American Journal of Medical Genetics Part A

WILEY

AMERICAN JOURNAL OF medical genetics PART A

CASE REPORT OPEN ACCESS

Recurrent Increased Nuchal Translucency Led to the Identification of Novel *NUP107* Variants

Isis Atallah[1] | Katarina Cisarova[1] | Cécile Guenot[2] | Estelle Dubruc[3] | Andrea Superti-Furga[1] | Belinda Campos-Xavier[1] | Sheila Unger[1]

[1]Division of Genetic Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland | [2]Materno-Fetal and Obstetrics Research Unit, Department of Obstetrics and Gynecology, University Hospital, Lausanne, Switzerland | [3]Service of Clinical Pathology, Institute of Pathology, Lausanne University Hospital, Lausanne, Switzerland

Correspondence: Isis Atallah (isis_atallah@hotmail.com)

Received: 12 March 2023 | **Accepted:** 12 October 2024

Funding: The authors received no specific funding for this work.

Keywords: Galloway-Mowat syndrome | nuchal translucency | *NUP107* | steroid-resistant nephrotic syndrome | whole exome sequencing | whole genome sequencing

ABSTRACT

Five percent of fetuses presents increased fetal nuchal translucency. It is a well-known marker for aneuploidy (T21, Turner syndrome) and a variety of monogenic syndromes such as Noonan syndrome and certain skeletal dysplasias, as well as associated with structural malformations such as congenital heart disease. Current diagnostic algorithms for increased nuchal translucency include a rapid test for aneuploidy (fluorescence in situ hybridization, FISH, or quantitative PCR), a cytogenetic analysis (karyotype or chromosomal microarray, CMA) followed by or concurrent with targeted gene panel analysis for RASopathies/Noonan syndrome. Some centers now propose whole exome sequencing as an adjunct, but its usefulness in isolated increased nuchal translucency remains debated. We describe the recurrence of apparently isolated increased nuchal translucency in 2 euploid fetuses. Whole genome sequencing identified two compound heterozygous variants in the *NUP107* gene in both fetuses. Biallelic variants in *NUP107* are responsible for severe steroid-resistant nephrotic syndrome, either isolated or syndromic (Galloway-Mowat syndrome); in addition to the renal phenotype, the latter also includes intellectual deficiency and dysmorphic features. Pregnancy termination made it impossible to assess whether the *NUP107* variants found would have resulted in isolated or syndromic steroid-resistant nephrotic syndrome. However, identifying the responsible gene improved the accuracy of the genetic counseling. This family is an example of the added benefit of introducing WES/WGS in standardized protocols for prenatal diagnosis of euploid fetuses in "isolated" increased nuchal translucency.

1 | Introduction

Fetal nuchal translucency (NT) is commonly used to screen for fetal abnormalities in early pregnancy. According to the Fetal Medicine Foundation, increased NT is defined as a subcutaneous fluid accumulation in the posterior neck of the fetus above the 95th centile for the crown-rump length. It occurs per definition in about 5% of fetuses (Shakoor et al. 2017). Increased NT is associated with a large number of diagnoses ranging from isolated malformations to a variety of genetic syndromes, including Noonan syndrome (Sinajon et al. 2020), skeletal dysplasias (Clementschitsch et al. 2003; Ngo et al. 2007) and neurodevelopmental disorders (Roozbeh, Azizi, and Darvish 2017).

Belinda Campos-Xavier and Sheila Unger contributed equally to this work as last authors.

Invasive prenatal testing is proposed for fetuses with NT values above 2.5 mm. Rapid screening for chromosomal aneuploidy (13, 18, 21, X, and Y), chromosomal microarray (CMA), and sequencing of the genes involved in Noonan syndrome and RASopathies are routinely performed. CMA detects an additional 5% of pathogenic copy number variants (CNV) than karyotyping (Grande et al. 2015; Wapner et al. 2012), and targeted gene analysis further increases the diagnostic yield by 1.4%–2.9% (Mastromoro et al. 2022; Sinajon et al. 2020).

