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Transvaginal neurosonography-based phenotypic stratification guides whole exome sequencing in fetal ventriculomegaly: diagnosis, evaluation, and management

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Transvaginal neurosonography-based phenotypic stratification guides whole exome sequencing in fetal ventriculomegaly: diagnosis, evaluation, and management

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ABSTRACT

Objective

To assess whether transvaginal neurosonography (TVNS) improves detection of cerebral abnormalities in fetuses with ventriculomegaly (VM) and whether TVNS phenotypes are associated with whole-exome sequencing (WES) diagnostic yield.

Methods

This retrospective study included 128 fetuses with VM undergoing invasive prenatal diagnosis between January 2022 and May 2025. Fetuses with abnormal karyotypes, chromosomal microarray analysis (CMA) findings, or TORCH infection were excluded. All underwent transabdominal sonography (TAS) followed by TVNS and were classified as isolated VM or VM with additional abnormalities. WES was performed in all fetuses, and pathogenic/likely pathogenic (P/LP) variant rates were compared between groups.

Results

TVNS identified additional abnormalities in 48/128 fetuses (37.5%), including 22 missed by TAS. Before 22 weeks, TVNS detected abnormalities more frequently than TAS (44.9% vs. 20.5%, P<0.001), with the earliest at 16+2 weeks. WES identified P/LP variants in 20 fetuses (15.6%), including 12 (9.4%) VM-related variants. P/LP yield was higher with additional TVNS abnormalities than isolated VM (33.3% vs. 5.0%, P<0.001). TVNS showed higher sensitivity for P/LP-associated phenotypes than TAS (80.0% vs. 45.0%).

Conclusions

TVNS improves early detection of cerebral abnormalities in fetal VM. TVNS phenotypes are associated with WES diagnostic yield and may refine prenatal genetic risk stratification after negative chromosomal testing.

Keywords

Fetal ventriculomegaly; transvaginal neurosonography; whole-exome sequencing; prenatal diagnosis; phenotypic stratification; genetic counseling

1 Introduction

Fetal ventriculomegaly (VM) is one of the most common fetal cerebral abnormalities detected by prenatal ultrasound, with a reported prevalence of 0.03%–2.2% [1].Lateral ventricular width is an important measure of fetal brain development and is used to guide further genetic assessment and perinatal management [2].The normal lateral ventricular width between 16 and 38 weeks of gestation has been reported to be approximately 7.8 ± 0.8 mm [3,4].Severe VM is associated with an increased risk of postnatal neurodevelopmental impairment [5].Mild-to-moderate VM, however, has a heterogeneous clinical course, with outcomes influenced by additional cerebral abnormalities, genetic abnormalities, intrauterine infection, and other structural anomalies [6]. Identifying fetuses with VM who may have an increased risk of adverse outcomes is therefore an important part of prenatal diagnosis.

Transabdominal sonography (TAS) is routinely used to screen for fetal cerebral abnormalities. Detailed visualization of intracranial structures, including the midline structures, corpus callosum, cerebellar vermis, and periventricular regions, may be affected by fetal position, maternal abdominal characteristics, and ultrasound penetration [7].These factors can be particularly relevant in early gestation, when subtle cerebral abnormalities may be difficult to characterize. Fetal magnetic resonance imaging (MRI) provides complementary information for fetal brain assessment but is generally performed later in pregnancy, limiting its role in early characterization of fetal cerebral phenotypes.

Transvaginal neurosonography (TVNS), using a high-frequency transvaginal probe positioned closer to the fetal head, enables detailed multiplanar assessment of fetal brain structures. Previous studies have shown that TVNS can identify subtle central nervous system abnormalities that may not be apparent on routine TAS, particularly in early gestation [8,9]. Whether the detailed phenotypes identified by TVNS are associated with genetic findings and can help identify fetuses who may benefit from further genomic testing remains to be established.

