Clinical Genetics

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CLINICAL GENETICS

ORIGINAL ARTICLE OPEN ACCESS

First-Tier Versus Last-Tier Trio Whole-Genome Sequencing for the Diagnosis of Pediatric-Onset Rare Diseases

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Received: 21 February 2025 | **Revised:** 11 April 2025 | **Accepted:** 16 April 2025

Funding: This work was funded by a Telethon Foundation grant awarded to the Medical Genetics Laboratory of ASST Papa Giovanni XXIII in Bergamo, Italy as part of the project "Responsible Implementation of Newborns Genome Sequencing (RINGS): a technical and interpretative feasibility study," grant number GSP21003. The work was partially supported by the "PG23/FROM 2017 Call for Independent Research" as part of the RARE - Rapid Analysis for Rapid carE - project. This work was also funded by the National Plan for NRRP Complementary Investments (PNC, established with the decree-law 6 May 2021, n. 59, converted by law n. 101 of 2021) in the call for the funding of research initiatives for technologies and innovative trajectories in the health and care sectors (Directorial Decree n. 931 of 06-06-2022) - project n. PNC0000003 - AdvaNced Technologies for Human-centrEd Medicine (project acronym: ANTHEM), Pilot 1.4 Timely genomic diagnosis for children with rare diseases toward precision medicines. This work was also partially supported by Illumina, Inc. through a collaboration agreement.

Keywords: critical illness | delayed diagnosis | neonatal intensive care units | newborn infant diseases | outpatients | pediatric intensive care units | public health practice | rare diseases | whole genome sequencing

ABSTRACT

Despite advances in diagnostics, children with rare genetic disorders still face extended diagnostic odysseys, delaying appropriate clinical management, and placing burdens on families and healthcare resources. Whole-genome sequencing (WGS) offers a more comprehensive interrogation of the genome than other genetic tests, but its use in clinical practice remains limited. This study compared diagnostic rates, turnaround times, and clinical utility of first-tier versus last-tier trio-WGS for patients with suspected genetic pediatric-onset conditions, including 97 critical and 104 non-critical patients. Eighty-five patients (42.3%), including 57 (58.8%) critical and 28 (26.9%) non-critical patients, received a molecular diagnosis. The diagnostic rate was higher for first-tier (57%) than for last-tier (32.8%) trio-WGS. Of 121 causative variants identified, 19.8% would have been missed by whole-exome

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sequencing. Laboratory processing time was 4 days for all patients. The clinical setting had the greatest impact on time to reporting, averaging 5 days for critical patients versus 74 days for outpatients. WGS results impacted clinical decision-making for 34% of all critical and 14.3% of WGS-positive non-critical patients. This is the first Italian clinical study to demonstrate the diagnostic and clinical utility of a genome-first approach for both critical and non-critical patients with suspected genetic pediatric-onset disorders and feasibility in a public healthcare system.

1 | Introduction

Infants and children with suspected genetic disorders frequently face a succession of referrals, clinical evaluations, and diagnostic procedures, often lasting years. These aptly named diagnostic odysseys occur regardless of clinical context—involving both inpatients and outpatients—and have a major impact on patient care and outcomes, as well as substantial financial and emotional consequences for families and far-reaching effects on healthcare resource utilization [1–3]. Early molecular diagnosis of suspected genetic disorders can mitigate these effects by avoiding unnecessary invasive diagnostic procedures for patients, ending the diagnostic odyssey for families, and impacting clinical decision making, resulting in an overall improvement in the efficiency of care [1, 3–6].

Widely accessible genetic methodologies such as chromosomal microarrays, single-gene tests, multi-gene panels, and repeat expansion analyses are commonly used to investigate suspected genetic etiologies for patients. Each method has demonstrated analytical and clinical utility for identifying specific genetic anomalies, but the types of variations detectable by each technique are limited. Whole-exome sequencing (WES) can identify a broader range of genetic abnormalities than variant-centric approaches, but it is restricted to investigating the genome's protein-coding regions and, therefore, does not capture certain types of alterations such as structural rearrangements, noncoding variants, and many repeat expansions [5–8].

In a consolidated laboratory workflow, whole-genome sequencing (WGS) has the capacity to interrogate nearly all regions of the genome, including coding and noncoding regions, nuclear and mitochondrial DNA, and areas difficult to sequence using other genetic tests. WGS also allows simultaneous detection of single-nucleotide variants (SNVs), small insertions and deletions (indels), copy-number variants (CNVs) and structural variants (SVs) repeat expansions, and runs of homozygosity [6–12]. In addition, WGS is as etiologically agnostic as WES, allowing analysis of genomes without the hypothesis-driven limitations presented by target gene or variant-based approaches. Furthermore, using parent–child trios and reverse phenotyping reduces the rate of inconclusive results [13–15]. Additional advantages of WGS include fast laboratory processing times and evolving platforms for secondary and tertiary analysis that offer efficient and accurate variant calling, annotation, and interpretation. Prior studies have documented high rates of concordance between WGS and other technologies as well as the ability of WGS to improve diagnostic rates (DRs) through detection of variants missed by other platforms [1, 6, 8, 9, 11, 12, 16–26].

