nature portfolio

Peer Review File

Citywide implementation of a rapid whole genome sequencing program for critically ill pediatric patients

Corresponding Author: Professor Ahmad Abou Tayoun

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Thank you for the opportunity to review this manuscript which describes the outcomes of a rapid WGS program for critically ill newborns and children in Dubai. 100 participants were enrolled, with 53% receiving a diagnosis. rWGS was performed with an average turnaround of 3.4 days. The results were of high clinical utility. Diagnostic and clinical utility, including time to diagnosis are compared against previous standard of care at the same centres in a historical cohort.

The results are comparable to two previous studies, one describing rWGS in a cohort of 184 newborns enrolled through 5 hospitals in California, with 40% diagnostic yield and 3 day TAT (Dimmock et al); and a second describing rWGS in a cohort of 290 infants and children enrolled through a national network in Australia, with 54% diagnostic yield, and 2.9 day TAT (Lunke et al).

The main novelty of the manuscript lies in the setting in a Middle Eastern healthcare system and the comparison with a historical cohort.

The authors appropriately highlight some of the resulting differences, particularly in the proportion of diagnoses due to autosomal recessive conditions, which are related to the higher degree of consanguinity in the population. However, apart from this, I wonder if it is possible to comment on any local implementation challenges either for the research program or for how they see this may transition to standard of care. For example, can you comment on what the cost of the test was? Do you foresee cost/funding as being the principal implementation barrier or was it more a question of previous lack of local capacity/capability?

I am also unclear on the role of clinical genetics services in your model. Were all patients assessed by a clinical geneticist and seen by genetic counsellors? Or is your model more of a 'mainstreaming' model, where testing and result return rest with NICU/PICU physicians? If so, is your workforce skilled and ready to integrate rWGS? What steps were taken to prepare/support them? There is literature from other healthcare systems suggesting that while NICU/PICU physicians are supportive of rWGS, they feel underprepared and prefer models that closely integrate with genetics professionals.

How exactly was 'clinical utility' defined? There are now specific instruments for defining clinical utility of genomic testing such as the C-Guide (including specific versions for rapid testing in newborns) and it would have been good to see those being used.

I am also unclear on the purpose of reporting carrier screening results in this cohort. Was it to inform the child's future reproductive risk? Or the reproductive risk of the parents? Many would argue that reporting unrelated carrier status in young children is not appropriate as it is not actionable. If it was done for the benefit of the parents, then was reporting restricted to situations where both parents (as well as the child) were identified to be carriers?

Are you in the process of or planning to ascertain the views and experiences of families?

Do you have data on time elapsed between hospital admission/initial presentation and test initiation? Do you have any sense about the level of uptake? Was there a process of vetting referrals or were all referrals accepted?

Minor point: the bar chart showing diagnostic yield by clinical indication for testing should probably be restricted to just the commoner indications. With indications that had 1-5 patients tested, results are unlikely to be representative.

Reviewer #2

(Remarks to the Author)
Review of Manuscript# NMED-A141001A

In this study, the Little Falcon, the authors present their data from the first 100 cases who underwent rapid whole genome sequencing in the neonatal and peditric critical case setting in the Dubai health care system and show the significant diagnostic and therapeutic and management implications of early diagnosis in the critical care setting with a single rapid and comprehensive genomic assay.

This study is one of a kind in a Middle Eastern population with unique genetic architecture notable for its the high rate of consanguinity and the abundance of autosomal recessive genetic disorders. The high diagnostic yield approaching 80% in those with history of parental consanguinity show the remarkably potential for the wide application in of WGS in all health care setting on early diagnosis, management, treatment and eventually prevention in the highly inbred population of the Middle East.