Studies evaluating the utility of adding whole exome sequencing (WES) and/or whole genome sequencing (WGS) to the diagnostic algorithm suggested an additional diagnostic yield ranging from 3.2% to 21% for WES/WGS (Drury et al. 2015; Lord et al. 2019) in CMA negative samples. However, the diagnostic yield of prenatal WES is low for fetuses with isolated increased NT (1.8%) but significantly higher when there are also structural anomalies (26%) (Mellis et al. 2022). The diagnostic yields of chromosomal analysis and WES/WGS increased with NT thickness (Ji et al. 2023; Zhou et al. 2023). In fetuses with negative standard karyotype and CMA analysis, WES analysis yielded 44.70% when NT thickness was between 3.0 and 5.5 mm and 55.3% when NT was higher than 5.5 mm (Di Girolamo et al. 2023).

Thus, a nuchal translucency thickness greater than 2.5 mm is associated with a 3.3-fold increased risk of adverse fetal outcomes (Reischer et al. 2022). Noteworthy, the adverse fetal outcome rate did not increase further with increasing NT thickness greater than 2.5 mm (Reischer et al. 2022). Despite extensive research, the etiology of non-immune hydrops fetalis may remain unknown in up to 16.4%–19.8% of cases (Bellini et al. 2015; Reischer et al. 2022).

We report a case of recurrence of isolated increased NT in two euploid fetuses diagnosed by WGS. Although limited to one family, this study is an example of the significant contribution of WES/WGS in prenatal diagnosis. It highlights the need for standardized protocols for applying these techniques in the prenatal diagnostic workflow.

Consequently, there is less traumatic termination of pregnancy for those couples who choose this option.

2 | Clinical Report

A 34-year-old woman (G3P0) was referred for genetic evaluation due to a recurrence of increased nuchal translucency in two fetuses. The couple were healthy and unrelated. The first pregnancy was a blighted ovum. During the second pregnancy, the first-trimester ultrasound (12 + 5/7 SA) showed an increased NT at 4.6 mm associated with subcutaneous thoracic and abdominal edema (fetal hydrops). FISH for chromosomal aneuploidy (13, 18, 21, X, and Y) and CMA, performed on chorionic villus sampling, yielded normal results. Pregnancy interruption took place at 12 + 5/7 weeks of pregnancy by suction curettage. The histology analysis of the curettage material showed hydropic chorionic villi and normal fetal tissues.

The first-trimester ultrasound of the third pregnancy showed an NT at 8.7 mm at 12+ 2/7 SA. Pregnancy termination was performed at 16 SA. The fetal autopsy showed a female fetus with a collapsed hygroma colli, low implanted ears, and 11 pairs of ribs; no visceral or cerebral malformations were observed (Figure 1). Fetal biometry was normal (length at P50-P60). The placenta was small (P5) with some trophoblastic pseudo-inclusions. Parvovirus immunohistochemistry was negative. FISH for aneuploidies and CMA were normal. Because of the recurrence of increased nuchal translucency, a genetic etiology was suspected, and a whole genome was performed. Informed consent was obtained from the parents.