Time to diagnosis is a special priority in critical care settings where a molecular diagnosis may inform clinical management and early implementation of life-saving interventions and therapies. Prior studies have demonstrated increased diagnostic yield and clinical utility of WGS with a much shorter time to diagnosis than standard testing approaches [1, 4, 6, 17, 26, 27].

An important concern impacting the adoption of WGS in clinical practice has been cost. Recent studies exploring the cost-effectiveness of WGS in pediatric populations have found first-tier use of WGS shortened diagnostic odysseys and reduced costs, especially in critical care settings [28–33]. Taken together, these data support the use of WGS as a first-tier genetic test for a range of clinical indications.

Presently, in several European nations including Italy, WES or WGS, when used, are typically performed at the end of a multistep diagnostic approach to children with suspected genetic conditions. In Italy, the healthcare system is publicly funded with regional administration, resulting in heterogeneity in genetic test usage and access across regions. While genome-wide analyses are starting to be used for critically ill children, they are still rarely utilized in non-critical settings, except for patients at the end of a diagnostic odyssey; but, even then, predominantly in the context of research [34].

This one-year cohort study is the first clinical study in Italy to evaluate the performance and utility of WGS for rare disease diagnostics. Trio-WGS was performed for 97 critically ill neonatal/pediatric intensive care unit (NICU/PICU) patients and 104 non-critical outpatients with suspected pediatric-onset genetic disorders. We evaluated the variants identified by WGS and compared DRs, time to diagnosis, clinical utility, and feasibility of first- and last-tier WGS in critical and non-critical care settings within our publicly funded healthcare system.

2 | Materials and Methods

2.1 | Cohort

From January to December 2023, critical patients were referred to the medical genetics laboratory from the NICU/PICUs of six public hospitals across Lombardy, while outpatients were referred from the clinical genetics unit of ASST Papa Giovanni XXIII Hospital of Bergamo, Italy. Eligible patients were suspected of having a genetic condition with pediatric-onset, regardless of age at recruitment, whose biological parents were available for trio-WGS. Patients were excluded if they had a previous diagnosis that entirely explained their phenotype (e.g., metabolic disease identified at expanded newborn screening performed in Italy), a phenotype most likely related to an acquired etiology, or a suspected chromosomal aneuploidy (for instance trisomy 21). This study was approved by the Institutional

Review Board and Ethical Committee of ASST Papa Giovanni XXIII Hospital of Bergamo. Informed consent was obtained from both parents of enrolled children and from adult patients with legal capacity.

Patients were classified as critical (i.e., patients admitted to the NICU/PICU with acute presentations due to organ failure, systemic metabolic disease, or severe syndromic or neurological phenotypes), or non-critical (i.e., outpatients). Patients were also grouped by genetic testing history. Undiagnosed patients with prior genetic analyses capable of identifying variants detectable by WGS, for example, karyotype, array-comparative genomic hybridization, single- or multi-gene sequencing, or WES, were classified as last-tier WGS. All other patients were classified as first-tier WGS.

2.2 | Genome Sequencing, Variant Detection, and Annotation

All WGS analyses were performed at the ASST Papa Giovanni XXIII Medical Genetics Laboratory in Bergamo, Italy. Genomic DNA was extracted from peripheral blood following standard procedures. Samples from critical patients were processed as soon as they arrived at the laboratory; samples from non-critical patients were processed in order of arrival and to fill the flow cell of critical samples.

Libraries of seven trios were prepared simultaneously using the Illumina DNA PCR-Free Prep kit (Illumina Inc., San Diego, CA, USA). Sequencing was performed using an S4 flow cell on an Illumina NovaSeq 6000 system, with 150 bp paired-end reads (Illumina, PE 2x150). Alignment to reference genome GRCh38/USCShg38 and variant calling were performed with Illumina DRAGEN version 3.9.5. Trios with an average coverage of $\geq 30\times$ were considered suitable for analysis. Variant annotation, filtering, and visualization were conducted using the Illumina TruSight Software Suite version 2.6.3.

2.3 | Variant Interpretation, Reporting, and Patient Counseling

A gene-agnostic approach was applied, with phenotype serving as the primary criterion for data interpretation rather than gene selection. Variants were filtered in the context of the patient's phenotype and family history, the frequency of variants in the general population [35], and compatibility with publicly available information about disease incidence. Variants were classified according to published guidelines [36, 37] and reported in accordance with nomenclature standards [38, 39].

