Prenatal Diagnosis

WILEY

PRENATAL DIAGNOSIS

ORIGINAL ARTICLE OPEN ACCESS

The Clinical Utility of Sequence-Based Genetic Testing for Fetal Edema Following Non-Diagnostic Microarray Results: A Population-Based Cohort Study

Victoria M. Allen[1,2] | Heleen H. Arts[1,2] | Erica Schollenberg[1,2] | Samantha Kinley[1] | Erika Aberg[2] | Jo-Ann K. Brock[1,2]

[1]Dalhousie University, Halifax, NS, Canada | [2]IWK Health Center, Halifax, NS, Canada

Correspondence: Victoria M. Allen (victoria.allen@dal.ca)

Received: 9 September 2025 | **Revised:** 30 January 2026 | **Accepted:** 24 February 2026

ABSTRACT

Objective: Ultrasound findings of fetal edema may provide early evidence of a genetic disorder. Our objective was to evaluate the frequency and diagnostic yield of sequencing in a selected population after a non-diagnostic microarray result for fetal edema.

Method: Fetal edema (nuchal translucency > 3.5 mm, cystic hygroma, hydrops, other types) cases referred for genetic testing (2014–2022) were identified in our laboratory information system. Chart review confirmed associated ultrasound findings, findings from other imaging or pathology, multidisciplinary review, and genetic test utilization. Descriptive analyses determined diagnostic yields.

Results: There were 114 fetal edema cases with a non-diagnostic microarray result. Multidisciplinary review occurred in 79 (83%) cases, with targeted genes, gene panels, and/or exomes performed in 51 (54%) cases based on clinical assessment. Diagnoses were made in 17 (34%, with 59% providing a recurrence risk of ≥ 25%). RASopathy testing and exomes (with selective criteria) resulted in diagnostic yields of 18.5% and 41.2%, respectively. The highest diagnostic yields were in cases with fetal hydrops and other types of fetal edema (restricted to one body cavity).

Conclusion: Review of additional testing beyond CMA in this cohort did not attempt to assess the overall yield of sequencing; instead, the study used a selected approach in which cases of fetal edema underwent further testing beyond CMA. In cases with cystic hygroma or increased nuchal translucency, RASopathy panel sequencing provides a good diagnostic yield, and should be offered as standard of care with CMA. Correlation of new and developing prenatal and postnatal findings, along with multidisciplinary team review, may inform additional testing, counseling and pregnancy management options, and is particularly important to consider in cases presenting with non-immune fetal hydrops.

1 | Introduction

Fetal edema comprises conditions such as increased nuchal translucency (NT), cystic hygroma (CH), fetal hydrops, or isolated fetal edema, such as ascites, pleural or pericardial effusions, or skin edema. It is associated with a broad differential diagnosis and a range of outcomes, including normal variation, genetic disorders, congenital infections, structural abnormalities, fetal growth restriction, fetal demise and developmental delay. Genetic etiologies for prenatal ultrasound findings of fetal edema have been described using a variety of methodologies [1–9]. The diagnostic yield for rapid aneuploidy detection (RAD), karyotype, and chromosomal microarray analysis (CMA) following ultrasound findings of increased NT, CH, fetal hydrops, or isolated fetal edema is 30%–40% [5]. Sequence-based testing can provide an incremental diagnostic yield of 30% [4, 10, 11].

Summary

- What is already known about this topic?
  - Fetal edema is associated with a broad range of etiologies, including underlying genetic disorders.
  - Following non-diagnostic rapid aneuploidy detection (RAD) and chromosomal microarray analysis (CMA) results, next-generation sequencing (NGS) may identify additional causative variants.
  - The incremental diagnostic yields with sequence-based testing have an important contribution to clinical counseling and management.
- What does this study add?
  - Population-based comprehensive genome diagnostics and perinatal data.
  - Diagnostic yields of sequence-based testing using selective clinical criteria for cases with hydrops were improved in a clinical practice setting compared with reported studies.
  - Timely access to prenatal ultrasound and the clinical value of multidisciplinary teams have the potential to effectively provide patients with earlier diagnoses and appropriate NGS testing.

The current study is a population-based retrospective cohort evaluation of all fetuses that had genetic testing done for a prenatal ultrasound finding of non-specific fetal edema, and was designed to evaluate the use and yield of sequence-based testing following non-diagnostic testing results for RAD and CMA. This study involved a review of clinical data already available in a population-based genomics laboratory information system and health records, including consultant, laboratory, and imaging reports. Specifically, the objectives were to assess the use of targeted gene panels and exome analysis in cases of fetal edema, to determine the diagnostic yield of this testing based on selected clinical criteria, to examine the clinical value of review by multidisciplinary teams, and to clarify the specific fetal edema indications (increased NT, CH, non-immune hydrops, or other types of edema restricted to one body cavity) that would benefit best from sequence-based methods in a low resource clinical practice setting, to optimize counseling and management for patients.