Chromosomal microarray analysis (CMA) is recommended for the genetic evaluation of fetuses with VM [10].With the increasing clinical use of next-generation sequencing, whole-exome sequencing (WES) has expanded the detection of monogenic disorders in fetuses with normal chromosomal findings. The diagnostic value of WES is closely related to the fetal phenotype, while the cost of testing and interpretation of variants of uncertain significance (VUS) remain relevant considerations in clinical practice. Detailed prenatal phenotyping may therefore help identify fetuses with a higher likelihood of clinically relevant genetic variants and inform the selection of candidates for WES.

In this study, we retrospectively analyzed 128 fetuses with VM who had negative karyotype and CMA results and no evidence of TORCH infection. We aimed to (1) compare the detection of cerebral abnormalities by TVNS and TAS in fetuses with VM and (2) determine whether TVNS-based phenotypic classification was associated with differences in WES diagnostic yield.

2 Materials and Methods

2.1 Study design and population

This retrospective cohort study was conducted at the Prenatal Diagnosis Center of the General Hospital of Northern Theater Command, Chinese PLA. Fetuses diagnosed with VM between January 2022 and May 2025 were consecutively assessed. Inclusion criteria were: (1) lateral ventricular width ≥10 mm on initial TAS; (2) TVNS performed during the same visit or within 1 week; (3) normal fetal karyotype and CMA results obtained by amniocentesis; (4) negative TORCH screening; and (5) complete clinical, imaging, genetic, and follow-up data. Exclusion criteria were multiple pregnancy, abnormal karyotype or CMA, confirmed intrauterine infection, and incomplete TVNS or inadequate image quality.

A total of 128 fetuses met the criteria. After genetic counseling, written informed consent was obtained for amniocentesis and genetic testing. Maternal clinical characteristics, ultrasound and MRI findings, genetic results, pregnancy outcomes, and postnatal developmental data were collected. The study workflow is shown in Figure 1.

2.2 Ultrasound assessment

TAS was performed using a GE Voluson E10 system with a 3.5–5.0 MHz transducer according to International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) guidance. Standard transventricular, transthalamic, and transcerebellar planes were obtained.Ventricular width, cavum septum pellucidum (CSP), corpus callosum, cerebellum, posterior fossa, and other intracranial and extracranial structures were assessed. The maximal atrial width was measured according to established criteria.

TVNS was performed during the same visit or within 1 week by two fetal medicine specialists, each with >5 years of fetal neurosonography experience, using the same ultrasound system with a 5.0–9.0 MHz transvaginal probe. After bladder emptying, the patient was placed in the lithotomy position and the transducer was gently positioned in the posterior vaginal fornix. Systematic axial, coronal, and sagittal examinations were performed according to ISUOG recommendations, with particular attention to the ventricular system, corpus callosum, CSP, cerebellar vermis, cortical development, midline structures, and posterior fossa [11].

2.3 Image interpretation and reference standard

TAS and TVNS images were independently reviewed by two senior fetal medicine specialists blinded to genetic results. Disagreements were resolved by consensus. All included fetuses underwent fetal MRI, which served as the reference standard for assessment of fetal cerebral structural abnormalities. Diagnostic findings obtained by TAS and TVNS were compared with MRI findings.

2.4 Definition of TVNS-based phenotypes

VM was defined as a lateral ventricular width ≥10 mm between 15 and 40 weeks of gestation [13].Based on the final TVNS assessment, fetuses were classified as having isolated VM or VM with additional ultrasound abnormalities. Isolated VM indicated ventricular enlargement without additional intracranial or extracranial structural abnormalities detected by TVNS. The additional-abnormality group included fetuses with VM plus structural findings such as corpus callosum abnormalities, abnormal CSP, cerebellar vermian hypoplasia, delayed cortical maturation, subependymal cysts, or other cerebral abnormalities. VM severity was classified as mild (10–<12 mm), moderate (12–<15 mm), or severe (≥15 mm).