Initial WGS results were discussed with the patient's clinical team for reverse phenotyping. Variants of unknown significance (VUS) were only considered if they had convincing evidence to explain, and occurred in genes closely related to, the patient's phenotype, in accordance with recommendations endorsed by the European Society of Human Genetics (ESHG) [40]. When available, clinical and biochemical tests were performed to confirm the presumed molecular diagnosis. After reverse phenotyping, variants initially classified as VUS were reevaluated and reinterpreted as conclusive or excluded from the final report. Herein, pathogenic, likely pathogenic, and reevaluated VUS that led to a conclusive result are considered causative variants.

After reverse phenotyping, a final report was written. Geneticists and clinical teams delivered the final report and provided post-test counseling to patients and families.

2.4 | Data Collection and Outcomes

Data collected included patient sex, age at time of analysis, family and genetic testing history, and detailed clinical description. Comprehensive deep phenotyping was provided by clinical geneticists. Dates of arrival of samples in the laboratory and start of sample processing, trio-WGS results, and dates of first communication of results to the clinical team and final report were also recorded. Primary outcomes included DRs and laboratory turnaround times (TAT).

Conclusive tests identified one or more diagnoses; tests that explained part of the patient's phenotype were considered a partial diagnosis. DR was calculated as the number of trio-WGS analyses that resulted in causative variants divided by the total number of analyses performed. DRs were compared between critical and non-critical patients and first- versus last-tier WGS.

Average workflow times for WGS were calculated, including elapsed time from sample arrival in the laboratory to the start of sample processing, time of initial communication of WGS results to the referring clinician, elapsed time for reverse phenotyping, and time from sample acceptance in the laboratory to final reporting. TAT and time to final report were defined as the period from sample acceptance in the laboratory to discussion of preliminary results with the referring clinician and to the emission date of the final report, respectively.

Clinical utility of WGS was inferred from patients' identified diagnoses and clinical reports and sought to identify patients for whom WGS, whether conclusive or inconclusive, led to targeted treatment (e.g., medical or surgical therapy, indication/non-contraindication for organ transplantation); patients for whom a diagnosis guided toward palliative care; and patients for whom conclusive analyses led to specific follow-up, such as cardiological follow-up in patients with RASopathies or connective tissue disorders or specific surveillance for conditions with a higher cancer risk (e.g., PTEN hamartoma tumor syndrome). The assessment of clinical utility did not consider the benefits of diagnosis in setting up individualized follow-up or managing reproductive risk for parents and other family members, nor its impact on the psychological burden of families.

2.5 | Statistical Analysis

Continuous variables were summarized as mean, median, and range, while categorical variables were presented as counts and percentages. Group differences were assessed using the non-parametric Mann–Whitney test for continuous variables and the Chi-square test (or Fisher's exact test, when appropriate) for categorical variables.

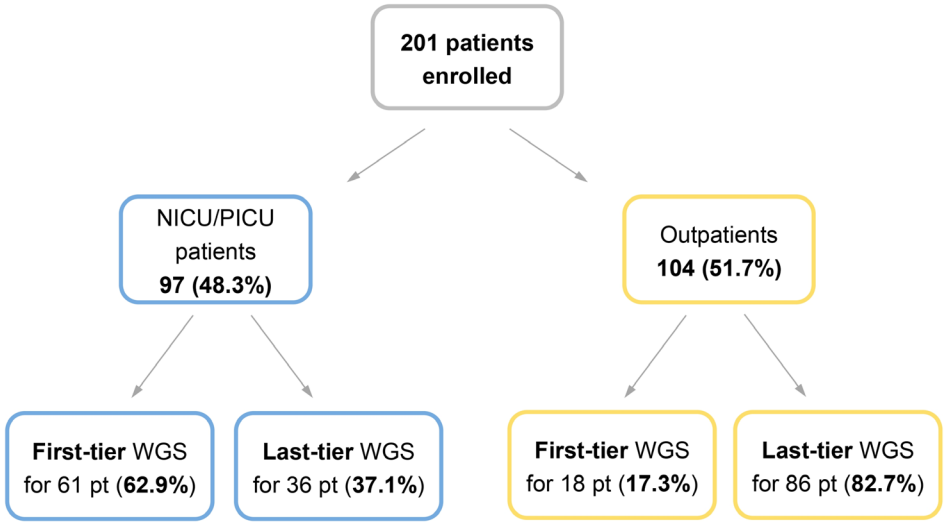

FIGURE 1 | Study flow chart. Abbreviations: NICU/PICU, neonatal intensive care unit/pediatric intensive care unit; pt., patients; WGS, whole-genome sequencing.