2 | Methods

Data from the IWK Health Clinical Genomics Laboratory Information System (CGLIS) in Halifax, Nova Scotia, was combined with health records review from January 1, 2014 to December 31, 2022 to conduct a retrospective, population-based cohort study. The CGLIS performs or coordinates all publicly funded genetic testing in the Canadian Maritime Provinces (including Nova Scotia, New Brunswick and Prince Edward Island) for anomalies detected by fetal ultrasound, and securely stores cytogenetic and molecular genetic information related to testing indications, types of samples, and testing results.

For the duration of the study, RAD (QF-PCR for common aneuploidy), karyotype (during earlier years of the study), and CMA (for causative copy number variants, CNV) comprised first-tier genetic testing at IWK Health, with sequence-based testing (genes, gene panels, or exomes) offered for selected cases meeting the specific testing criteria developed collaboratively by IWK Health Maritime Medical Genetics and Clinical Genomics services. Testing criteria during the study period at IWK Health included a standard 17-gene RASopathy panel for NT greater than 5 mm, single gene or gene panels based on assessed clinical phenotype, and exomes based on one or more of the following findings: two or more affected organ systems, progressive clinical course, and recurrence of phenotype in a subsequent pregnancy. Following review of ultrasound findings of fetal edema and structural anomalies, patients have the opportunity for consultation with the Medical Genetics service, who offer additional sequence-based testing within the clinical presentation and the IWK laboratory criteria; for ongoing pregnancies during the study period, referral to the Medical Genetics service was required to access the opportunity for genetic testing beyond assessment for aneuploidy.

All fetal samples with abnormal ultrasound findings of fetal edema (chorionic villus sampling (CVS), amniocentesis, fetal blood, and products of conception (POC)) were identified by CGLIS, as well as cord blood or newborn samples within 4 weeks of delivery, based on prenatal fetal ultrasound detection of fetal edema with or without structural anomalies. Genetic testing using RAD, karyotype and CMA was done in-house. For gene sequencing, testing was done at either locally or through accredited commercial referral laboratories. A panel was restricted to one or more genes, while exome analysis involved phenotype-driven testing. Single genes and small gene panels, such as RASopathy, were performed in-house, whereas larger gene panels (> 200 genes) and exomes were performed by referral laboratories. Classification of variants for testing done locally followed the America College of Medical Genetics (ACMG) criteria [12], and for other testing, classification was done according to the referral laboratory procedure. The clinicians involved in the care of the patient provided interpretation of the variants as clinically relevant pathogenic variants or variants of uncertain significance.

Findings of fetal edema included cases with NT $\geq$ 3.5 mm, CH, hydrops, and other types of fetal edema restricted to one body cavity (such as ascites, effusions, or skin edema). Eligible samples were those identified by the CGLIS as having undergone RAD and CMA and had non-diagnostic results; health records review was performed for information on patient demographics, such as maternal age and gestational age at initiation of fetal testing, multidisciplinary review (including consultation and counseling with Medical Genetics service, Maternal Fetal Medicine, and other relevant subspecialists as needed, such as cardiology, neurosurgery, orthopedics), associated ultrasound findings and findings from other imaging modalities, autopsy, newborn examination, and the results of genetic testing.

A descriptive analysis of the study population was performed and evaluated the number of genetic tests and the diagnostic yield of causative microarray and single gene variants in a cohort of fetuses with edema, as well as the clinical collaboration with a multidisciplinary team. Chi-square (or similar test for small numbers) and *t*-test statistical analyses were performed (for categorical and continuous variables, respectively, significance set at

$p < 0.05$) using Open Epi (Version 3.01, 2013). Aggregate cell sizes < 5 were suppressed to protect patient privacy in compliance with local data management principles. IWK Health Research Ethics Board approval was obtained (IWK REB Project #1024606).

3 | Results

The CGLIS identified 593 prenatal and neonatal cases eligible for chromosomal microarray testing after non-diagnostic RAD results, based on prenatal detection of structural abnormalities, 127 of which involved fetal edema as a primary feature (Figure 1). Cases with anemia and congenital infections were previously excluded. The majority of samples tested were from CVS, amniocentesis, fetal blood, and POC (103/114, 90.4%). Of these 127 cases, 13 (10.2%) had a diagnostic CMA result and 105 (82.6%) participated in a multidisciplinary review either before or after CMA testing. Table 1 summarizes the demographic characteristics of the study population, grouped by presentation type (increased NT, CH, hydrops, or other types of fetal edema). Sixteen (61.5%) other types of fetal edema had skin edema. A difference in mean maternal age was noted for cases presenting with increased NT ($p = 0.04$) and hydrops ($p = 0.047$), but not CH or other types of fetal edema ($p > 0.05$), compared to the overall mean maternal age. Cases with increased NT were less likely to be associated with nulliparity ($p = 0.01$), compared to overall nulliparity, with no differences noted for CH, hydrops, or other types of fetal edema ($p > 0.05$). There was no difference in mean body mass index (BMI) in any of the groups compared with overall BMI ($p > 0.05$). The mean gestational age at the time of initiating genetic testing was 19.0 weeks overall, with lower gestational ages for cases with increased NT and CH, and with higher gestational ages for cases presenting with hydrops and other types of fetal edema ($p < 0.001$ for all groups) compared to the overall gestational age. One or more structural anomalies were present in 55/127 (43.3%) cases of fetal edema, the majority of which were cardiac and/or renal anomalies, and were more commonly seen in cases with hydrops (51.6%) and other types of fetal edema (69.2%) compared to the total ($p = 0.01$ and $p < 0.001$, respectively).