The study is novel, well-written and is generalizable to the health care system Middle Eastern region and other inbred populations. I have some major comments on the scientific content that can be addressed:

• I have a major issue with the inclusion criteria, especially if this cohort is to be replicated elsewhere. Did the inclusion criteria all need to be satisfied or a specific number of them for inclusion? Apart from congenital anomalies, each inclusion criteria in isolation is very broad and nonspecific for a genetic condition. For example, what kind of ECG abnormality was considered for inclusion? What does lack of response to standard therapy here mean? Also, would any lab abnormality qualify for enrollment such as abnormal CBC for example? I suggest that the authors should be very specific in their inclusion criteria, and specific how many criteria had to be met for enrollment.
• In the workflow diagram, it is better to use "diagnostic and non-diagnostic result" instead of "receiving and not receiving molecular diagnosis" as receiving and not receiving could be misinterpreted (result not delivered, data not analyzed, etc..)
• While the reason for excluding non-viable neonate in this study is understandable as it is a clinical study concerned about the proband, this approach risks missing genetic conditions leading to early lethality tha could be preventable in future pregnancies in a consanguineous family. Such limitation should be mentioned.
• What single systems were mostly affected? Perhaps these can be specified in order under the results section or included in Figure 2B?
• For the cases with multiple molecular findings including dual molecular diagnoses, were there any diagnosis detected pre-symptomatically/incidentally? For example, for the cases with SLC19A3 and BTD, where the variants accounting for the reason for admission or were any detected pre-symptomatically and lead to implementation of preventative therapeutic intervention?
• Also, for the multiple molecular findings, how many accounted for blended/overlapping phenotype and how many underlying a distinct phenotype? (PMID: 27959697)
• For the incidental carrier findings, what was the protocol for reporting the carrier findings? were only known pathogenic variants reported
• For the diagnostic and carrier findings, how often was the variant a founder variant? Perhaps the authors can specific if there were any founder or recurrent variants or conditions in this cohort.
• Why were the pharmacogenomic variants not reported and utilized for therapeutic guidance?
• For the case with ABCC8, what was the surgical invention carried out?
• In lines 243-245, it should be clarified that negative rWGS does not fully exclude genetic etiology as there still remains a lot of limitation to WGS. Thus, instead of saying "confirming an non-genetic etiology", the author could say "ruling out a list of suspected or implicated genes and thus confirming a diagnosis of a non-genetic transient neonatal hyperinsulinemia"
• In lines 438-440, the sentence "Project Baby Bear,33 the NSIGHT Consortium, 17,34,35 the Australian Acute Care Genomics Program,36,37 NHS England's national rWGS service, 38 GEMINI Study,39 and NICUSeq Study.40" is incomplete.
• One big limitation for the wide implementation of WGS besides the manpower and technical limitations is perceived high cost. Would it be possible for the authors show the contrary by calculating the cost saved (cost effectiveness) of implementing rWGS from early diagnosis in this cohort against the standard of care testing in the retrospective cohort?

Reviewer #3

(Remarks to the Author)

In this study, the authors describe the diagnostic yield and clinical impact of 100 instances of rapid GS for critically ill patients within the health system of Dubai. They identified a diagnostic yield of 53%, higher in families with consanguinity; 11% had multiple diagnoses, 5% had newborn screen-relevant results or ACMG secondary findings. About half of patients had impact on clinical care.

The strengths of this article include the clear descriptions of the diagnostic yield and patient population, and helpful figures illustrating study workflow and diagnostic yield. The inclusion of a comparator cohort is helpful.

My main critique relates to novelty and how the findings from this study advance the field of implementation of rGS in the critical care setting. The authors state that the novelty of this study is implementation in the Middle East, and the presentation of a framework for city-wide implementation that can be adapted elsewhere. However, the details of such a framework are lacking. In particular, the authors purport that this study demonstrated feasibility of rGS incorporation into the health system, although it's not clear what the measure of feasibility is.

Based on the wealth of literature that exists on use of rGS in critical care settings (as cited in the Discussion), insight into a new implementation would be very useful. However, the authors actually provide very little detail in to the implementation process. For example, who was responsible for the cost of this sequencing and analysis? What did it take to shift from the prior testing approach to this one? Key stakeholder perspectives?

Otherwise, it seems that the diagnostic yield and genomic landscape is similar to other prior, large studies of genomic sequencing in Middle Eastern populations (e.g. PMID: 37344829). The impact on clinical care in critical care units has also been well-established.