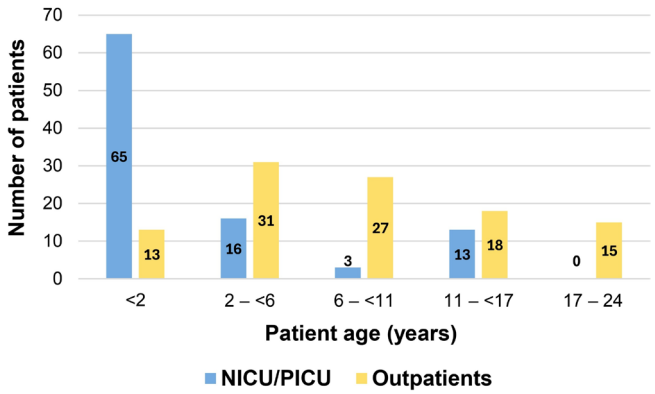

FIGURE 2 | Distribution of cohort by age groups and practice setting. Abbreviations: NICU/PICU, neonatal intensive care unit/pediatric intensive care unit.

3 | Results

3.1 | Cohort

Two hundred one unrelated patients were enrolled in this study, including 97 critically ill children admitted to a NICU or PICU and 104 non-critical individuals receiving outpatients. Seventy-nine (39.3%) patients received WGS as a first-tier test, with a much higher proportion of first-tier use of WGS in critical (62.9%) versus non-critical (17.3%) patients ($p < 0.001$) (Figure 1). The remaining 122 patients (60.7%) received WGS as a last-tier test, with 2/97 (2.1%) NICU/PICU patients and 61/104 (58.7%) outpatients having previously received trio-WES with negative results.

Overall, median age at the time of WGS was 4 years (range newborn to 24 years); male to female ratio was 1.39:1. Distribution of the cohort by age groups and practice setting is shown in Figure 2. While most NICU/PICU patients were younger than 2 years of age at the time of analysis (65/97, 67.0%), outpatients were more evenly divided across age groups, with 60/104 (57.7%) being 6 years of age or older and 15/104 (14.4%) receiving trio-WGS after 17 years of age and a diagnostic odyssey that for 11/15 (73.3%) included negative trio-WES. All included patients older than 17 years had a clinical onset of symptoms at birth or in childhood. Parental consanguinity was reported in 4.5% of patients. The primary clinical indications for referral for WGS are shown in Figure 3.

3.2 | Coverage, DRs, and Types of Variants Identified by WGS

By sequencing 21 samples per flow cell, an average sequencing coverage of 44× per sample was achieved (range 24×–70×). Six samples (2 probands, 4 parents) demonstrated mean coverage < 30× and were successfully re-sequenced.

A conclusive molecular diagnosis was obtained for 85/201 (42.3%) patients, including 57/97 (58.8%) critical patients and 28/104 (26.9%) non-critical patients ($p < 0.001$) (Table 1). The DR was higher for first-tier than last-tier WGS for both critical and non-critical patient subcohorts, with 63.9% versus 50.0% of critical patients and 33.3% versus 25.6% of non-critical patients receiving molecular diagnoses, although these differences were not statistically significant. Among the most frequently observed phenotypic categories (with more than 20 patients per category), the highest DR, 46.2%, was observed for patients with malformative syndromes, followed by a 39.7% DR for patients with a neurodevelopmental/neurological phenotype, and a 36.4% DR for those with a metabolic phenotype (Table 2).

Three patients (1.5%) received two different molecular diagnoses and six (3.0%) received partial diagnoses that did not fully explain their phenotypes (Table 1, Table S1). For seven patients (three critical and four non-critical), WGS analysis identified variants initially classified as VUS that, after reverse phenotyping and additional clinical investigations, were clinically interpreted as causative. Notably, 20% of patients with prior negative trio-WES conducted within the last 2 years received a molecular