Table 2 summarizes the yield of diagnostic microarray results, participation in multidisciplinary review, and additional sequence-based testing among fetal edema cases according to presentation group. Overall, 10.2% of cases had a diagnostic CMA result; there was no difference in diagnostic yield in each of the groups of fetal edema compared to the total ($p > 0.05$), although cases presenting with other types of fetal edema had a clinically lower diagnostic yield (3.9%). Multidisciplinary review, including consultation with the Maritime Medical Genetics Service, occurred in 82.7% of the cases overall; those presenting with increased NT were more likely to undergo multidisciplinary review compared to the total ($p < 0.01$), while those cases with fetal hydrops were less likely to undergo multidisciplinary review compared to the total ($p < 0.01$); there was no difference for CH and other types of fetal edema ($p > 0.05$). A total of 95 cases that did not have a diagnostic CMA result underwent multidisciplinary review and assessment for additional sequence-based testing. Overall, 51 of these cases (53.7%) had additional sequence-based testing ranging from

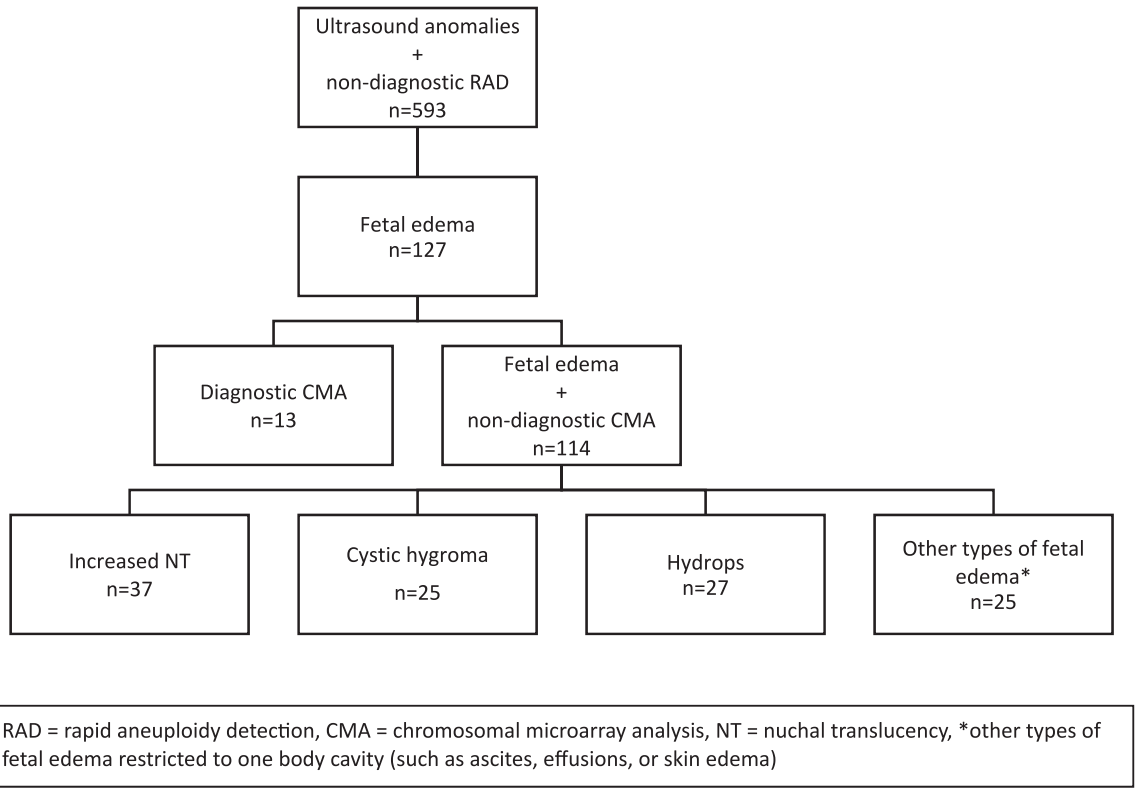

FIGURE 1 | Flow diagram determination of study population, IWK health clinical genomics laboratory information system, 2014–2022. RAD = rapid aneuploidy detection, CMA = chromosomal microarray analysis, NT = nuchal translucency, *other types of fetal edema restricted to one body cavity (such as ascites, effusions, or skin edema).