2.5 Whole-exome sequencing and variant interpretation

Amniotic fluid and peripheral blood samples from both parents were collected for WES and familial verification. Genomic DNA was extracted, fragmented, amplified, and subjected to hybridization capture. WES was performed on the Illumina HiSeq 2000 platform using paired-end sequencing (2 × 150 bp), with an average depth of approximately 100×. Sequencing data underwent quality control, alignment, variant calling, annotation, and interpretation. Variants were annotated using the Human Gene Mutation Database and dbSNP and interpreted according to the 2015 ACMG/AMP guidelines [14]. P/LP variants were confirmed by Sanger sequencing and evaluated for parental segregation.

2.6 Postnatal neurodevelopmental follow-up

For live-born children whose pregnancies continued, longitudinal neurodevelopmental assessment was performed using the Chinese version of the Griffiths Mental Development Scales (GMDS-C) [15].Developmental quotients (DQs) were calculated for six domains. Normal development was defined as DQ ≥85, borderline development as DQ 70–84, and developmental delay as DQ <70. Assessments were performed at 6, 12, and 24 months, followed by annual follow-up until school age.

2.7 Outcome measures

Primary outcomes were the detection of additional fetal cerebral abnormalities by TAS and TVNS and the association between TVNS-defined phenotype and WES P/LP yield. Secondary outcomes included diagnostic performance according to gestational age (<22 vs. ≥22 weeks), earliest detection by TVNS, diagnostic time gained relative to TAS, genetic yield among abnormalities detected by TVNS but missed by TAS, and postnatal neurodevelopmental outcomes.

2.8 Statistical analysis

Analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± standard deviation or median (interquartile range), as appropriate; categorical variables were expressed as numbers and percentages. Paired detection rates between TAS and TVNS were compared using the McNemar test. Pearson's chi-square or Fisher's exact test was used to compare WES yields between phenotype groups, with Bonferroni correction for pairwise comparisons. Using WES-confirmed P/LP variants as the reference standard, sensitivity, specificity, positive predictive value, and negative predictive value were calculated for TAS and TVNS. All tests were two-sided, and $P<0.05$ was considered statistically significant.

3 Results

3.1 TVNS detected additional cerebral abnormalities

Among the 128 fetuses with VM, additional ultrasound abnormalities were identified in 48 (37.5%) by TVNS and in 26 (20.3%) by TAS. Twenty-two fetuses (17.2%) had abnormalities detected only by TVNS, and no fetus was positive on TAS but negative on TVNS (Tables 1–3). The abnormalities included partial agenesis or hypoplasia of the corpus callosum, abnormal CSP, cerebellar vermian hypoplasia or underdevelopment, periventricular pseudocysts, irregular ventricular walls or subependymal heterotopia, delayed cortical maturation, and choroid plexus cysts (Table 1).

TVNS detected several abnormalities earlier than TAS. The earliest was an abnormal CSP, detected at 16+2 weeks by TVNS compared with 20+5 weeks by TAS. Earlier detection was also observed for abnormalities of the corpus callosum, ventricular walls, cerebellar vermis, and cortical development (Table 2). Before 22 weeks, additional abnormalities were identified in 35 of 78 fetuses (44.9%) by TVNS compared with 16 of 78 (20.5%) by TAS (P<0.001). At ≥22 weeks, the corresponding rates were 26.0% and 20.0%, respectively (P=0.302) (Table 4).

3.2 WES findings according to ultrasound phenotype and VM severity

WES identified P/LP variants in 20 of 128 fetuses (15.6%), including 12 (9.4%) considered related to the VM phenotype. The VM-related variants involved *L1CAM, AP1S2, FLNA, TMEM67, PDHA1, DCHS1, KAT5, PEX1, RELN, AKT3, CC2D2A*, and *GALC*. Eight additional P/LP variants, involving *OTC, SYNGAP1, ANGPT2, KIF21A, CHD2, SCN1A, DMD*, and *COL4A1*, were considered unrelated to the VM phenotype. VUS were identified in 14 fetuses (10.9%).