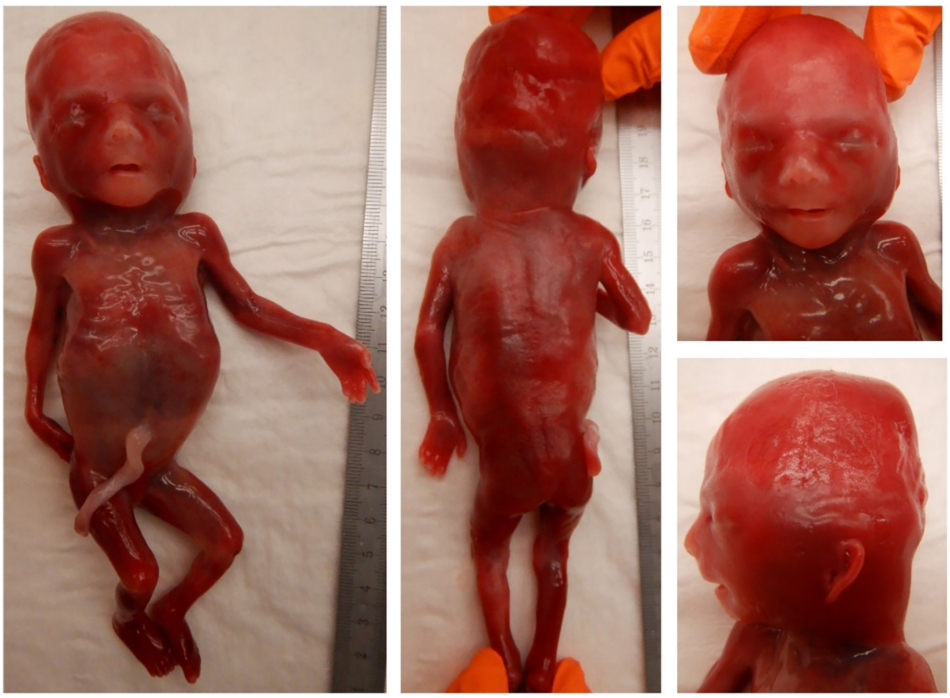

FIGURE 1 | Fetal autopsy of fetus 2 showing markedly low-set ears.

3 | Materials and Methods

Whole Genome sequencing was performed for both fetuses. Genomic DNA was extracted from the chorionic cells for fetus 1 and skin fibroblasts for fetus 2 and sequenced on an Illumina Nova-Seq instrument by Novogene, UK. We used Novoalign software (V3.08.00, Novocraft Technologies) to map the raw reads to the human reference genome (hg19/GRCh37), and Picard (version 2.14.0-SNAPSHOT) was used to remove duplicate reads. Single nucleotide variants and small insertions and deletions (indels) were detected using the Genome Analysis Tool Kit (GATK v4.0) software package, following the Best Practice Guidelines (DePristo et al. 2011). Copy number variants and larger structural variants were detected using Parliament2 (Zarate et al. 2020) and merged using Survivor (Jeffares et al. 2017). All the SNVs and indels were annotated with ANNOVAR (Wang, Li, and Hakonarson 2010) in combination with *in-house* scripts and databases. AnnotSV (Geoffroy et al. 2018) was used to annotate structural variant calls. Variant annotation was verified using VariantValidator (Freeman et al. 2018). Sanger sequencing was used for variant confirmation and segregation analysis.

4 | Results

WGS identified compound heterozygous missense variants in *NUP107* (Nucleoporin 107kd, OMIM *607617) in both fetuses. Both variants were unreported; the variant c.440G>C; p.Gly-147Ala in exon 5 was classified as likely pathogenic, and the variant c.827 T>C; p.Ile276Thr in exon 10 was classified as a variant of uncertain significance (VUS) according to international guidelines (ACMG) (Figure 2a). Both variants arose in highly conserved amino acids (Figure 2b) and are damaging according to five prediction programs (SIFT, Polyphen, CADD, VEST4 and Mutation Taster). We found two heterozygous carriers of the variant p.Ile276Thr in the Genome Aggregation Database (gnomAD). In contrast, we did not observe the variant p.Gly147Ala in gnomAD nor any other internal or external databases of patients or controls. We did not identify any other pathogenic or likely pathogenic SNVs or CNVs. Table 1 summarizes the frequencies of these two variants reported in the genetic databases and their pathogenicity according to several prediction programs. The parental DNA study confirmed that the two variants identified in the two fetuses are in different alleles (trans); the variant in exon 5 was inherited from the mother, and the variant in exon 10 was inherited from the father (Figure 2a). Table S1 summarizes the rare genetic variants shared by the two affected fetuses. Following the genetic results, the histology of the kidneys of foetus 2 was reevaluated. Kidneys were normal and did not reveal any sign of glomerulosclerosis.

Considering the segregation analysis (trans configuration), the co-segregation with the disease in the two affected fetuses, and the lack of alternative explanation, it was highly suggested that both *NUP107* variants likely contributed to both fetuses' phenotypes. However, further studies should be performed to validate the pathogenicity of the two variants in *NUP107* observed in our family. One possible approach is the use of CRISPR/Cas9 editing technology to test the viability of the variants in a homozygous state (Paquet et al. 2016).