TABLE 1 | Summary characteristics of the study population, 2014–2022.

	Increased NT $n = 41$	Cystic hygroma $n = 29$	Hydrops $n = 31$	Other[a] $n = 26$	Total $n = 127$
Mean maternal age (SD), range	33.0 (4.6), 24–42	32.2 (5.2), 20–38	29.0 (5.4), 18–42	29.7 (4.1), 23–38	31.1 (5.2), 18–42
Nulliparous, %	25.6	44.4	35.7	52.0	40.7
Mean BMI (SD), range	25.0 (4.7), 19–38	25.4 (4.9), 19–34	28.0 (9.5), 17–51	27.4 (7.7), 18–49	26.2 (6.6), 17–51
Mean GA at testing (SD), range	15.0 (2.2), 12–21	15.0 (2.7), 12–21	24.0 (7.7), 13–40	24.4 (6.2), 17–40	19.0 (6.8), 12–40
One or more structural anomalies, %	31.7	27.6	51.6	69.2	43.3

Abbreviations: BMI = body mass index, GA = gestational age, NT = nuchal translucency, SD = standard deviation.
[a]Other types of fetal edema restricted to one body cavity (such as ascites, effusions, or skin edema).

TABLE 2 | Diagnostic microarray copy number variants (CNV), multidisciplinary review, and additional genetic testing among fetal edema cases undergoing genetic testing, IWK health clinical genomics laboratory information system, 2014–2022.

	Increased NT $n = 41$	Cystic hygroma $n = 29$	Hydrops $n = 31$	[a]Other $n = 26$	Total $n = 127$
One or more structural anomalies, %	22.0	27.6	58.1	73.1	52.0
Diagnostic CMA result, %	9.8	13.8	12.9	3.9	10.2
Multidisciplinary review, %	95.1	86.2	64.5	80.8	82.7
Additional sequence-based testing, %[b]	36.1	76.2	66.7	50.0	53.7

Abbreviations: CMA = chromosomal microarray analysis, NT = nuchal translucency.
[a]Other types of fetal edema restricted to one body cavity (such as ascites, effusions, or skin edema).
[b]The denominator for additional sequence-based testing is based on those seen in a multidisciplinary review with diagnostic CMA results that did not provide a diagnosis.

targeted single genes, targeted gene panels, and exomes. Additional testing was higher in cases presenting with CH ($p < 0.001$) and hydrops ($p = 0.01$), but not with increased NT or other types of fetal edema ($p > 0.05$); additional testing with increased NT was clinically lower than in the other groups. For those cases undergoing additional testing, aside from cases with increased NT where structural anomalies may have been more difficult to detect, 84% of testing was done in the context of fetal phenotype, including the presence of structural anomalies, consistent with established institutional testing criteria during the study period.

Supporting Information S1: Table 1 summarizes the targeted sequence-based testing used in the study. Table 3 summarizes the types of additional sequence-based genetic testing that were performed, categorized by fetal edema group, following a CMA result that was non-diagnostic; in 12 cases (24% of tested samples), more than one additional sequence-based test was performed. A RASopathy gene panel was the most frequently used sequence-based test, performed for 27 cases, representing 53.0% of all cases having additional testing. RASopathy testing was used more often in the setting of increased NT (33.3%) and CH (42.9%), while other types of fetal edema (5.0%) less commonly had RASopathy testing. There was no difference in the types of additional sequence-based testing in cases presenting with fetal hydrops (27.8% for all). Other targeted sequencing included single genes (9 cases) and defined gene panels (10 cases) based on the presenting phenotype, the most common of which was the fetal arthrogryposis/akinesia panel. Targeted testing other than the RASopathy panel was rarely used in cases presenting with increased NT (5.6%), and more consistently used in the setting of CH (23.8%), hydrops (27.8%), and other types of fetal edema (35.0%). Exome analysis was used in 18 cases (20%) overall, either as an initial sequencing test (10 cases) or after other sequencing (8 cases).

Table 3 also shows the diagnostic yields according to type of sequencing test (RASopathy panel, other targeted gene/panel sequencing, and exome analysis) and total diagnostic yields of additional sequence-based testing. Supporting Information S1: Table 2 summarizes the gene variants that were considered diagnostic. In this study, exome analysis included 9 singleton exomes and 9 trio or quad exomes with parental samples (including, e.g., samples from a second affected fetus in the case of quad analyses); it was a clinical practice setting where both parents were not always available for trio analysis, or where the geneticist had made a clinical decision to proceed only with a singleton analysis. For those cases tested following review by the Maritime Medical Genetics Service because of a non-diagnostic CMA result, higher diagnostic yields were observed more frequently for cases presenting with hydrops (50.0%) and other types of fetal edema (50%), and less frequently for increased NT (7.7%) and CH (31.3%). While RASopathy testing did not provide a diagnosis for cases with other types of edema, a diagnosis was provided by RASopathy panel testing in 7.7% of cases presenting with increased NT, 12.5% with CH, and 16.7% with hydrops (relative to the total number of RASopathy panel tests performed, $n = 27$). Targeted single genes or gene panels other than RASopathy infrequently provided a diagnosis for cases presenting with increased NT (0%), CH (6.3%), or hydrops (8.3%), and more frequently provided a diagnosis for cases presenting with other types of fetal edema (30%, $p < 0.001$ compared to both CH and

