Review Article

Prenatal Diagnosis of Euploid Increased Nuchal Translucency on Fetal Ultrasound (II): RASopathy Disorders – Prenatal Ultrasound Findings and Genotype–phenotype Correlations

CME Credits

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Abstract

Prenatal diagnosis of euploid increased nuchal translucency (NT) remains a challenge to obstetricians and genetic counselors, although increased euploid NT at prenatal diagnosis can be associated with a favorable outcome. Prenatal diagnosis of euploid increased NT should include a differential diagnosis of pathogenetic copy number variants and RASopathy disorders (RDs) including Noonan syndrome. Therefore, chromosomal microarray analysis, whole-exome sequencing, RASopathy-disorder testing, and protein-tyrosine phosphatase nonreceptor type 11 gene testing may be necessary under such a circumstance. In this report, a comprehensive review of RDs with its prenatal ultrasound findings and genotype-phenotype correlations is presented.

Keywords: Increased nuchal translucency, Noonan syndrome, prenatal diagnosis, RASopathy disorder, ultrasound

Introduction

Prenatal diagnosis of euploid increased nuchal translucency (NT) remains a challenge to obstetricians and genetic counselors, although euploid increased NT at prenatal diagnosis can be associated with a favorable outcome. Prenatal diagnosis of euploid increased NT should include a differential diagnosis of pathogenetic copy number variants, Noonan syndrome (NS), and/or RASopathy disorders (RDs). Therefore, chromosomal microarray analysis (CMA), whole-exome sequencing (WES), RD testing, and protein-tyrosine phosphatase nonreceptor type 11 (*PTPN11*) gene testing may be necessary under such a circumstance.

RASopathy

RASopathy is used for a clinically defined group of disorders caused by germline pathogenic variants in components of the RAS protein/mitogen-activated protein kinase (RAS/MAPK) pathway.[1-3] The RAS/MAPK pathway is a signal transduction cascade associated with cellular processes such as proliferation, survival, differentiation, and metabolism, and is composed of the RAS proteins, RAS guanine nucleotide exchange factors (GEFs), RAS GTPase-activating proteins, RAS effector proteins and their targets, and other pathway modulators.[4] The common mutations of RASopathy-associated genes include the genes of (1) *HRAS*, *NRAS*, *MRAS*, *RRAS,* and *RIT1* for RAS isoforms; (2) *BRAF*, *RAF1* (*CRAF*), *MAP2K1* (*MEK1*), and *MAP2K2* (*MEK2*) for kinases; (3) *SOS1* and *SOS2* for GEFs; (4) *CBL* and *LZTR1* for ubiquitination machinery; (5) *SHOC2* for scaffolds; and (6) *PPP1CB* and *PTPN11* for phosphatases.[4]

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The reported RDs include NS, neurofibromatosis type 1 (NF1), cardiofaciocutaneous syndrome (CFC), NS with multiple lentigines, Costello syndrome (CS), Legius syndrome (LS), central conducting lymphatic anomaly syndrome, SYNGAP1 syndrome, and capillary malformation-arteriovenous malformation syndrome with overlapping features of short stature, facial dysmorphism, congenital heart defect (CHD), and lymphatic dysfunction.[1-4]

Genetic Counseling of Euploid Increased Nuchal Translucency

Genetic counseling of increased NT in the presence of normal chromosomes remains a challenge to obstetricians and genetic counselors.[5-8] Souka *et al*.[5] in a review concluded that the adverse perinatal outcome of the fetuses with euploid increased NT dose not statistically increase until the NT measurement ≥ 3.5 mm. They also concluded that if the fetus survives until midgestation without ultrasound abnormalities at 20–22 weeks of gestation, the risk of an adverse perinatal outcome and postnatal developmental delay is not statistically increased. Bilardo *et al*.[6] in a review concluded that in the fetuses with euploid increased NT, the adverse outcome is proportional to the degree of NT enlargement, and the majority of the babies with normal detailed ultrasound examination and echocardiography will have an uneventful outcome with no increased risk for developmental delay when compared to the general population. Alamillo *et al*.[7] in a review of euploid increased NT concluded that NS is the only molecular genetic condition that has a clear association with the finding of increased NT in the first trimester, and further prenatal testing is recommended. Bakker *et al*.[8] in a review of euploid increased NT concluded that CMA and mutational analysis for NS are recommended in the fetuses with euploid increased NT as well as a detailed sonographic investigation of the fetal heart and dysmorphic features, and the chance of a favorable fetal outcome is high if the above examinations are normal.