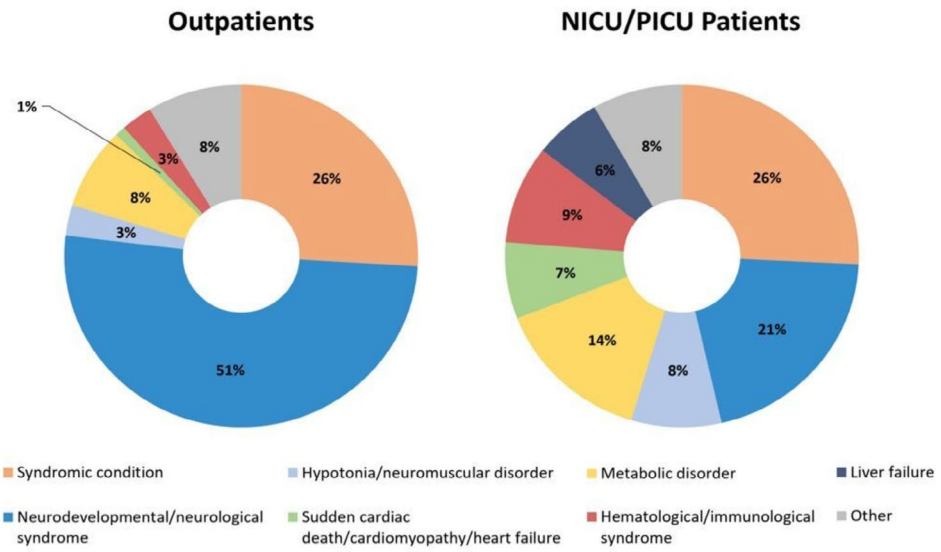

FIGURE 3 | Distribution of clinical indications for WGS among critical and non-critical subcohorts. For NICU/PICU patients, "other" includes cancer (3.1%), connective tissue disease, and genodermatosis (2% each), and skeletal dysplasia (1%). For outpatients, "other" includes connective tissue disease, cancer, and skeletal dysplasia (2% each), and growth disorder and genodermatosis (1% each). Abbreviations: NICU/PICU, neonatal intensive care unit/pediatric intensive care unit; WGS, whole-genome sequencing.

TABLE 1 | Detection rates for first- and last-tier WGS.

	NICU/PICU patients			Outpatients		
	First-tier WGS	**Last-tier WGS**	**Overall**	**First-tier WGS**	**Last-tier WGS**	**Overall**
Number of patients (%)	61 (62.9)	36 (37.1)	97	18 (17.3)	86 (82.7)	104
Diagnostic rates (%)						
Conclusive tests	**39 (63.9)**	**18 (50.0)**	**57 (58.8)**	**6 (33.3)**	**22 (25.6)**	**28 (26.9)**
Single diagnosis	36 (59.0)	16 (44.4)	52 (53.6)	6 (33.3)	18 (20.9)	24 (23.1)
Double diagnosis	2 (3.3)	0	2 (2.1)	0	1 (1.2)	1 (1.0)
Partial diagnosis	1 (1.6)	2 (5.6)	3 (3.1)	0	3 (3.5)	3 (2.9)
Inconclusive tests	22 (36.1)	18 (50.0)	40 (41.2)	12 (66.7)	64 (74.4)	76 (73.1)
After negative trio-WES (%)	N/A	2/4 (50.0)	2/4 (50.0)	N/A	11/61 (18.0)	11/61 (18.0)

Note: Numbers of conclusive test results (and %) are shown in bold.
Abbreviations: N/A, not applicable; NICU/PICU, neonatal intensive care unit/pediatric intensive care unit; WES, whole-exome sequencing; WGS, whole-genome sequencing.

diagnosis by WGS, including 2/4 (50%) critical and 11/61 (18%) non-critical patients (Table 1).

Of 121 causative DNA variants reported to clinicians, SNVs were the most common, accounting for 76.0%; small deletions/duplications and CNVs comprised another 20.7%. Based on variant type and genomic location, 24/121 (19.8%) causative variants reported, including 14 small deletion/duplication variants ranging from 50 bp to the size of a single gene, would likely have been missed by current WES methodologies (Table S2). De novo autosomal dominant inheritance and autosomal recessive inheritance were by far the most common modes of transmission observed (Table S3).

3.3 | Workflow and Timelines for WGS

The process flow from a patient's clinical indication to the final report is illustrated in Figure 4. Samples from critical patients were processed within 1 day of arrival in the laboratory and

TABLE 2 | Detection rates for difference clinical indications.

Clinical indication	NICU/PICU (%)	Outpatients (%)	Total (%)
Malformative syndrome	16/25 (64)	8/27 (29.6)	24/52 (46.2)
Neurological/neurodevelopmental disorder	15/20 (75)	14/53 (26.4)	29/73 (39.7)
Metabolic disorder	7/14 (50)	1/8 (12.5)	8/22 (36.4)
Hypotonia/neuromuscular disorder	7/8 (87.5)	1/3 (33.3)	8/11 (72.7)
Genodermatosis	2/2 (100)	0/1 (0)	2/3 (66.7)
Skeletal dysplasia	0/1 (0)	2/2 (100)	2/3 (66.7)
Sudden cardiac arrest/cardiomyopathy	5/7 (71.4)	0/1 (0)	5/8 (62.5)
Cancer	2/3 (66.7)	0/2 (0)	2/5 (40)
Connective tissue disease	0/2 (0)	1/2 (50)	1/4 (25)
Hematological/immunological syndrome	2/9 (22.2)	1/3 (33.3)	3/12 (25)
Liver disease	1/5 (20)	0/0 (0)	1/5 (20)
Other organ failure	0/1 (0)	0/0 (0)	0/1 (0)
Growth disorder	0/0 (0)	0/2 (0)	0/2 (0)
Total diagnoses	57/97 (58.8)	28/104 (26.9)	85/201 (42.3)

sequenced immediately. To contain costs, non-critical samples were used to fill the flow cells of critical samples and sequenced as flow cell capacity permitted, resulting in a longer and more variable elapsed time between sample arrival in the laboratory and the start of sample processing.