TABLE 3 | Additional sequence-based testing and exome analysis after non-diagnostic microarray analysis (CMA) results and diagnostic yields from additional genetic testing among fetal edema cases undergoing genetic testing, IWK health clinical genomics laboratory information system, 2014–2022.

	Increased NT $n = 36$	Cystic hygroma $n = 21$	Hydrops $n = 18$	[a]Other $n = 20$
One or more structural anomalies, %	25.0	33.3	61.1	85.0
Additional sequence-based testing, %[b]	36.1	77.2	66.7	50.0
RASopathy testing	33.3	42.9	27.8	5.0
Other targeted sequencing[c]	5.6	23.8	27.8	35.0
Exome analysis	2.8	23.8	27.8	30.0
	$n = 13$	$n = 16$	$n = 12$	$n = 10$
One or more structural anomalies, %	30.8	43.8	58.3	90.0
Diagnostic yields from additional testing, %[d]	7.7	31.3	50.0	50.0
RASopathy testing	7.7	12.5	16.7	0
Other targeted sequencing[c]	0	6.3	8.3	30.0
Exome analysis	0	12.5	25.0	20.0

Abbreviations: CMA = chromosomal microarray analysis, NT = nuchal translucency.
[a]Other types of fetal edema restricted to one body cavity (such as ascites, effusions, or skin edema).
[b]The denominator for additional sequence-based testing is based on those seen in a multidisciplinary review with CMA results that did not provide a diagnosis.
[c]Other targeted sequencing included targeted single genes and defined gene panels.
[d]The denominator for additional sequence-based testing is based on the cases that had additional testing performed.

hydrops). Testing by exome analysis provided a diagnosis in 12.5% of cases with CH, 25.0% of cases with hydrops, and 20% of cases with other types of edema.

4 | Discussion

The current study evaluated the use of sequence-based testing following a non-diagnostic microarray result, for different presentations of fetal edema detected by prenatal ultrasound, in a low-resource clinical practice setting. During the study time frame, CMA and sequence-based testing were offered for cases based on clinical phenotype and/or family history meeting specific institutional testing criteria.

Most cases of fetal edema accessed multidisciplinary review, including consultation with the IWK Health Maritime Medical Genetics Service, either before or after CMA, with additional sequence-based testing providing diagnostic yields comparable to published literature. A RASopathy gene panel was the most frequently used sequence-based test in the study cohort. Sequence-based testing had the highest diagnostic yield in cases of fetal hydrops and other types of fetal edema restricted to one body cavity compared with increased NT or CH.

The current study demonstrated diagnostic yields from additional sequence-based testing, including RASopathy testing, other targeted sequencing, and exome analysis, for cases presenting with either increased NT ≥ 3.5 (7.7%) or CH (31.3%) that are similar to recent published literature. RASopathies are common genetic causes of fetal edema; prenatal findings are not specific but may include increased NT, CH, hydrops, pleural effusions, and polyhydramnios [13, 14] While additional structural anomalies may be present, 20% of fetuses found to have RASopathies have isolated fetal edema on ultrasound. In a systematic review and meta-analysis, Makhamreh et al. [15] demonstrated that the rate of RASopathy diagnoses among cases with non-immune fetal hydrops with a genetic diagnosis was 23% following non-diagnostic RAD and CMA results, while in a review of fetuses with NT ≥ 3.5 mm, a RASopathy panel provided a diagnosis in 1.44% [5]. Current literature shows that a limited gene panel for RASopathies with fetal edema has a diagnostic yield of 9%–30% following non-diagnostic RAD and CMA results, although limited gene panels may not typically be part of a standardized assessment for fetal edema [4, 15, 16]. The current study demonstrated a similar diagnostic yield for RASopathies, including 16.7% for cases presenting with fetal hydrops and 7.7% and 12.5% for those presenting with increased NT and CH cases, respectively. The higher yield for increased NT and CH in the current study is likely due to the institutional testing criteria during the study period (i.e., RASopathy testing was primarily used in the setting of NT greater than 5 mm), resulting in a smaller subset of cases eligible for sequence-based testing than has been examined in the literature.