Increased Nuchal Translucency, Cystic Hygroma, Pleural Effusion, Hydrops Fetalis, Polyhydramnios, Congenital Heart Defect, and Renal Anomaly on Prenatal Ultrasound and Genotype-Phenotype Correlations

NS has been reported to be the most common single-gene disorder associated with increased NT. Pergament *et al*.[9] reported an incidence of 6.7% (8/120) for NS in the fetuses with euploid increased NT in the first trimester. Lee *et al*.[10] reported an incidence of 9% (12/134) for NS with *PTPN11* mutations in the fetus with cystic hygroma, increased NT and/or hydrops fetalis, and in the fetuses with cystic hygroma, 16% had *PTPN11* mutations, whereas in the fetuses with increased NT, only 2% had *PTPN11* mutations. Ali *et al*.[11] reported an incidence of 10.3% (4/39) for NS in the fetuses with euploid increased NT. Sinajon *et al*.[12] applied microarray and RD testing in 226 fetuses with increased NT and found that 51.3% (116/226) had aneuploidy, and 48.7% (110/226) had normal karyotypes, of which microarray detected abnormalities in 8.2% (9/110) of the cases, and RD testing detected pathogenic variants in 2.9% (3/103) of the cases. Mellis *et al*.[13] in a systematic review and meta-analysis to determine the diagnostic yield of WES, found a diagnostic yield of 2% (95% confidence interval [CI] 0%–5%, $P = 0.04$) for isolated increased NT. Wald *et al*.[14] in a review of prenatal screening for serious CHD using NT suggested that prenatal screening for serious CHD using NT measurement is likely to be effective. Clur *et al*.[15] found major CHD in 4.9% (34/693) fetuses with euploid increased NT (median: 5.2 mm, 2.5–9.6 mm) including conotruncal defects, branchial arch derivative defects, and left and right obstructive lesions and shunts. Houweling *et al*.[16] detected gene mutations in three euploid fetuses. One fetus had a KRAS gene mutation and was associated with increased NT, pleural effusion, and dilated renal pelvis. Another fetus had a *PTPN11* gene mutation and was associated with increased NT. The third fetus had a *PTPN11* gene mutation and was associated with increased NT, pericardial effusion, and complete atrioventricular septal defect.

In a study of prenatal features of NS in 47 patients with molecular diagnosis of NS, Baldassarre *et al*.[17] found that 41% of the cases had increased NT, 38% had polyhydramnios, and 21% had fetal ultrasound anomalies. Bakker *et al*.[18] used targeted ultrasound examination and DNA testing for NS and detected three cases of NS (two with *PTPN11* mutations and one with *RAF1* mutation) associated with euploid increased NT. They suggested that DNA testing for NS is indicated in fetuses with euploid increased NT if there are persistent nuchal fold or cystic hygroma and at least one of the following findings of hydrops fetalis, pleural effusion, cardiac anomalies, polyhydramnios, or specific facial abnormalities. Croonen *et al*.[19] in a study of prenatal diagnostic testing of the NS genes in 75 euploid fetuses with increased NT distended jugular lymphatic sacs (JLS), pleural effusion, ascites, hydrops fetalis, cystic hygroma, polyhydramnios, and cardiac anomalies, found that 17.3% (13/75) had mutated NS genes including *PTPN11* ($n = 9$), *RAF1* ($n = 3$), and *KRAS* ($n = 1$). They also found mutated NS genes including *PTPN11* ($n = 2$) and *RAF1*, *BRAF,* and *MAP2K1* (each $n = 1$) in five cases among 60 other euploid fetuses with sonographic abnormalities such as increased NT, cardiac anomaly, hydrops fetalis, cystic hygroma, distended JLS, hydrothorax, and/or polyhydramnios.

Hakami *et al*.[20] in a retrospective study of prenatal ultrasound findings in 46 newborns with Noonan spectrum disorder (NSD) found that 67.4% (31/46) had only one ultrasound abnormality ranging from CHD ($n = 12$) (26.1%), cystic hygroma ($n = 9$) (19.6%), increased NT ($n = 4$) (8.7%), polyhydramnios ($n = 2$) (4.3%), pleural effusion ($n = 2$) (4.3%), hydrops ($n = 2$) (4.3%) to CHD and cystic hygroma ($n = 5$) (10.9%), and two more above ultrasound abnormalities ($n = 10$) (21.8%), and

suggested that prenatal molecular testing for NSD should be considered even in the presence of a single associated abnormal ultrasound finding. Pierpont and Digilio[21] in a review of cardiovascular disease in NS found that the most common forms of cardiac defects include pulmonary stenosis, hypertrophic cardiomyopathy (HCM), and atrial septal defect, of which HCM is associated with an increased risk of mortality and morbidity. *PTPN11*, *KRAS*, *RAF1*, *SOS1,* and *SHOC2* are associated with a higher incidence of CHD, whereas RIT1, *BRAF,* and *SOS2* are associated with HCM.