P/LP variants were more frequent in fetuses with additional ultrasound abnormalities than in those with isolated VM (16/48 [33.3%] vs 4/80 [5.0%], P<0.001). VM-related P/LP variants were identified in 9 of 48 fetuses (18.8%) and 3 of 80 (3.8%), respectively. VUS were identified in 8 of 48 fetuses (16.7%) with additional abnormalities and 6 of 80 (7.5%) with isolated VM (Table 5).

Overall genetic abnormalities, defined as P/LP variants or VUS, were identified in 24 of 48 fetuses (50.0%) with additional abnormalities and 10 of 80 (12.5%) with isolated VM (P=0.012). According to VM severity, P/LP variants were identified in 4 of 78 fetuses (5.1%) with mild VM, 6 of 34 (17.6%) with moderate VM, and 10 of 16 (62.5%) with severe VM (P<0.001). The corresponding rates of overall genetic abnormalities were 14.1%, 29.4%, and 81.3%, respectively. The P/LP detection rate was higher in severe than in mild or moderate VM, whereas the difference between mild and moderate VM was not statistically significant (P=0.057).Within the isolated-VM group, overall genetic abnormalities were identified in 6.9%, 22.2%, and 50.0% of fetuses with

mild, moderate, and severe VM, respectively. Among fetuses with additional ultrasound abnormalities, the corresponding rates were 35.0%, 37.5%, and 91.7%. No statistical comparison was performed within the severe isolated-VM subgroup because of the small number of cases (Table 5).Based on the association between TVNS-defined phenotypes and genetic diagnostic yield, we proposed a TVNS-based risk stratification framework to guide WES application and prenatal counseling (Figure 2).

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3.3 Diagnostic performance of TVNS and TAS for P/LP variants

For the detection of P/LP variants, TVNS had a sensitivity of 80.0%, specificity of 70.4%, positive predictive value of 33.3%, negative predictive value of 95.0%, and accuracy of 71.9%. The corresponding values for TAS were 45.0%, 84.3%, 34.6%, 89.2%, and 78.1%, respectively. For VM-related P/LP variants, the sensitivity and negative predictive value were 75.0% and 96.2% for TVNS, compared with 50.0% and 94.1% for TAS. For VUS, sensitivity was 57.1% for TVNS and 21.4% for TAS. Agreement between TVNS and TAS was modest for P/LP variants (κ=0.321) (Table 6).

3.4 P/LP variants among fetuses with abnormalities detected only by TVNS

Among the 22 fetuses with abnormalities detected by TVNS but not by TAS, 8 (36.4%) had P/LP variants. These included *AKT3* and *L1CAM* variants in fetuses with corpus callosal abnormalities; *DCHS1* and *PEX1* variants in those with abnormal CSP; *PDHA1* and *RELN* variants in those with periventricular cystic or ventricular wall abnormalities; a *KAT5* variant in a fetus with cerebellar vermian hypoplasia; and a *GALC* variant in a fetus with delayed cortical maturation.

3.5 Postnatal outcomes

Among the 12 fetuses with VM-related P/LP variants, 11 pregnancies were terminated and 1 continued to delivery. The child with a *DCHS1* variant had developmental quotients (DQs) ≥85 in all assessed domains at 8 months.Among the 8 fetuses with P/LP variants considered unrelated to the VM phenotype, 5 pregnancies were terminated and 3 continued. One infant with an *OTC* variant developed severe metabolic decompensation and died shortly after birth. A child with a de novo *SYNGAP1* variant had global developmental delay at 12 months, whereas a child with an *ANGPT2* variant had DQs ≥90 in all assessed domains at 24 months. All children with VUS had normal GMDS-C assessments at the last available follow-up (Table 7).