Beyond RASopathy testing, reports in the literature of other sequence-based testing using targeted single genes or gene panels to investigate fetal edema were rare, and were more likely to evaluate associated findings (such as arthrogryposis/akinesia) [17]. Low diagnostic yield of targeted gene sequencing other than RASopathy testing was also demonstrated in the current study, and did not provide a diagnosis for cases presenting with increased NT (case numbers were also small). However, in the context of typical clinical practice, exomes performed for cases meeting specific criteria, which included the presence of fetal anomalies, provided high diagnostic yields comparable to those in published literature. A recent systematic review investigating increased NT in the early first trimester showed a high prevalence of chromosomal, genetic and structural abnormalities in fetuses with persistent and resolved

increased NT, and in particular, they demonstrated that diagnostic yields with exome sequencing for single gene disorders with increased NT were 7.6% [18]. Studies have shown diagnostic yields for exome sequencing to be low in cases of isolated increased NT ≥ 3.5 mm (1.8%–4.8%) [5, 19, 20], but significantly higher for increased NT in the presence of additional structural anomalies (17%–37.5%) [10, 19, 20]. Another study [21] demonstrated a diagnostic yield of 34.3% using exome analysis for NT thickening > 95th percentile and CH; however, this was in a selected subgroup of ongoing pregnancies, including the possibility of other structural abnormalities. In the current study, exome analysis was restricted to specific institutional testing criteria; thus, for increased NT, exome testing was rare, and no diagnostic variants were reported.

Prospective and meta-analysis information in the literature related to non-immune fetal hydrops with or without concurrent anomalies demonstrated diagnostic yields from exome sequencing ranging from 17% to 50% [3, 4, 9, 22, 23]. Gulrajani et al. [10] also demonstrated diagnostic yields for fetal hydrops (26%) and single compartment effusions (22%) in their prospective study with exome sequencing. With lower rates of multidisciplinary review for cases with fetal hydrops (and potentially less opportunities for sequence-based testing), the current study demonstrated similar diagnostic yields from exome analysis in cases with fetal hydrops and other fetal edema (33% and 20% respectively), and higher diagnostic yields when including RASopathy testing and other targeted sequencing as well as exome sequencing (50% and 50%, respectively).

Collaboration with and assessment by multidisciplinary teams with genetic testing is a recognized and valued resource [24–26]. While a high percentage of cases of increased NT, CH and other cases of fetal edema were reviewed by multidisciplinary teams in the current study, cases with fetal hydrops were less likely to have had this involvement (64%), despite the finding that this group had the highest diagnostic yields from additional sequence-based testing. This is likely a consequence of later timing of diagnosis of hydrops and subsequent pregnancy loss (spontaneous or induced) that may have resulted in these cases undergoing genetic testing at autopsy; local protocol for fetal genetic testing in the setting of demise with hydrops includes CMA as a standard test, but additional sequence-based testing requires consultation with the medical genetics service. Importantly, access to this service may facilitate postnatal phenotyping genetic reanalysis as variants are further defined, which can provide additional information about etiologies and recurrence risk. A key recommendation by the Canadian College of Medical Geneticists [27] in the care of pregnant patients in Canada included comprehensive access to consultation with the medical genetics service, which was identified as an area of improvement in our population.

The current study employed data from a population-based data source to assess the diagnostic yield of sequence-based testing following a non-diagnostic CMA result. It demonstrated higher diagnostic yields than those reported in the literature in some groups of fetal edema (hydrops, and other types of fetal edema) based on clinical presentation; this may be attributed to the fact that testing was representative of a clinical practice setting with testing requested based on clinical phenotype within the limits of laboratory testing criteria during the study period. The medical genetics team that provides service to Nova Scotia and the other Maritime provinces is based at IWK Health. Limitations included a small amount of missing data from health record review, particularly for those cases not from Nova Scotia. In addition, the differences in mean maternal age and gestational age at genetic testing likely represented the challenges in universal access to dating ultrasounds, NT assessment, and the timing of the clinical presentation of fetal hydrops and other cases of fetal edema.

5 | Conclusion

In the context of fetal edema, the presence of additional clinical features can inform appropriate genetic testing strategies to optimize diagnostic results. This study showed that sequencing of a RASopathy gene panel and whole exomes has a high diagnostic yield in cases with non-specific findings of increased NT, CH, or hydrops following a non-diagnostic CMA result. This testing is therefore warranted for such cases, and targeted RASopathy gene panel testing should be offered in parallel with CMA if exome analysis cannot be offered due to limited health care resources, or until there is a clear indication for performing exome analyses. Correlation of new and developing prenatal and postnatal findings, along with multidisciplinary team review, may inform additional testing, counseling and pregnancy management options, and is particularly important to consider in cases presenting with non-immune fetal hydrops.

Acknowledgments

The authors acknowledge the IWK Health Clinical Genomics Laboratory Information System for providing access to the data.

Funding

This work was supported by Genome Canada.

Ethics Statement

IWK Health Research Ethics Board approval was obtained for this study on May 21, 2019 (File #1024606) with annual renewal approval. IWK Health REB approved the waiver of consent due to impracticability.