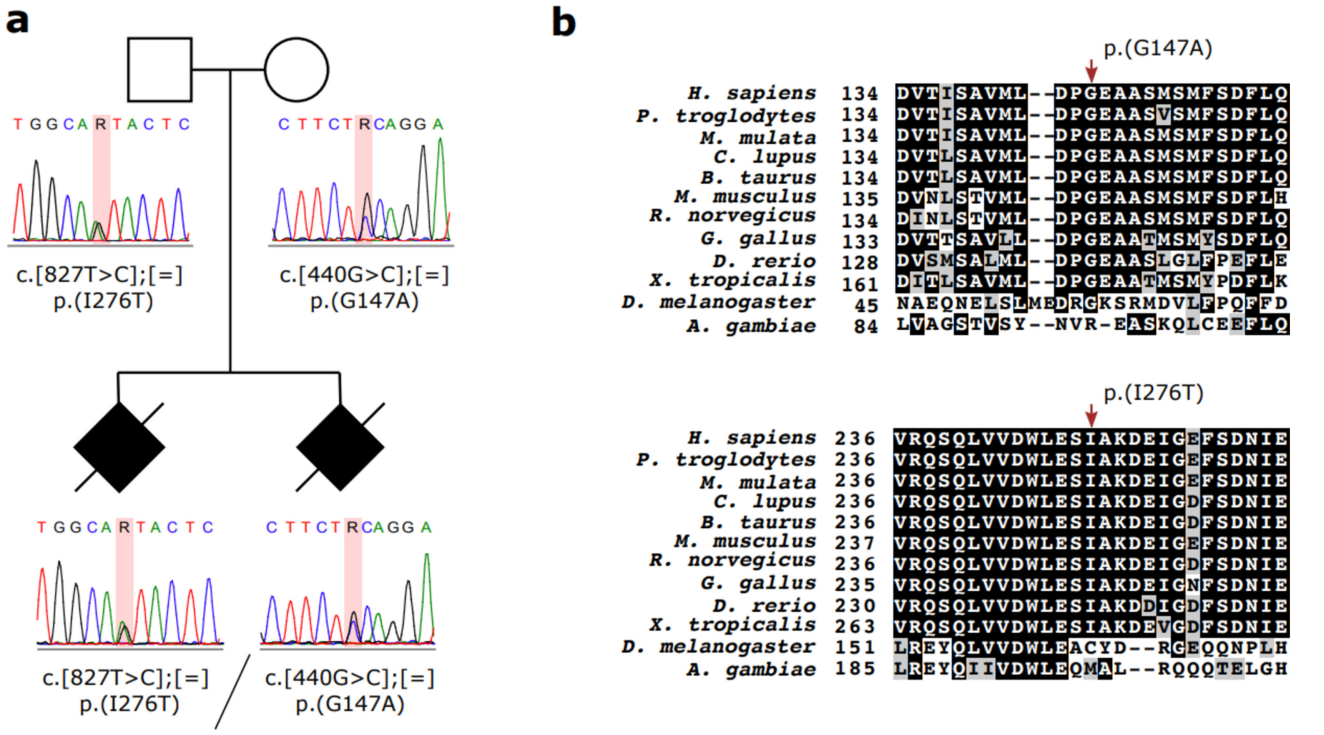

FIGURE 2 | (a) Pedigree of the family. Both fetuses carried two compound heterozygotes pathogenic variants in *NUP107* (NM_020401.4; c.440G>C; p.G147A in exon 5 and c.827T>C; p.I276T in exon 10) while their parents are simple heterozygotes with no clinical manifestations (carriers). (b) Both missense variants affect strongly conserved amino acids in *NUP107* orthologs.

TABLE 1 | Pathogenicity prediction of the two *NUP107 variants found in the two affected fetuses* using bioinformatics tools.