A more minor detail - there is a part of the discussion that seems to be out of place, and I wonder if some of the text was inadvertently deleted (line 303 jumps to line 438).

Overall, this is a nicely written report on implementation of rGS in a city that was not previously using it as a first-line test, but the study design falls short on revealing the aspects of the study and results that would inform other places looking to do the same thing.

Reviewer #4

(Remarks to the Author)
Authors present a well written, well-designed study of rapid WGS sequencing in pediatric care setting. As they note, the middle east is underrepresented in studies of this kind and thus represents a new and interesting population to examine. Authors identify a high diagnostic rate in their population due, in large part, to know rates of consanguinity. They make a convincing case for rWGS in their population. It is not clear if the authors think these conclusions apply to the middle east generally or whether this could be unique to the UAE.

A major weakness of this study is a failure to examine changes in care cost as resulted by rWGS. In this population, especially one where metabolic disease is so prevalent and readily (and inexpensively comparted to rWGS) testable do the incurred cost of sequencing outweigh the cost savings (if any) by changes in management?
This study is also complicated by the fact that it is a mxi of PICU and NICU populations. Statistics (dx rate, homozygous rate, etc etc etc) should be broken out for each care setting and presented as a supplement. Differences should be noted in the main text.

Minor issues:
Because this is a unique population, rates of de novo, homozygous, recessive vs. dominant disease rates should be compared to European and/or north American rates. It would also be interesting to note which families had a previously affected child and/or a family history of the same disease.

All identified variants should be listed in a supplementary table (genomic coordinate, p. and c.) as well as any ClinVar ID or MAF in gnomad or similar database. And whether the variant was considered P or LP. I would note in the text the proportion of novel vs. known variants identified.

Comparison of patient demographics of cohort to controls (line ~262) must all have p-values. Further, I suggest having all demographics (cohort, control, p-values) represented in a table.

Paper should include a description of how a result was determined to be diagnostic or not. (physician review, all P/LP?)

What is the sensitivity of CNV calling? What is the smallest and/or largest CNV called?

Supplemental figure 3 is redundant to figure 4D.

Matthew Bainbridge, Rady Children's

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Thank you for the opportunity to review a revised version of this manuscript. The authors have satisfactorily addressed my previous comments and suggestions.

Reviewer #2

(Remarks to the Author)
We thank the authors for addressing all of our comments. The additions and clarifications have improved the manuscript further and all my comments were well-addressed. However, there remains minor small points that may be clarified further to avoid confusion if possible

1) In line 224 and Figure 4E, where did the 63 molecular diagnoses come from when cases with diagnostic results are 53? Even when we add those with dual diagnoses, the number is still 64? the list in S4-S6 are long with many pathogenic and likely pathogenic variant creating more confusion on the numbers.
2) Line 237 indicate that "70 diagnostic variants were identified" and line 240 states "monogenic diagnosis encompasses 64 variants'"); yet tables S5-6 list many more pathogenic and likely pathogenic variants. Can you kindly clarify to avoid confusion on the numbers?

One minor suggestion: Figure 1C 47 should be "non-diagnostic or inconclusive" to match Figure 4A.

I have no other suggestions or edits. The author did well in addressing the comments from the prior version.

Reviewer #3

(Remarks to the Author)
Thank you to the authors for providing this revision of their manuscript. They have done an admirable job describing the cohort and sequencing approach in addition to the genomic analysis. I think that these data describing the genomic aspects of their process will be a helpful benchmark for future centers looking to implement rapid genomic sequencing.

They did not address my concern about a measure of feasibility. The outcomes reported are generally effectiveness outcomes. It is difficult to conclude that their data demonstrate the feasibility of an rGS program in their health system without defining how they are measuring feasibility or defining success. When I think about feasibilty, I think about ways they could demonstrate that their approach is implementable. One way to do it would be to ask the clinicians or hospital administrators not only if the diagnoses were useful, but if they think the rGS system is feasible and why/why not. There are other outcomes measures that could indirectly reflect feasibility. For example: 100 probands were enrolled. What if this was only 10% of patients who met eligibility criteria? does that meet a threshold for implementation success? Please correct me if this information (reach of rGS) is reported and was overlooked by me.