Consent

Patient consent for this study was not obtained due to impracticability. Waiver of Consent was approved by the IWK Health Research Ethics Board.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the IWK Health Center. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the author(s) with the permission of IWK Health Center.

References

1. S. Tano, T. Kotani, M. Yoshihara, et al., "A Case of Non-immune Hydrops Fetalis With Maternal Mirror Syndrome Diagnosed By Trio-Based Exome Sequencing: An Autopsy Case Report and Literature Review," *Molecular Genetics and Metabolism Reports* 33 (2022): 100925, https://doi.org/10.1016/j.ymgmr.2022.100925.

2. Y. Lajmi, L. Loeuillet, G. Petrilli, et al., "Two Novel Variations p.(Ser1275Thr) and p.(Ser1275Arg) in FLT4 Causing Prenatal Hereditary Lymphedema Type 1," *Birth Defects Research* 115, no. 5 (2023): 563–571, https://doi.org/10.1002/bdr2.2141.

3. M. E. Norton, J. Van Ziffle, B. R. Lianoglou, U. Hodoglgil, W. P. Devine, and T. Sparks, "Exome Sequencing vs Targeted Gene Panels for the Evaluation of Nonimmune Hydrops Fetalis," *American Journal of Obstetrics and Gynecology* 226, no. 1 (2022): 128.e1–128.e11, https://doi.org/10.1016/j.ajog.2021.07.014.

4. T. N. Sparks, B. R. Lianoglou, R. R. Adami, et al., "Exome Sequencing for Prenatal Diagnosis in Nonimmune Hydrops Fetalis," *New England Journal of Medicine* 383, no. 18 (2020): 1746–1756, https://doi.org/10.1056/nejmoa2023643.

5. G. Mastromoro, D. Guadagnolo, H. N. Khaleghi, et al., "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* 13, no. 1 (2022): 48, https://doi.org/10.3390/diagnostics13010048.

6. M. Favier, J. Delanne, G. Gorincour, et al., "Early Prenatal Diagnosis of Causative Homozygous Variants in ASCC1 in a Fetus With Cystic Hygroma and Additional Homozygous Variants of Unknown Significance Associated With a Neurological Phenotype Not Visible in Early Gestation: Dual Diagnosis or Not?," *Prenatal Diagnosis* 44, no. 3 (2024): 352–356, https://doi.org/10.1002/pd.6519.

7. Q. X. Yu, L. Zhen, X. M. Lin, Y. J. Wen, and D. Z. Li, "Prenatal Diagnosis of Autosomal Recessive Noonan Syndrome Associated With Biallelic LZTR1 Variants Presented With Thick Nuchal Translucency and Cardiac Abnormalities," *Prenatal Diagnosis* 43, no. 13 (2023): 1662–1665, https://doi.org/10.1002/pd.6462.

8. W. J. Wu, G. C. Ma, T. Y. Chang, M. H. Lee, W. H. Lin, and M. Chen, "Outcome and Etiology of Fetal Pleural Effusion, Fetal Ascites and Hydrops Fetalis After Fetal Intervention: Retrospective Observational Cohort From a Single Institution," *Ultrasound in Obstetrics and Gynecology* 63, no. 4 (2024): 536–543, https://doi.org/10.1002/uog.27501.

9. D. Rogerson, A. Alkelai, J. Giordano, et al., "Investigation Into the Genetics of Fetal Congenital Lymphatic Anomalies," *Prenatal Diagnosis* 43, no. 6 (2023): 703–716, https://doi.org/10.1002/pd.6345.

10. N. B. Gulrajani, B. R. Lianoglou, K. Tick, et al., "Genetic Diseases Underlying a Spectrum of Fetal Effusions," *American Journal of Obstetrics and Gynecology* 233, no. 4 (2025): 333.e1–333.e11, https://doi.org/10.1016/j.ajog.2025.04.072.

11. R. Mellis, K. Oprych, E. Scotchman, M. Hill, and L. S. Chitty, "Diagnostic Yield of Exome Sequencing for Prenatal Diagnosis of Fetal Structural Anomalies: A Systematic Review and Meta-Analysis," *Prenatal Diagnosis* 42, no. 6 (2022): 662–685, https://doi.org/10.1002/pd.6115.

12. S. Richards, N. Aziz, S. Bale, 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* 17, no. 5 (2015): 405–424, https://doi.org/10.1038/gim.2015.30.

13. E. A. Croonen, W. M. Nillesen, K. E. Stuurman, et al., "Prenatal Diagnostic Testing of the Noonan Syndrome Genes in Fetuses With Abnormal Ultrasound Findings," *European Journal of Human Genetics* 21, no. 9 (2013): 936–942, https://doi.org/10.1038/ejhg.2012.285.

14. K. E. Stuurman, M. Joosten, I. van der Burgt, et al., "Prenatal Ultrasound Findings of RASopathies in a Cohort of 424 Fetuses: Update on Genetic Testing in the NGS Era," *Journal of Medical Genetics* 56, no. 10 (2019): 654–661, https://doi.org/10.1136/jmedgenet-2018-105746.