GeneName	AAChange	ProtChange	ID	ClinVar	GnomAD_V4	SIFT	Polyphen	CADD	MutationTaster	Vest4	Mutscore
NUP107	c.440G>C	p.Gly147Ala	rs1592504030	—	—	Deleterious	0.99	23.9	Deleterious	0.74	0.305
NUP107	c.827T>C	p.Ile276Thr	rs1592504030	—	0.000003197	Deleterious	0.95	23.5	Deleterious	0.73	0.203

5 | Discussion

The diagnostic algorithm's first step in a fetus with increased nuchal translucency is a rapid aneuploidy test. However, once this has been excluded, the direction and extent of subsequent investigations vary from center to center. There are no universally accepted protocols concerning further analysis in euploid fetuses with apparently isolated increased NT. Often, a detailed ultrasound is proposed to search for cardiac malformations and/or sequencing of a RASopathy gene panel.

We describe a couple with isolated increased NT in two subsequent pregnancies where WGS analysis identified biallelic pathogenic variants in *NUP107* in both affected fetuses. Pathogenic variants in *NUP107* have been associated with a severe steroid-resistant nephrotic syndrome (Braun et al. 2018; Park et al. 2017) with a median age of end-stage renal disease at 58.9 months (Park et al. 2017). Although increased nuchal translucency seems to be a common presentation of congenital or infantile nephrotic syndrome (Dorval, Servais, and Boyer 2020; Souka et al. 2002), it has not been previously described for *NUP107* pathogenic variants.

Biallelic *NUP107* pathogenic variants have also been reported in patients with the Galloway-Mowat syndrome, which is a genetically heterogenous condition caused by mutations in one of these seven genes: *OSGEP* (Domingo-Gallego et al. 2019; Lin et al. 2018), *WDR73* (Colin et al. 2014; El Younsi et al. 2019; Rosti et al. 2016), *NUP107* (Rosti et al. 2017) and four genes coding for subunits of Kinase, Endopeptidase and Other Proteins of small Size (KEOPS) (Braun et al. 2017). A few cases of Galloway-Mowat syndrome have already been described with a thickened nuchal fold (Horton, Smith, and Strauss 2009) (Choy et al. 2010). Horton et al. even suggested that a thickened nuchal fold should be considered a sonographic marker for Galloway-Mowat syndrome (Horton, Smith, and Strauss 2009).

Galloway-Mowat syndrome (OMIM*618348) is a pleiotropic autosomal recessive disorder associated with global developmental delay, progressive microcephaly, early-onset steroid-resistant nephrotic syndrome, and dysmorphic features, including microcephaly, bitemporal narrowing, sloping forehead, low set ears, and micrognathia. Although most patients reported in the literature have been diagnosed postnatally, prenatal findings include late-onset intrauterine growth retardation, oligohydramnios, microcephaly, and micrognathia, which usually appear in the second or third trimester of pregnancy (22–32 weeks) (Chen et al. 2005; Chen et al. 2006; Horton, Smith, and Strauss 2009; Kang et al. 2005). Despite some minor dysmorphic features in fetus 2, we were unable to assess if the *NUP107* variants found would have resulted in an isolated or syndromic form of steroid-resistant nephrotic syndrome.

This article raises questions on the importance of WES/WGS versus targeted gene panel analysis in prenatal diagnosis of euploid fetuses with increased NT. Should it be generally recommended? It is challenging to conclude reports of single families, and indeed, the addition of this diagnostic test may not increase largely the diagnostic yield. However, given the importance and

time-sensitive nature of decision-making during pregnancy, we would argue that any improvement in diagnostic yield is significant. The time may have come to systematically offer WES/WGS as an integral part of the diagnostic algorithm in the prenatal setting.

Author Contributions

I.A., C.G. and S.U. acquired and interpreted clinical data. K.C. and B.C.-X. performed genetic analysis and K.S., B.C.-X., A.S.-F., S.U. and I.A. interpreted results. E.D. performed and interpreted the fetus autopsy. I.A. and S.U. wrote the initial manuscript. All authors critically reviewed and edited the manuscript and approved the submitted version.

Acknowledgments

We thank the parental couple described here for her willingness to share the clinical and sequence data to advance science and clinical care. Open access funding provided by Universite de Lausanne.

