal analysis results [26]. This approach has proven to be an important tool for prenatal diagnosis. In a study where they evaluated the PTPN11 gene analysis results in 134 fetuses based on prenatal ultrasound findings (NT and cystic hygroma), the mutation prevalence was reported as 16% for cystic hygroma and 2% for NT [27]. In our entire study group, the mutation rate for the PTPN11 gene was 2%, but this rate reached 13.3% in cases with cystic hygroma. When evaluating the PTPN11 gene mutation rate according to ultrasound findings, mutations were detected in 2 of 88 cases with isolated NT/nuchal fold thickening (2.3%) and 3 of 132 cases with multiple ultrasound findings (2.3%), consistent

with the literature. Another study performed molecular analysis on 75 fetuses with normal karyotype and ultrasound findings, identifying the most frequent mutations in 4 genes, reaching a rate of 17.3% [28]. Another study in their next-generation sequencing (NGS) study of 845 fetuses involving 10 genes identified pathogenic/likely pathogenic variants in 8.5% of cases (72 cases). In this series, PTPN11 gene mutations represented 37.8% of all mutations, followed by SOS1 gene mutations at 27% [29]. In our series, SOS1 gene mutations were detected in 2 cases and RAF1 gene mutation in 1 case in individuals excluded for PTPN11 gene mutations, which was found to agree with the literature. Based on prenatal and postnatal findings, a prospective study in a panel test of 13 genes in 212 neonates suspected of having NS found mutations in 46 cases, 31 of which were in the PTPN11 gene (67.4%) [30]. This study reported that 31 out of 46 cases with mutations had isolated ultrasound findings. The mutation rate was 64.5% in cases with isolated cystic hygroma and 19.3% in cases with increased NT. Based on these results, it was recommended that, in prenatal cases, the PTPN11, RAF1, and KRAS genes, and if possible, the BRAF and MAP2K1 genes, should also be examined. Literature reviews suggest that pathogenic variants can be detected in approximately 90% of post-natal cases through target exon analyses that include exons 3, 4, 7, 8, 13, and 14 of the PTPN11 gene. In our study, all mutations detected in the PTPN11 gene through both target exon analysis and whole gene sequencing were found in the target exons, supporting the view that analyzing the six targeted exons of the PTPN11 gene using Sanger sequencing in the first phase of prenatal diagnosis is a correct approach. In five cases with PTPN11 mutations, two had isolated increased nuchal translucency/nuchal fold, three had lymphatic system findings such as pleural effusion, and one had cystic hygroma. One case each showed cardiac, renal, and extremity findings. In the two cases with isolated NT/nuchal fold, different changes were detected in exon 13. Among these changes, the p.Q510P change had been previously reported in postnatal NS cases but had not been reported in prenatal cases. In the other isolated NT/nuchal fold case, the p.M504T change detected in exon 13 was identified as a novel variant with paternal inheritance. It was observed that the father had a normal phenotype. This change was classified as pathogenic according to the ACMG-2015 criteria (American College of Medical Genetics) from (The Human Genomics Community) open database: (PM1-strong) located in a hot spot region where mutations are frequently detected [31], (PM2-moderate) the variant was not found in the Genome Ad database, (PM5-moderate) the clinical relevance of valine and leucine changes at the same position has been reported, (PP2-supporting) 95.5% of disease-associated variants in PTPN11 are pathogenic, and 51% of these pathogenic variants are nonsense mutations, (PP3-supporting) all 21 different prediction programs indicated the variant as pathogenic. These criteria suggest a disease association, and the variant was classified as pathogenic. As with other autosomal dominant (AD) inherited diseases, clinical heterogeneity caused by expression differences among individuals carrying the same PTPN11 mutation from the same or different families is a well-known phenomenon. In this family, clinical follow-up during the postnatal and adolescent periods will contribute to better assessing the phenotypic effects of the identified variant. The other novel change we identified (c.319C>T,

p.P107S) was found in a case with multiple USG findings and led to the conversion of proline to serine in the SH2 domain it encodes. This variant was not detected in either the fetus's mother or father, and although gonadal mosaicism cannot be excluded, it is considered to be inherited de novo. According to the ACMG-2015 criteria [32] , this variant was classified as a variant of uncertain significance (VUS) due to the following reasons: (PM2-moderate) the variant was not found in the GenomeAd database, (PP2-supporting) 95.5% of disease-associated variants in PTPN11 are pathogenic, and 51% of these pathogenic variants are nonsense mutations, (PP3-supporting) 17 out of 21 different prediction programs (excluding FTHMM, MVP, MutPred, and REVEL) predicted the variant as pathogenic.

Since proline-serine changes carry the possibility of phosphorylation, the NetPhos 3.1 Server phosphorylation prediction tool was used. For serine at position 107, it predicted non-specific kinase activity with a score of 0.797 and Casein kinase phosphorylation with a score of 0.541 (probability; low 0 → high 1). When examining the location of the amino acid with a nonsense mutation, it is reported that the amino acids between positions 103 and 111 play a role in the folding of two SH domains (N-SH2/C-SH2 linker) [33]. Previously, Noonan syndrome has been associated with mutations at positions 106 and 110, such as p.D106A, p.D106G, p.E110A, and p.E110K. The prediction programs indicate pathogenic potential, the clinical and USG findings are consistent with PTPN11 pathologies, and the de novo occurrence of the change supports the likelihood of p.P107S being disease-related.

The p.T73I change, detected in a case with multiple USG pathologies, had been reported in a prenatal case with isolated hydrops fetalis findings. The p.E139D change detected in a case with multiple USG pathologies was reported in two prenatal cases, one with isolated hydrops and the other with multiple USG findings. When examining the USG findings in prenatal cases with PTPN11 mutations in the literature, it has been observed that the same mutation is associated with both isolated and multiple findings. Regarding USG findings, no specific genotype-phenotype correlation has been reported [34].

The USG findings of the two cases with the same pathogenic variant in the SOS1 gene were different. One case had an isolated NT increase (>3.5 mm). In contrast, in the other case, in addition to the NT increase (12 mm), there were findings of cystic hygroma, ascites, right aortic arch, and pyelectasis. This situation is consistent with the forms emerging due to the expression differences of autosomal dominant (AD) disorders. Furthermore, the fact that both PTPN11 and SOS1 pathogenic variants can be responsible for similar clinical presentations is also consistent with the concept of locus heterogeneity in AD disorders, where genes located at different chromosomal loci can manifest with the same or different inheritance patterns, producing a range of clinical spectra.

Conclusion

According to the study results, in pregnancies with increased NT findings and invasive procedures, the analysis of selected exons of the Rasopathy PTPN11 gene in all cases where no chromosomal anomaly was detected in fetal chromosomal analysis contributes at least 2% to the diagnosis, depending on the USG findings, when certain exons of specific genes are sequenced in light of literature information. In the next phase, the examination of known other genes using NGS methods, conducting whole exome and whole

genome analyses for cases without a molecular diagnosis, and investigating the genome for large deletions, duplications, and rearrangements to assess possible copy number variations will contribute to diagnostic research.

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