4 Discussion

1. TVNS improves fetal CNS phenotyping and facilitates genetic risk assessment in

fetuses with ventriculomegaly

Prenatal ultrasound remains the primary modality for screening and diagnosing fetal structural abnormalities, whereas fetal magnetic resonance imaging (MRI) is mainly used as a complementary examination for complex CNS abnormalities or inconclusive ultrasound findings. With advances in ultrasound resolution and fetal neurosonography, the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) has emphasized the distinction between routine and targeted neurosonographic assessment in fetuses at increased risk of CNS abnormalities [16,17]. Ventriculomegaly (VM) is a heterogeneous finding that may occur in isolation or in association with abnormalities of the corpus callosum, cavum septi pellucidi, periventricular structures, cortical development, and posterior fossa [18,19]. Assessment based solely on ventricular width may therefore not fully reflect the fetal neurological phenotype.In the present study, transvaginal neurosonography (TVNS) improved the detection of additional intracranial abnormalities in fetuses with VM, particularly before 22 weeks of gestation, and enabled diagnosis approximately 2.3 weeks earlier than transabdominal ultrasound (TAS). The clinical relevance of TVNS lies not only in identifying additional abnormalities but also in improving the accuracy of fetal CNS phenotyping. In our cohort, TVNS demonstrated a sensitivity of 80.0% and a negative predictive value (NPV) of 95.0% for detecting additional intracranial abnormalities. Furthermore, no fetus with positive TAS findings was missed by TVNS. These findings support the use of TVNS as a complementary examination following routine TAS rather than as a replacement for conventional ultrasound. TAS remains essential for its accessibility and applicability in prenatal screening, whereas TVNS may provide additional anatomical information when VM or other CNS abnormalities are suspected.

The value of detailed neurosonographic assessment is illustrated by the fetus carrying an L1CAM variant associated with X-linked corpus callosum agenesis. At 17+1 weeks, TVNS identified agenesis of the corpus callosum genu, whereas TAS demonstrated only mild ventricular enlargement (10.8 mm). This case illustrates that some monogenic neurological disorders may initially present with subtle structural abnormalities rather than prominent ventricular dilatation, and ventricular measurement alone may not fully capture the complexity of fetal CNS development.

The association between TVNS findings and molecular outcomes further supports refined fetal phenotyping. TVNS showed higher sensitivity for detecting P/LP variants than TAS (80.0% vs. 45.0%), with a higher NPV (95.0% vs. 89.2%). Similar differences were observed for VM-related P/LP variants. TVNS also identified 22 additional intracranial abnormalities not detected by TAS, eight of which (36.4%) harbored P/LP variants identified by WES. Fetuses with additional abnormalities detected by TVNS had a significantly higher P/LP detection rate than those with

isolated VM (33.3% vs. 5.0%, P<0.001). These findings indicate that detailed neurosonographic assessment can distinguish fetuses with isolated VM from those with additional CNS abnormalities and provide useful information for genetic risk assessment.

2. TVNS-guided WES improves the selection of VM fetuses for genomic testing

Fetal VM has a heterogeneous etiology, including genetic abnormalities, congenital infections, and structural brain lesions. Genetic causes range from chromosomal abnormalities and pathogenic CNVs to single-gene disorders [20–22]. Although karyotyping and CMA detect chromosomal abnormalities and clinically significant CNVs, their ability to identify single-gene disorders is limited [23,24]. WES therefore provides an important complementary approach, particularly after negative conventional cytogenetic testing [25,26].In our cohort, fetuses with additional intracranial abnormalities identified by TVNS had a substantially higher WES P/LP detection rate than those with isolated VM (33.3% vs. 5.0%). TVNS also showed an NPV of 95.0% for WES-detected P/LP variants. Some fetuses initially classified as having isolated VM by TAS were reclassified after TVNS identified additional CNS abnormalities, highlighting the importance of comprehensive neurosonographic phenotyping in genetic risk assessment.In contrast, the P/LP detection rate in TVNS-confirmed isolated VM was 5.0%, lower than that reported in previous studies [27]. Differences in phenotypic classification may partly account for this discrepancy, as TAS-based studies may have included fetuses with subtle abnormalities that were not initially detected. The overall WES P/LP detection rate was 15.6%, supporting the value of WES in fetuses with abnormal neurosonographic findings after negative conventional chromosomal testing.