Ethics Statement

We obtained written informed consent from the parents for genetic testing and publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

References

Bellini, C., G. Donarini, D. Paladini, et al. 2015. "Etiology of Non-Immune Hydrops Fetalis: An Update." *American Journal of Medical Genetics. Part A* 167A, no. 5: 1082–1088. https://doi.org/10.1002/ajmg.a.36988.

Braun, D. A., S. Lovric, D. Schapiro, et al. 2018. "Mutations in Multiple Components of the Nuclear Pore Complex Cause Nephrotic Syndrome." *Journal of Clinical Investigation* 128, no. 10: 4313–4328. https://doi.org/10.1172/JCI98688.

Braun, D. A., J. Rao, G. Mollet, et al. 2017. "Mutations in KEOPS-Complex Genes Cause Nephrotic Syndrome With Primary Microcephaly." *Nature Genetics* 49, no. 10: 1529–1538. https://doi.org/10.1038/ng.3933.

Chen, C. P., T. Y. Chang, S. P. Lin, et al. 2005. "Prenatal Magnetic Resonance Imaging of Galloway-Mowat Syndrome." *Prenatal Diagnosis* 25, no. 6: 525–527. https://doi.org/10.1002/pd.1183.

Chen, C. P., S. P. Lin, T. Y. Chang, J. D. Tsai, J. K. Huang, and W. Wang. 2006. "Recurrent Galloway Mowat Syndrome Associated With Abnormal Prenatal Sonographic Findings." *Genetic Counseling* 17, no. 1: 87–89.

Choy, K. W., K. F. To, A. W. H. Chan, T. K. Lau, and T. Y. Leung. 2010. "Second-Trimester Detection of Mowat-Wilson Syndrome Using Comparative Genomic Hybridization Microarray Testing." *Obstetrics and Gynecology* 115, no. 2 Pt 2: 462–465. https://doi.org/10.1097/AOG.0b013e3181c9c8f8.

Clementschitsch, G., G. Hasenohrl, H. Steiner, and A. Staudach. 2003. "Early Diagnosis of a Fetal Skeletal Dysplasia Associated With Increased Nuchal Translucency With 2D and 3D Ultrasound." *Ultraschall Medicine* 24, no. 5: 349–352. https://doi.org/10.1055/s-2003-42910.

Colin, E., E. Huynh Cong, G. Mollet, et al. 2014. "Loss-Of-Function Mutations in WDR73 Are Responsible for Microcephaly and Steroid-Resistant Nephrotic Syndrome: Galloway-Mowat Syndrome." *American Journal of Human Genetics* 95, no. 6: 637–648. https://doi.org/10.1016/j.ajhg.2014.10.011.

DePristo, M. A., E. Banks, R. Poplin, et al. 2011. "A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data." *Nature Genetics* 43, no. 5: 491–498. https://doi.org/10.1038/ng.806.

Di Girolamo, R., G. Rizzo, A. Khalil, et al. 2023. "Whole Exome Sequencing in Fetuses With Isolated Increased Nuchal Translucency: A Systematic Review and Meta-Analysis." *Journal of Maternal-Fetal & Neonatal Medicine* 36, no. 1: 2193285. https://doi.org/10.1080/14767058.2023.2193285.

Domingo-Gallego, A., M. Furlano, M. Pybus, et al. 2019. "Novel Homozygous OSGEP Gene Pathogenic Variants in Two Unrelated Patients With Galloway-Mowat Syndrome: Case Report and Review of the Literature." *BMC Nephrology* 20, no. 1: 126. https://doi.org/10.1186/s12882-019-1317-y.

Dorval, G., A. Servais, and O. Boyer. 2020. "The Genetics of Steroid-Resistant Nephrotic Syndrome in Children." *Nephrology, Dialysis, Transplantation* 37: 648–651. https://doi.org/10.1093/ndt/gfaa221.

Drury, S., H. Williams, N. Trump, et al. 2015. "Exome Sequencing for Prenatal Diagnosis of Fetuses With Sonographic Abnormalities." *Prenatal Diagnosis* 35, no. 10: 1010–1017. https://doi.org/10.1002/pd.4675.