Relatedly, I think it's an oversimplification to state that the main barrier is cost of the sequencing (which was partially covered by Illumina for this study). Even if rGS was completely free, there were workforce considerations to implement it -- were new genetic counselors hired? How many hours per week of their time were required? What about the genetics MD workforce, any considerations regarding the costs of their time? This is certainly not to say that I don't support this initiative; I actually do, but I feel that in order to realistically justify implementation across other health systems, a reflection of the whole "cost" of implementation is needed. This may be out-of-scope for this paper, but suggesting that reduced cost of GS alone means that other centers could feasibly do what was done here is a vast oversimplification. There are some very nice reports describing the layers of barriers (particularly for private payer healthcare systems) that are involved in this implementation context. Here's one example: https://pubmed.ncbi.nlm.nih.gov/40367948/

Another interesting consideration is how much having an 'internal' lab system contributed to the success of this program. Is it advised for future health systems to also build their own CLIA lab? Or, if another ICU system wanted instead to work with an external diagnostic lab for test ordering, would that impact the workflow described here? Maybe this is inconsequential and ties into the cost consideration (e.g. estimate of $3500 total cost) but it could be worth considering especially as related to turnaround time and lab processing when sending samples externally.

Overall, I do think the authors mostly responded to the critiques. I still think that there could be more to learn about what was unique in this approach that led to their success so that other centers could learn from it. We have known about diagnostic yield, clinical utility, turnaround time for awhile now and yet many high-level ICUs still face substantial barriers that I don't think entirely related to just the flat cost of GS itself.

Reviewer #4

(Remarks to the Author)
I appreciate the author's feedback and the inclusion of the new data tables, I feel it greatly enhances the value of the paper for the audience. This is an important piece of research and will contribute to the body of work in the field of rapid sequencing in critically ill infants/children.

Response to referees

	Comment	**Response**
Editorial team	section related to the impact on clinical management is further strengthened, for example by providing information on the type of pharmacologic and dietary interventions provided and outcomes	We highly appreciate this feedback. To further strengthen the clinical management section we did several analyses, added 3 supplementary tables and edited this section accordingly. Specifically, 1- Using the clinical utility C-GUIDE ICU tool (PMID: 40542646), recommended by one reviewer, we quantified management changes across 10 clinical domains in all 200 patients (100 rWGS and 100 controls). This analysis, shown in detail in **a new supplementary Table S9 and in manuscript**, confirmed the higher clinical utility associated with rWGS relative to standard of care testing in controls. 2- We provide more information on the type of pharmacologic and dietary interventions in a **new supplementary Table S8**. 3- Beyond the positive impact on care trajectories, we also highlight cases where the rWGS-prompted management changes had an impact on disease trajectories (outcomes), either leading to favourable outcomes or to de-escalation and end of life decisions. **This information is added to manuscript as well as in a new supplementary table S10**.

		4- Finally, beyond direct patient care, the recessive nature of diseases in our context (70% of all diagnoses across the cohort and 97% in consanguineous families) adds a high "preventive" clinical utility to families in this population. All those parents had reproductive counselling and were offered pre-implementation genetic testing or prenatal diagnosis to mitigate the 25% recurrence risk for future affected pregnancies. This information is also now emphasized under clinical utility.
	Data and Code Availability Statement with details on how researchers can request access to the data and code generated and used in the manuscript (what data will be available for sharing, under which conditions, and how to request it)	To address this, we updated the Data and code Availability Statement: "The raw whole-genome sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1359111 and are publicly available without restriction. Other data supporting the findings of this study are provided in the main Article, Figures and Supplementary Files. These materials include clinical, demographics, genomic, diagnostic and clinical utility data underlying the reported analyses and figures. No custom code was generated for this study. Sequence alignment and variant calling were performed using Illumina DRAGEN software, and variant annotation and filtering were performed using Emedgene (Illumina), which are commercially available proprietary software platforms".