15. M. M. Makhamreh, K. Shivashankar, S. Araji, et al., "RASopathies Are the Most Common Set of Monogenic Syndromes Identified by Exome Sequencing for Nonimmune Hydrops Fetalis: A Systematic Review and Meta-Analysis," *American Journal of Medical Genetics* 194A, no. 5 (2024): e63494, https://doi.org/10.1002/ajmg.a.63494.

16. A. Scott, N. Di Giosaffatte, V. Pinna, et al., "When to Test Fetuses for RASopathies : Proposition From a Systematic Analysis of 352 Multicenter Cases and a Postnatal Cohort," *General Medicine* 23, no. 6 (2021): 1116–1124, https://doi.org/10.1038/s41436-020-01093-7.

17. A. Arduç, J. I. P. De Vries, M. B. Tan-Sindhunata, Q. Waisfisz, E. Pajkrt, and I. H. Linskens, "Perinatal Genetic Diagnostic Yield in a Population of Fetuses With the Phenotype Arthrogryposis Multiplex Congenita: A Cohort Study 2007–2021," *European Journal of Human Genetics* (2025), https://doi.org/10.1038/s41431-025-01848-3.

18. B. Matarrelli, A. Khalil, M. Bernassola, et al., "Outcome of Fetuses With Early Increased Nuchal Translucency: Systematic Review and Meta-Analysis," *Ultrasound in Obstetrics and Gynecology* 66, no. 2 (2025): 147–154, https://doi.org/10.1002/uog.29260.

19. R. Mellis, R. Y. Eberhardt, S. J. Hamilton, et al., "Fetal Exome Sequencing for Isolated Increased Nuchal Translucency: Should We Be Doing it?," *BJOG* 129, no. 1 (2022): 52–60, https://doi.org/10.1111/1471-0528.16869.

20. C. Cao, F. Liu, Y. Yang, Q. Zhang, J. Huang, and X. Liu, "Prenatal Whole-Exome Sequencing in Fetuses With Increased Nuchal Translucency," *Molecular Genetics & Genomic Medicine* 1, no. 11 (2023): e2246, https://doi.org/10.1002/mgg3.2246.

21. J. Zheng, T. Wang, H. Sun, et al., "Genetic Correlation Between Fetal Nuchal Translucency Thickening and Cystic Hygroma and Exploration of Pregnancy Outcome," *Scientific Reports* 14, no. 1 (2024): 27191, https://doi.org/10.1038/s41598-024-76628-y.

22. F. Mone, R. Y. Eberhardt, M. E. Hurles, et al., "Fetal Hydrops and the Incremental Yield of next Generation Sequencing Over Standard Prenatal Diagnostic Testing (FIND) Study: Prospective Cohort Study and Meta-Analysis," *Ultrasound in Obstetrics and Gynecology* 58, no. 4 (2021): 509–518, https://doi.org/10.1002/uog.23652.

23. H. B. Al-Kouatly, M. M. Makhamreh, S. M. Rice, et al., "High Diagnosis Rate for Nonimmune Hydrops Fetalis With Prenatal Clinical Exome From the Hydrops-Yielding Diagnostic Results of Prenatal Sequencing (HYDROPS) Study," *Genetics in Medicine* 23, no. 7 (2021): 1325–1333, https://doi.org/10.1038/s41436-021-01121-0.

24. B. Schuler, M. Mosera, L. D. Hatch, A. Grochowsky, and F. Wheeler, "Collaborative Efforts to Improve Genetic Testing in the Neonatal Intensive Care Unit," *Journal of Perinatology* 43, no. 12 (2023): 1500–1505, https://doi.org/10.1038/s41372-023-01817-y.

25. E. Wall, E. Petley, F. Mone, et al., "Molecular Autopsy for Fetal Structural Anomaly: Diagnostic and Clinical Utility of Multidisciplinary Team Approach," *Ultrasound in Obstetrics and Gynecology* 64, no. 3 (2024): 381–387, https://doi.org/10.1002/uog.27647.

26. H. McInnes-Dean, R. Mellis, M. Daniel, et al., "'Something That Helped the Whole Picture': Experiences of Parents Offered Rapid Prenatal Exome Sequencing in Routine Clinical Care in the English National Health Service," *Prenatal Diagnosis* 44, no. 4 (2024): 465–479, https://doi.org/10.1002/pd.6537.

27. J. Lazier, T. Hartley, J. K. Brock, et al., "Clinical Application of Fetal Genome-Wide Sequencing During Pregnancy; Position Statement for the Canadian College of Medical Geneticists," *Journal of Medical Genetics* 59, no. 10 (2022): 931–937, https://doi.org/10.1136/jmedgenet-2021-107897.

Supporting Information

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

Supporting Information S1: pd70116-sup-0001-suppl-data.docx.