El Younsi, M., L. Kraoua, R. Meddeb, et al. 2019. "WDR73-Related Galloway Mowat Syndrome With Collapsing Glomerulopathy." *European Journal of Medical Genetics* 62, no. 9: 103550. https://doi.org/10.1016/j.ejmg.2018.10.002.

Freeman, P. J., R. K. Hart, L. J. Gretton, A. J. Brookes, and R. Dalgleish. 2018. "VariantValidator: Accurate Validation, Mapping, and Formatting of Sequence Variation Descriptions." *Human Mutation* 39, no. 1: 61–68. https://doi.org/10.1002/humu.23348.

Geoffroy, V., Y. Herenger, A. Kress, et al. 2018. "AnnotSV: An Integrated Tool for Structural Variations Annotation." *Bioinformatics* 34, no. 20: 3572–3574. https://doi.org/10.1093/bioinformatics/bty304.

Grande, M., F. A. Jansen, Y. J. Blumenfeld, et al. 2015. "Genomic Microarray in Fetuses With Increased Nuchal Translucency and Normal Karyotype: A Systematic Review and Meta-Analysis." *Ultrasound in Obstetrics & Gynecology* 46, no. 6: 650–658. https://doi.org/10.1002/uog.14880.

Horton, A. L., J. K. Smith, and R. A. Strauss. 2009. "Recurrence of Galloway Mowat Syndrome and Associated Prenatal Imaging Findings." *Prenatal Diagnosis* 29, no. 3: 280–282. https://doi.org/10.1002/pd.2216.

Jeffares, D. C., C. Jolly, M. Hoti, et al. 2017. "Transient Structural Variations Have Strong Effects on Quantitative Traits and Reproductive Isolation in Fission Yeast." *Nature Communications* 8: 14061. https://doi.org/10.1038/ncomms14061.

Ji, X., Q. Li, Y. Qi, et al. 2023. "When NIPT Meets WES, Prenatal Diagnosticians Face the Dilemma: Genetic Etiological Analysis of 2328 Cases of NT Thickening and Follow-Up of Pregnancy Outcomes." *Frontiers in Genetics* 14: 1227724. https://doi.org/10.3389/fgene.2023.1227724.

Kang, L., P. L. Kuo, K. H. Lee, et al. 2005. "Late-Onset Growth Restriction in Galloway-Mowat Syndrome: A Case Report." *Prenatal Diagnosis* 25, no. 2: 159–162. https://doi.org/10.1002/pd.1099.

Lin, P. Y., M. H. Tseng, M. Zenker, et al. 2018. "Galloway-Mowat Syndrome in Taiwan: OSGEP Mutation and Unique Clinical Phenotype." *Orphanet Journal of Rare Diseases* 13, no. 1: 226. https://doi.org/10.1186/s13023-018-0961-9.

Lord, J., D. J. McMullan, R. Y. Eberhardt, et al. 2019. "Prenatal Exome Sequencing Analysis in Fetal Structural Anomalies Detected by Ultrasonography (PAGE): A Cohort Study." *Lancet* 393, no. 10173: 747–757. https://doi.org/10.1016/S0140-6736(18)31940-8.

Mastromoro, G., D. Guadagnolo, N. Khaleghi Hashemian, et al. 2022. "A Pain in the Neck: Lessons Learnt From Genetic Testing in Fetuses Detected With Nuchal Fluid Collections, Increased Nuchal Translucency Versus Cystic Hygroma-Systematic Review of the Literature, Meta-Analysis and Case Series." *Diagnostics (Basel)* 13, no. 1: 48. https://doi.org/10.3390/diagnostics13010048.

Mellis, R., R. Y. Eberhardt, S. J. Hamilton, et al. 2022. "Fetal Exome Sequencing for Isolated Increased Nuchal Translucency: Should We Be Doing It?" *BJOG* 129, no. 1: 52–61. https://doi.org/10.1111/1471-0528.16869.

Ngo, C., G. Viot, M. C. Aubry, et al. 2007. "First-Trimester Ultrasound Diagnosis of Skeletal Dysplasia Associated With Increased Nuchal Translucency Thickness." *Ultrasound in Obstetrics & Gynecology* 30, no. 2: 221–226. https://doi.org/10.1002/uog.4028.