		Note: Please let us know if you would like us to release the data immediately, or if you would like to coordinate a specific date for release.
	Additional information on how the approach was implemented in the Dubai healthcare system, including challenges encountered, so that it can inform future initiatives.	Thank you. We have expanded the first Results section, "Implementation of Patient Referral and Rapid Whole Genome Sequencing Workflow", to add information on implementation aspects of this approach, including: - referral network, - multidisciplinary clinical team, - selection criteria (**with more details in Methods and Figure 1**), - patient uptake and time from admission to rWGS referral (**new figure S1**), - genomic capacity (analysis, facility, team), - funding model. - This is in addition to other implementation aspects such as turnaround time, diagnostic yield, and impact on care trajectories in this setting, which are all detailed in other Results sections.
Reviewer #1	Comment on any local implementation challenges either for the research program or for how they see this may transition to standard of care.	Thank you for this great comment. We have expanded the first Results section, "Implementation of Patient Referral and Rapid Whole Genome Sequencing Workflow", to add information on implementation aspects of this approach, including: - referral network, - multidisciplinary clinical team, - selection criteria (**with more details in Methods and Figure 1**),

		- patient uptake and time from admission to rWGS referral (**new figure S1**), - genomic capacity (analysis, facility, team), - funding model. - This is in addition to other implementation aspects such as turnaround time, diagnostic yield, and impact on care trajectories in this setting, which are all detailed in other Results sections.
	For example, can you comment on what the cost of the test was? Do you foresee cost/funding as being the principal implementation barrier or was it more a question of previous lack of local capacity/capability?	As we now mention in results and discussion, our study was intentionally designed to focus on feasibility, clinical utility, and capacity building rather than formal cost-effectiveness analysis. A major historical barrier to implementation of rWGS in critical care has been the perceived high cost of sequencing. However, we contend that sequencing cost is no longer a primary limitation in this context. As sequencing and analytical technologies continue to advance, costs also continue to decline substantially. Earlier studies reported a median rWGS cost of $9,239 per proband (rang: $6,300-$16,063) and a median cost per diagnosis of $23,602 (range: $14,072-$37,480)[1]. In contrast, the current cost of rWGS in our setting–benchmarked against commercial laboratories in the United States–is approximately $3,500 per case, resulting in a cost per diagnosis of $6,603 in our cohort. This is markedly lower than prior reports even though those earlier studies still demonstrated overall cost savings per patient despite significantly higher sequencing expenses.

		[1]Kingsmore, S.F., Nofsinger, R. & Ellsworth, K. Rapid genomic sequencing for genetic disease diagnosis and therapy in intensive care units: a review. *npj Genom. Med.* **9**, 17 (2024). https://doi.org/10.1038/s41525-024-00404-0.
	Comment on the role of clinical genetics services in your model. Were all patients assessed by a clinical geneticist and seen by genetic counsellors? Or is your model more of a 'mainstreaming' model, where testing and result return rest with NICU/PICU physicians? If so, is your workforce skilled and ready to integrate rWGS? What steps were taken to prepare/support them? There is literature from other healthcare systems suggesting that while NICU/PICU physicians are supportive of rWGS, they feel underprepared and prefer models that closely integrate with genetics professionals.	We have added more information in the first section of results to illustrate this and other implementation aspects. In essence, NICU/PICU specialists were directly supported by genetic counselors who provided additional input from pediatric specialists (including clinical or metabolic geneticists) or genomics team as needed. Specific to this point we added the following: "This referral workflow integrated a multidisciplinary clinical team comprising neonatologists, pediatricians, and genetic counselors who jointly identified and referred eligible patients from a centralized NICU and PICU (~110 beds). Case selection was guided by predefined inclusion criteria, focusing on patients with suspected monogenic disorders where a rapid diagnosis was anticipated to inform acute clinical management (**Figure 1B** and **Methods**). In addition to formal referral pathways, ad hoc case discussions between clinicians and the genetics team were frequently undertaken to rapidly assess eligibility. Genetic counselors were involved in all cases, while clinical or metabolic geneticists were consulted