The processing time from DNA isolation to reporting of preliminary results to clinicians, including library preparation, sequencing, data analysis, and interpretation of results, was completed in 4 days for all samples (T_1–T_2 in Figure 4). The laboratory TAT, which was defined as the time from sample arrival in the laboratory to preliminary results (T_0–T_2 in Figure 4), averaged 5 days (median: 5, range: 5–10) for critical samples. The longer TATs among critical samples were caused by two samples that had to be re-sequenced due to suboptimal coverage or a delay in acquiring enough samples to fill the flow cell. In contrast, the laboratory TAT for non-critical samples averaged 74 days (median: 78, range: 5–128). The laboratory TAT was significantly longer ($p < 0.001$) for non-critical samples compared with critical samples and was primarily due to the prioritization of urgent samples for flow cell processing, which often increased T_0–T_1 times for non-critical samples.

Production of final reports required reversing phenotyping and was impacted by clinical phenotype and care setting. For some patients, reverse phenotyping involved only one clinical evaluation, while for others it required multiple targeted tests. Additionally, reverse phenotyping was often prolonged for outpatients due to the need to bring these patients back to the clinic. The time from sample arrival in the laboratory to final reporting averaged 11 working days (median: 10, range: 5–19) for critical patients and 109 working days (median: 117, range: 10–165) for non-critical outpatients. This time was significantly longer ($p < 0.001$) for non-critical samples compared with critical samples.

3.4 | Clinical Utility of WGS

Clinical utility of WGS was assessed through patient records. All conclusive cases benefited from information related to familial reproductive risk and individualized follow-up. Otherwise, WGS results had the most profound impact for critical patients, with 33/97 (34%) receiving condition-specific treatments, organ transplantation, or multidisciplinary care tailored to the molecular diagnosis (Table S4 shows illustrative cases). Notably, for 16 critical patients with cardiac, liver, or bone marrow failure, negative WGS resulted in a no-contraindication for transplantation. Additionally, for five critical patients (8.8% of critical patients with conclusive WGS results) diagnosis of a condition with an unfavorable prognosis avoided unnecessary treatment and guided transfer to palliative care. Among non-critical patients, clinical management was changed for four patients (14.3% of patients receiving a molecular diagnosis).

4 | Discussion

Prior studies have demonstrated the diagnostic efficacy of WGS across a range of phenotypes and the ability of WGS to improve DRs by identifying variants that lie beyond the capacity of other methods, including WES [1, 6, 8, 11, 16, 19–26]. In this study, trio-WGS yielded a molecular diagnosis for 42.3% of patients, similar to diagnostic ranges observed by others [4, 6, 8, 9, 14, 16, 19–27, 29, 41–43]. The highest subcohort DR was observed with first-tier use of trio-WGS for critically ill patients, many of whom presented with complex or confounding clinical phenotypes often associated with high genetic heterogeneity. The lowest subcohort DR was observed with last-tier WGS in non-critical patients and was likely impacted by the diagnostic odysseys most outpatients had experienced, leaving only variants