Paquet, D., D. Kwart, A. Chen, et al. 2016. "Efficient Introduction of Specific Homozygous and Heterozygous Mutations Using CRISPR/Cas9." *Nature* 533, no. 7601: 125–129. https://doi.org/10.1038/nature17664.

Park, E., Y. H. Ahn, H. G. Kang, N. Miyake, H. Tsukaguchi, and H. I. Cheong. 2017. "NUP107 Mutations in Children With Steroid-Resistant Nephrotic Syndrome." *Nephrology, Dialysis, Transplantation* 32, no. 6: 1013–1017. https://doi.org/10.1093/ndt/gfw103.

Reischer, T., B. Muth, A. Catic, et al. 2022. "Clinical Course and Outcome of Non-Immune Fetal Hydrops in Singleton Pregnancies." *Journal of Clinical Medicine* 11, no. 3: 702. https://doi.org/10.3390/jcm11030702.

Roozbeh, N., M. Azizi, and L. Darvish. 2017. "Pregnancy Outcome of Abnormal Nuchal Translucency: A Systematic Review." *Journal of Clinical and Diagnostic Research* 11, no. 3: QC12–QC16. https://doi.org/10.7860/JCDR/2017/23755.9384.

Rosti, R. O., E. Dikoglu, M. S. Zaki, et al. 2016. "Extending the Mutation Spectrum for Galloway-Mowat Syndrome to Include Homozygous Missense Mutations in the WDR73 Gene." *American Journal of Medical Genetics. Part A* 170A, no. 4: 992–998. https://doi.org/10.1002/ajmg.a.37533.

Rosti, R. O., B. N. Sotak, S. L. Bielas, et al. 2017. "Homozygous Mutation in NUP107 Leads to Microcephaly With Steroid-Resistant Nephrotic Condition Similar to Galloway-Mowat Syndrome." *Journal of Medical Genetics* 54, no. 6: 399–403. https://doi.org/10.1136/jmedgenet-2016-104237.

Shakoor, S., D. Dileep, S. Tirmizi, S. Rashid, Y. Amin, and S. Munim. 2017. "Increased Nuchal Translucency and Adverse Pregnancy Outcomes." *Journal of Maternal-Fetal & Neonatal Medicine* 30, no. 14: 1760–1763. https://doi.org/10.1080/14767058.2016.1224836.

Sinajon, P., D. Chitayat, M. Roifman, et al. 2020. "Microarray and RASopathy-Disorder Testing in Fetuses With Increased Nuchal Translucency." *Ultrasound in Obstetrics & Gynecology* 55, no. 3: 383–390. https://doi.org/10.1002/uog.20352.

Souka, A. P., H. Skentou, L. Geerts, S. Bower, and K. H. Nicolaides. 2002. "Congenital Nephrotic Syndrome Presenting With Increased Nuchal Translucency in the First Trimester." *Prenatal Diagnosis* 22, no. 2: 93–95. https://doi.org/10.1002/pd.121.

Wang, K., M. Li, and H. Hakonarson. 2010. "ANNOVAR: Functional Annotation of Genetic Variants From High-Throughput Sequencing Data." *Nucleic Acids Research* 38, no. 16: e164. https://doi.org/10.1093/nar/gkq603.

Wapner, R. J., C. L. Martin, B. Levy, et al. 2012. "Chromosomal Microarray Versus Karyotyping for Prenatal Diagnosis." *New England Journal of Medicine* 367, no. 23: 2175–2184. https://doi.org/10.1056/NEJMoa1203382.

Zarate, S., A. Carroll, M. Mahmoud, et al. 2020. "Parliament2: Accurate Structural Variant Calling at Scale." *GigaScience* 9, no. 12: giaa145. https://doi.org/10.1093/gigascience/giaa145.

Zhou, H., X. Yang, C. Yi, et al. 2023. "Prenatal Diagnosis and Early Childhood Outcome of Fetuses With Extremely Large Nuchal Translucency." *Molecular Cytogenetics* 16, no. 1: 22. https://doi.org/10.1186/s13039-023-00650-4.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.