3. Genetic risk stratification strategy for fetuses with ventriculomegaly

Genetic risk stratification is important for counseling and selection of prenatal genetic testing in fetuses with VM. Previous studies have reported a higher likelihood of genetic abnormalities when VM is accompanied by additional ultrasound abnormalities or soft markers [28,29]. In our cohort, fetuses with additional intracranial abnormalities detected by TVNS had a higher P/LP detection rate than those with isolated VM, supporting the value of detailed neurosonographic phenotyping in genetic risk assessment.Genetic abnormalities have been reported in both mild and moderate VM [30], while pathogenic CNV detection rates have not differed significantly between these groups [31,32]. Similarly, we found no significant difference in P/LP detection between mild and moderate VM. The detection rate was higher in moderate than mild VM (17.6% vs. 5.1%), indicating a higher genetic risk with increasing ventricular size.Ventricular size should be interpreted together with the overall neurosonographic phenotype. In our cohort, a ventricular width of ≥12 mm was used as a clinical risk indicator rather than a genetic cutoff. Additional CNS abnormalities identified by TVNS provided further information for genetic risk assessment, including in fetuses

with ventricular measurements below 12 mm. Integrating ventricular size and neurosonographic findings may therefore improve prenatal genetic counseling.

4. Incidental findings and VUS interpretation in WES

WES expands the ability to identify monogenic disorders but also introduces challenges related to incidental findings and VUS [33]. Genetic information obtained during pregnancy may influence parental decision-making despite incomplete knowledge of disease penetrance, severity, and prognosis.In this cohort, pathogenic variants in *L1CAM*, *DCX*, *RELN*, *ARX*, and *PDHA1* were identified, consistent with reported genetic causes of VM and neurodevelopmental disorders[34]. WES may also identify clinically significant variants unrelated to the original imaging indication. In our cohort, one male fetus carried an *OTC* c.583G>C missense variant associated with X-linked ornithine transcarbamylase deficiency [35]. Following genetic counseling, the parents continued the pregnancy, and the infant subsequently developed progressive metabolic abnormalities and died. This case highlights the importance of appropriate counseling and multidisciplinary interpretation of incidental findings.

The VUS detection rate was 10.9% in this study. Interpretation should incorporate fetal phenotype, parental segregation, functional evidence, and updated genomic databases. Long-term follow-up and periodic reassessment of uncertain variants may further improve the clinical utility of WES.

5. Strengths and limitations

This study combined detailed TVNS assessment with WES to characterize additional CNS abnormalities and their genetic findings in fetuses with VM, providing a basis for more individualized genetic risk assessment.

The study was conducted at a single center using a retrospective design, and the interpretation of TVNS may vary with operator experience. The relatively small subgroup sizes also limited the power of some comparisons. WES testing was based on clinical indications and parental decisions, which may have introduced selection bias. In addition, WES cannot capture all genetic mechanisms, and longer postnatal follow-up is needed to further characterize neurodevelopmental outcomes.

5 Conclusion

In conclusion, TVNS improves the early detection and phenotypic characterization of subtle brain abnormalities in fetuses with VM beyond routine TAS, with a particular clinical advantage before 22 weeks of gestation. Importantly, the refined neurosonographic phenotypes provided by TVNS may reclassify some fetuses initially considered to have isolated VM and improve genetic risk stratification. These findings support a sequential diagnostic approach integrating routine TAS, TVNS-based phenotypic refinement, genetic risk stratification, and WES. For fetuses with additional

brain abnormalities identified by TVNS or clinically significant ventricular dilatation, WES should be considered when karyotype and CMA results are negative to improve the detection of monogenic disorders. In fetuses with confirmed isolated mild VM, the relatively low yield of WES should be discussed, with testing decisions individualized according to clinical findings and parental preferences.