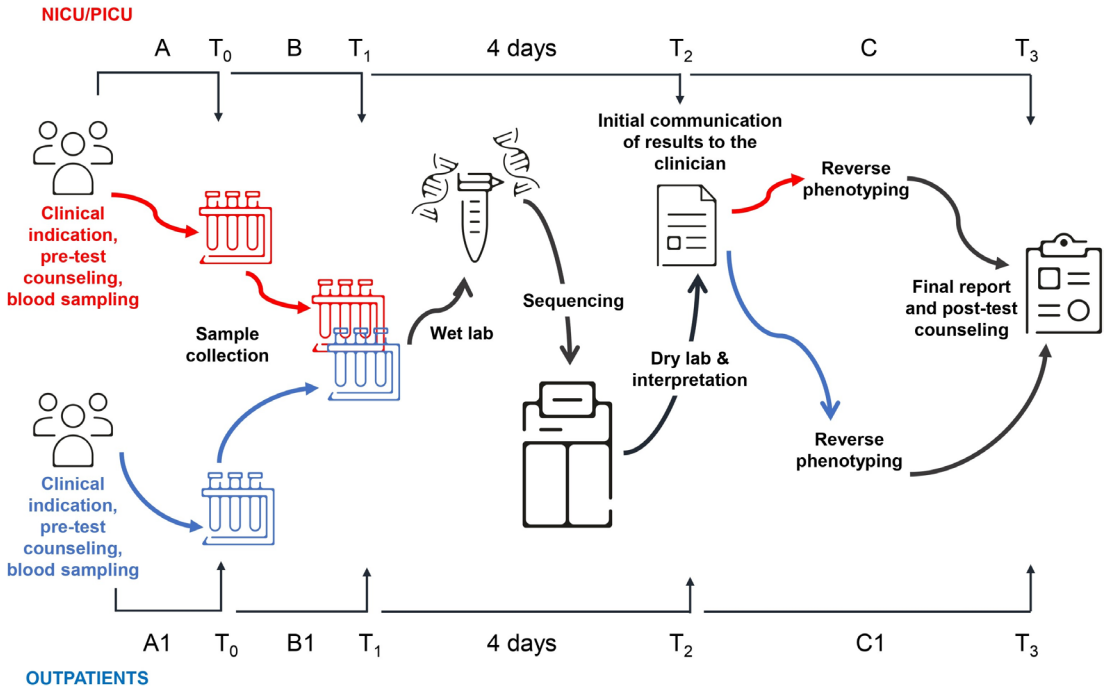

FIGURE 4 | Process flow from patient's clinical indication to the final report. Red text, tubes, and arrows represent critical samples; blue represents non-critical samples. A–C refer to critical samples; A1–C1 to non-critical samples. A and A1 correspond to elapsed time between onset of symptoms and blood sampling for WGS. T_0 corresponds to time of sample arrival in the laboratory. B and B1 correspond to elapsed time between sample arrival in the laboratory and accumulation of the 7 trios to be processed simultaneously. T_1 corresponds to the start time of sample processing and T_2 corresponds to time of communication of WGS results to the referring clinician. T_1–T_2 represents the processing time from DNA isolation up to the initial communication of results. T_0–T_2 represents laboratory TAT. C and C1 correspond to time for reverse phenotyping. T_0–T_3 corresponds to elapsed time from sample arrival in the laboratory to final report. Abbreviations used: NICU/PICU, neonatal intensive care unit/pediatric intensive care unit; TAT, turnaround time.

requiring WGS to be identified. Others have similarly reported higher DRs for first- versus last-tier WGS [6, 19, 23]. In this study, the detection of causative variants for patients with negative prior genetic testing showcases the resource- and time-saving advantages of WGS's consolidated workflow over a sequential genetic testing approach.

Notably, WGS identified a molecular etiology for 20% of WES-negative patients in this cohort. Furthermore, 19.8% of the causative variants identified in this study would have been missed by current WES methodologies due to intrinsic limitations in variant detection and bioinformatic pipelines. Like prior reports, these observations highlight the superior capacity of WGS to identify variants missed by other tests, including sequencing panels and WES [6, 11, 25, 26, 44, 45].

In our setting, the laboratory sample processing time—the execution of WGS, from DNA isolation to interpretation—took 4 days for all samples, but the time from symptom onset to final diagnosis was impacted by variability in the time from patient presentation to sample collection, laboratory sample volumes and flow cell capacities, and the time required for reverse phenotyping. The significant difference in laboratory TAT between critical patients (5 days) and outpatients (74 days) in our study was primarily due to the prioritization of urgent samples for sequencing. This delay could be mitigated by implementing a dedicated team and workflow in the laboratory.

Presently, WGS is not widely considered a first-tier test in routine pediatric clinical practice. Professional guidance that advocates for WGS as a first-tier genetic test would likely encourage earlier ordering of WGS tests. However, attention to factors impacting accessibility of the test and reverse phenotyping is needed to achieve earlier therapeutic intervention for patients and families, especially in non-critical care settings.

A molecular diagnosis impacts all patients who receive one by allowing a shift from the diagnostic pathway to evidence-based care including condition-specific follow-up and targeted therapies, where available. A molecular diagnosis also impacts the patient's family by providing information about reproductive risk and supporting early identification of at-risk relatives [6, 23, 24, 26, 27, 30, 41, 43, 46, 47]. In the short observational timeline of this study, the rate of immediate clinical utility of WGS results was demonstrably higher for critically ill patients than for outpatients; however, the NICU/PICU subcohort was characterized by a greater preponderance of metabolic disorders or genetically determined organ failures for which tailored therapies or transplantation recommendations could be offered. In contrast, outpatients had a higher prevalence of

neurodevelopmental disorders and malformation syndromes, which frequently lack specific treatments. For outpatients, the benefits of WGS may be less immediate but still valuable in guiding long-term management. Due to this study, the ASST Papa Giovanni XXIII hospital has adopted trio-WGS as a first-tier test for patients with suspected genetic pediatric-onset conditions.

This was the first study to evaluate use of WGS as a first-tier test for critically ill patients in Italy. The short laboratory TAT and high sample throughput and DRs demonstrate the feasibility of implementing WGS, even in a small- to mid-sized laboratory, within a publicly funded healthcare system. The required conditions are dedicated and trained staff, investment in laboratory resources and workflow standardization, and enough patients to build a high level of experience within the laboratory. In our experience, the interpretation phase for WGS in this study was no more complex than that of WES. However, establishing a sufficient weekly sample volume was important for cost optimization and sustainability. In our assessment, approximately seven trio-WGS samples per week and 300 trio-WGS samples per year are sufficient to establish technical expertise, contain costs, and ensure the ability to perform tests urgently when needed.