In a study of 424 euploid fetuses with prenatal ultrasound findings of increased NT and/or cystic hygroma, distended JLS, hydrops fetalis, polyhydramnios, pleural effusion, ascites, cardiac defects, and renal anomalies, Stuurman *et al*.[22] found that 9.4% (40/424) had RASopathies including mutations of the genes of *PTPN11* (n = 27) (6.4%), *RAF1* (n = 5) (1.2%), *RIT1* (n = 3) (0.7%), *SOS1* (n = 1) (0.2%), *HRAS* (n = 1) (0.2%), *MAP2K2* (n = 1) (0.2%), *BRAF* (n = 1) (0.2%), and *SHOC2* (n = 1) (0.2%). Stuurman *et al*.[22] suggested that RD testing including *A2ML1*, *BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2,* and *SOS1* is recommended when three is an isolated increased NT ≥5.0 mm or when there is an isolated increased NT of ≥3.5 mm with one of the following ultrasonographic findings of a distended JLS, hydrops fetalis, polyhydramnios, pleural effusion, ascites, cardiac defects, and renal anomalies.

In a study of 76 patients with RASopathies including NS (n = 59), CS (n = 9), CFC syndrome (n = 6), and NSML (n = 6), Lee *et al*.[23] found that 61.8% (47/76) had cardiac abnormalities including atrial septal defects, pulmonary stenosis, HCM, ventricular septal defect, and patent ductus arteriosus. The mutated genes in 59 cases of NS with CHD included *PTPN11* (n = 41), *RIT1* (n = 4), *KRAS* (n = 3), *NF1* (n = 3), *SOS1* (n = 2), *BRAF* (n = 2), *RAF1* (n = 2), *MAP2K2* (n = 1), and *SPRED1* (n = 1). The mutated genes in five cases of CS with CHD included *HRAS* (n = 4) and *RAF1* (n = 1). The mutated genes in six cases of CFC with CHD included *BRAF* (n = 3), *SHOC2* (n = 2), and *RAF1* (n = 1). The mutated gene in six cases of NSML with CHD was *PTPN11* (n = 6).

Sparks *et al*.[24] in a study of 127 fetuses with unexplained nonimmune hydrops fetalis by WES found that 29% (37/127) had diagnostic genetic variants including RASopathy (n = 11), inborn error metabolism (n = 4), musculoskeletal disorders (n = 4), lymphatic disorders (n = 3), neurodevelopmental disorders (n = 3), cardiovascular disorders (n = 3), hematological disorders (n = 3), immunological disorders (n = 2), renal disorders (n = 1), ciliopathy (n = 1), overgrowth disorders (n = 1), and CHARGE syndrome (n = 1). RASopathy accounted for 30% (11/37) of the abnormalities. Mangels *et al*.[25] in a study of 63 cases of polyhydramnios found that 33 cases had a single-gene disorder including 15 cases with RASopathy. Scott *et al*.[26] suggested that when any sonographic finding suggesting lymphatic dysplasia and/or CHD, RD testing should be considered following normal CMA. By use of RD testing including the tested genes of *BRAF*, *HRAS*, *KRAS*, *MAP2K1*, *MAP2K2*, *NRAS*, *PTPN11*, *RAF1*, *RIT1*, *SHOC2,* and *SOS1*, Scott *et al*.[26] performed a systematic analysis of 325 CMA-negative fetuses with RASopathy prenatal ultrasound feathers of increased NT, cystic hygroma, hydrops fetalis, effusions, CHD, polyhydramnios, and/or renal anomalies and found that 14% (50/352) had RASopathies including mutations in the genes of *PTPN11 (*30%, 15/50), *RIT1 (*16%, 8/50), *RAF1 (*14%, 7/50), *HRAS* (12%, 6/50), and *LZTR1 (*8%, 4/50) and others such as *BRAF*, *KRAS*, *NRAS*, *SHOC2*, *SOS1,* and *SOS2*. Scott *et al*.[26] reported the genotype–phenotype correlations between prenatal sonographic findings and the involved genes. In their study, *PTPN11*, *SOS1,* and *KRAS* variants showed a milder phenotype, and about 50% of the variants of *PTPN11*, *SOS1,* and *KRAS* showed no significant prenatal sonographic abnormalities. However, most *LZTR1* and *HRAS* variants showed a significant prenatal phenotype, and more than half of the *RAF1* variants had hydrops, effusions, and CHD of HCM.

In summary, in this review article, a comprehensive review of RD and its prenatal ultrasound findings and genotype-phenotype correlations is presented, and the information provided is useful for ultrasonographers as well as genetic counselors.

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Conflicts of interest

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