Data Availability Statement

Data are available from the first author or corresponding author upon reasonable request.

Conflict of Interest Statement

The authors declare no conflicts of interest related to this work.

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Ethics and Patient Consent

The study protocol was approved by the Ethics Committee of the General Hospital of Northern Theater Command (No. Y(2026)348). All procedures were performed in accordance with the principles of the Declaration of Helsinki.A waiver of informed consent has been granted forretrospective data collection from patient records, as all clinical data in this report have been de-identified.

Author Contributions

Ting'ai Liu and Jiaxi Jin contributed equally to this work and are co-first authors.Ting'ai Liu: Conceptualization, Methodology, Investigation, Formal analysis, Writing original draft.Jiaxi Jin: Methodology, Investigation, Data curation, Formal analysis, Writing original draft.Jingli Sun: Investigation, Resources, Validation, Writing review and editing.Zhenyu Chen: Conceptualization, Supervision, Funding acquisition, Project administration, Writing review and editing.Corresponding author.

All authors have read and agreed to the published version of the manuscript.

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Figure 1. Distribution of associated ultrasound abnormalities in fetuses with ventriculomegaly (n = 48)

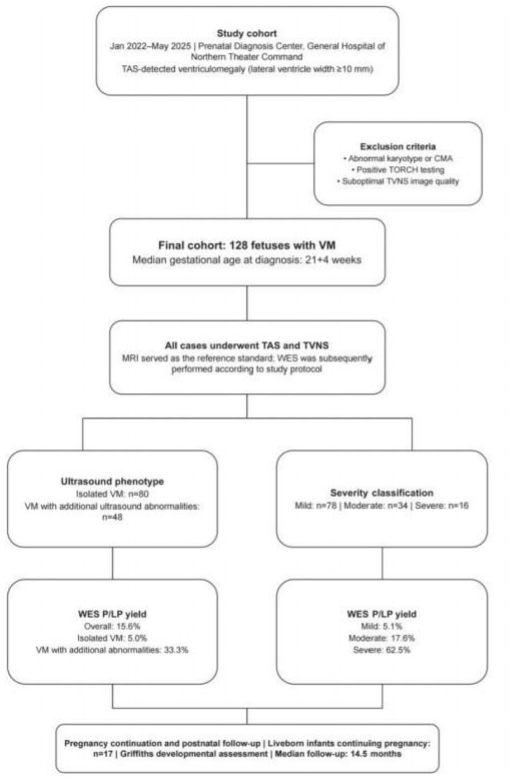
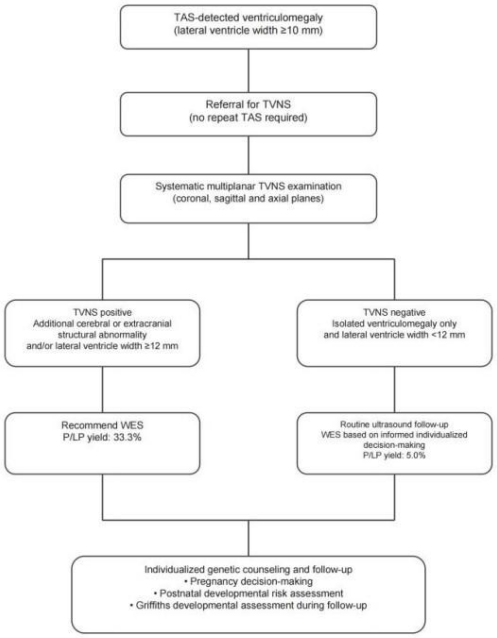


Figure 2. TVNS-based phenotypic stratification framework for guiding WES application and prenatal counseling in fetuses with ventriculomegaly.



Supplementary Files

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