Another key element in the success of WGS in our setting was close collaboration between referring clinicians and the laboratory. Clinical geneticists were key partners in test outcomes by conducting the initial deep phenotyping of patients, selecting patients for testing, and conducting reverse phenotyping.

Cost-effectiveness analyses were beyond the scope of this study. The multicenter nature of NICU/PICU recruitment and the lengthy diagnostic odysseys experienced by a large portion of the outpatient subcohort would have made it all but impossible to accurately assess base costs. However, prior Italian studies have shown the cost benefit of early utilization of WGS in support of clinical implementation [31–33].

This study has some limitations. The patient cohort, which included roughly equal numbers of critical and non-critical patients, is not proportionally reflective of the ratio of critical to non-critical patients in the overall pediatric patient population and could represent a source of bias. For this reason, we calculated DRs for the critical and non-critical subcohorts separately, as well as calculating the overall DR. The geographic localization of this study to the Lombardy region and the potential for referral of more severe cases could also impact the representativeness of this cohort. The short timeline for follow-up of this cohort likely led to an underestimation of clinical utility, and limitations of bioinformatic pipelines for detection and interpretation of certain variant types, especially non-coding variants, may have negatively impacted DRs.

5 | Conclusion

Trio-WGS demonstrates high rates of diagnostic and clinical utility in patients with suspected genetic disorders of pediatric-onset. These data contribute to emerging evidence of WGS as a superior technology for variant detection and the molecular diagnosis of complex phenotypes. Combined with the rapid elapsed time from sample intake to results in the laboratory, this study supports a genome-first approach to genetic assessment of pediatric patients in both critical and non-critical settings.

Author Contributions

Conceptualization and design: M.I., Li.P., D.P. Data acquisition, analysis, or interpretation: M.F.B., M.B., E.B., E.C., U.C., G.C., A.C., C.D., M.F., F.F., S.G., La.G., Lu.G., C.L., G.M., Da.M., Do.M., C.M., Li.P., La.P., A.P., E.R., A.S., L.S., D.T., M.I. Funding acquisition and supervision: M.I. Writing – original draft: C.L., E.R. Writing – review and editing: M.I., C.L., Da.M., Li.P., La.P., D.P., E.R. Guarantor: M.I. All authors critically reviewed the manuscript and approved the final version for publication. C.L. and E.R. contributed equally to this work.

Acknowledgements

The authors thank the patients and parents who participated in this study. The authors also thank Stacie Taylor and Raye Alford (Illumina Inc.) for editorial support, and Anna Rita Lincesso, Loredana Perego, Nunzia Della Malva, and Riccardo Rubini for the laboratory technical work. Finally, we thank Cinzia Giaccherini and FROM Research Foundation for their support in the statistical review and analysis. Francesca Furlan is a member of the European Reference Network for Hereditary Metabolic Disorders—MetabERN. Donatella Milani and Maria Francesca Bedeschi are members of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability ERN-ITHACA [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516]. Part of this work was presented in an abstract and oral presentation form at the XXVII SIGU Conference; October 2-4, 2024; Padova, Italy. This work was funded by a Telethon Foundation grant awarded to the Medical Genetics Laboratory of ASST Papa Giovanni XXIII in Bergamo, Italy as part of the project "Responsible Implementation of Newborns Genome Sequencing (RINGS): a technical and interpretative feasibility study", grant number GSP21003. The work was partially supported by the "PG23/FROM 2017 Call for Independent Research" as part of the RARE—Rapid Analysis for Rapid carE—project. This work was also funded by the National Plan for NRRP Complementary Investments (PNC, established with the decree-law 6 May 2021, n. 59, converted by law n. 101 of 2021) in the call for the funding of research initiatives for technologies and innovative trajectories in the health and care sectors (Directorial Decree n. 931 of 06-06-2022)—project n. PNC0000003—AdvaNced Technologies for Human-centrEd Medicine (project acronym: ANTHEM), Pilot 1.4 Timely genomic diagnosis for children with rare diseases toward precision medicines. This work reflects only the authors' views and opinions, neither the Ministry for University and Research nor the European Commission can be considered responsible for them. This work was also partially supported by Illumina Inc. through a collaboration agreement. Open access publishing facilitated by Aziende Socio Sanitarie Territoriale Papa Giovanni XXIII, as part of the Wiley - SBBL agreement.

Ethics Statement

This study was approved on May 17, 2022, by the Institutional Review Board and Ethical Committee of ASST Papa Giovanni XXIII Hospital of Bergamo (Reg. 22/22). Informed consent was obtained from both parents of enrolled children and from adult patients with legal capacity.

Conflicts of Interest

Maria Iascone reports receiving TruSight Software Suite access, technical and editorial support, and open access publication fees through a collaboration agreement between Medical Genetics Laboratory of ASST Papa Giovanni XXIII and Illumina Inc. Daniela Piazzolla is an employee and shareholder of Illumina Inc.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14760.

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Supporting Information

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