		selectively based on the clinical presentation and complexity of the case".
	How exactly was 'clinical utility' defined? There are now specific instruments for defining clinical utility of genomic testing such as the C-Guide (including specific versions for rapid testing in newborns) and it would have been good to see those being used.	Thank you for this comment. As we now clarify in Methods, clinical utility was evaluated by determining whether rWGS influenced patient management or care trajectory during their NICU/PICU admission period, either by prompting a change in clinical actions, including pharmacologic or dietary interventions, optimization of diagnostic evaluation, surgical or procedural decision-making, rapid specialty consultations, and/or initiation of palliative or end-of-life care, or by providing diagnostic clarity that informed ongoing care. The overall clinical utility was measured as the proportion of cases where rWGS had an impact on NICU/PICU care trajectories as defined above, detailed in **Table S1** and quantified in **Figure 5.** Based on your recommendation, we also now use a customized C-GUIDE version for rapid genetic testing (C-GUIDE ICU), to further evaluate utility across 10 clinical domains as described in PMID: 40542646. Using the C-GUIDE ICU tool we scored all 200 patients (100 rWGS and 100 controls) across the 10 questionnaire/clinical domains. Our findings, which are now added in the manuscript text and in a **new supplementary table S9**, confirm the impact of rWGS on clinical management and its higher clinical utility compared to standard testing in controls.

		Finally, beyond direct patient care, the recessive nature of diseases in our context (70% of all diagnoses across the cohort and 97% in consanguineous families) adds a high "preventive" clinical utility to families in this population. All those parents had reproductive counselling and were offered pre-implementation genetic testing or prenatal diagnosis to mitigate the 25% recurrence risk for future affected pregnancies. This information is also now emphasized under clinical utility. All the new analyses were highlighted in manuscript and the new tables.
	The purpose of reporting carrier screening results in this cohort. Was it to inform the child's future reproductive risk? Or the reproductive risk of the parents? Many would argue that reporting unrelated carrier status in young children is not appropriate as it is not actionable. If it was done for the benefit of the parents, then was reporting restricted to situations where both parents (as well as the child) were identified to be carriers?	We appreciate this comment. Carrier screening was included to extend the clinical value of rWGS beyond the immediate diagnostic question by providing families with additional relevant genetic information. In the UAE, where premarital screening for recessive conditions is mandatory (https://mohap.gov.ae/en/w/mohap-announces-mandatory-genetic-testing-as-part-of-premarital-screening-for-emiratis-starting-early-january-2025), this allowed for earlier optional determination of carrier status within families. In parallel, this approach is part of a broader effort to enable the collection of population-specific carrier frequency data, contributing to an important unmet need in Emirati genomic research.
	Are you in the process of or planning to ascertain the views and experiences of families?	Family perspectives were not formally assessed in this study due to the acute ICU context, where families are often under significant emotional burden, making it

		challenging for families to engage in additional research activities. This remains an important area for future work and will be considered in follow-up studies at a later, more appropriate timepoint.
	Do you have data on time elapsed between hospital admission/initial presentation and test initiation? Do you have any sense about the level of uptake?	Thank you for this comment. We agree that both patient referral/uptake and time between hospital admission and patient referral to rWGS are two important metrics of efficient implementation/integration of rWGS in ICU settings. Our data shows that uptake increased while the time from admission to referral decreased over time. We made a new figure (**Figure S1**) to show this data which we also describe in an expanded section about implementation in Results section.
	Was there a process of vetting referrals or were all referrals accepted?	We have added more details in first section of 'Results' on the referral pathway which involved a multidisciplinary team of neonatologists, paediatricians and genetic counsellors. Case selection was guided by predefined inclusion criteria (**detailed in Methods and in Figure 1**), focusing on patients (<18 years) with suspected monogenic disorders where a rapid diagnosis was anticipated to inform acute clinical management. In addition to formal referral pathways, ad hoc case discussions between clinicians and the genetics team were frequently undertaken to rapidly assess eligibility and reduce delays in referrals.
	The bar chart showing diagnostic yield by clinical indication for testing should probably be restricted to	Thank you. As suggested, we have changed **Figure 4C** to include indications with at least 5 patients.

	just the commoner indications. With indications that had 1-5 patients tested, results are unlikely to be representative.	
Reviewer #2	I have a major issue with the inclusion criteria, especially if this cohort is to be replicated elsewhere. Did the inclusion criteria all need to be satisfied or a specific number of them for inclusion? Apart from congenital anomalies, each inclusion criteria in isolation is very broad and nonspecific for a genetic condition. For example, what kind of ECG abnormality was considered for inclusion? What does lack of response to standard therapy here mean? Also, would any lab abnormality qualify for enrolment such as abnormal CBC for example? I suggest that the authors should be very specific in their inclusion criteria, and specific how many criteria had to be met for enrolment.	Thank you for this great comment. We agree that the inclusion criteria can be clearer and more specific. We have made several changes to address this. To clarify, our criteria were based on the Blue Shield of California Medical policy 2.04.102, which we now describe in more detail in the manuscript. Briefly, to meet eligibility criteria, critically ill infants or children had to be less than 18 years of age and admitted to the NICU/PICU with illness of unknown etiology with at least one inclusion criteria while not having any features/presentations on the exclusion list. We have now **added more details in Methods** to highlight the above and to be more specific on type of abnormalities (e.g., lab or ECG). We also **updated Figure 1B** to add more clarifications.
	In the workflow diagram, it is better to use "diagnostic and non-diagnostic result" instead of "receiving and not receiving molecular diagnosis" as receiving and not receiving could be misinterpreted (result not delivered, data not analyzed, etc..)	We have made the requested change.
	While the reason for excluding non-viable neonate in this study is understandable as it is a clinical study concerned about the proband, this approach risks missing genetic conditions leading to early lethality tha could be preventable in future pregnancies in a	This is a great point which we fully agree with. As you mention, non-viable neonates were not included since rWGS is not expected to have a direct impact on proband's care management. However, in discussion we highlight the possibility of missing lethal genetic

	consanguineous family. Such limitation should be mentioned.	conditions and missing valuable information for families to assess recurrence risks. Equally important is the opportunity to discover novel genes associated with novel life-threatening conditions in this population. We also address this in discussion.
	What single systems were mostly affected? Perhaps these can be specified in order under the results section or included in Figure 2B?	Most recurrent single system presentations were metabolic (n=15), neurologic (n=10) and cardiovascular (n=8). We have added this information in text, and it is also in **Figure 4C.**
	For the cases with multiple molecular findings including dual molecular diagnoses, were there any diagnosis detected pre-symptomatically/incidentally? For example, for the cases with SLC19A3 and BTD, where the variants accounting for the reason for admission or were any detected pre-symptomatically and lead to implementation of preventative therapeutic intervention?	This is a great point. We made a **new table** (**Table S4**) listing all patients with multiple molecular diagnoses and highlighted those that were symptomatic (or pre-symptomatic) at the time of testing as well as those with distinct or overlapping phenotypes. This table also highlights pre-symptomatic patients where a management plan was implemented.
	Also, for the multiple molecular findings, how many accounted for blended/overlapping phenotype and how many underlying a distinct phenotype? (PMID: 27959697)	Please see above.
	For the incidental carrier findings, what was the protocol for reporting the carrier findings? were only known pathogenic variants reported	Yes, only pathogenic variants were reported. We have clarified this in Methods.
	For the diagnostic and carrier findings, how often was the variant a founder variant? Perhaps the authors can specific if there were any founder or recurrent variants or conditions in this cohort.	For diagnostic variants, we encountered one recurring variant in the *TRAPPC12* gene in two patients. We also highlight the frequency of genes and variants associated with carrier or other findings in both results and a **new supplementary tables S5 and S7**.

	Why were the pharmacogenomic variants not reported and utilized for therapeutic guidance?	Pharmacogenomic variants were reported but were not utilized for therapeutic guidance in this cohort. We highlight this in results.
	For the case with ABCC8, what was the surgical invention carried out?	Thank you. We clarified that the surgical intervention was to remove focal pancreatic lesions as suggested by the *ABCC8* genetic diagnosis (focal type).
	In lines 243-245, it should be clarified that negative rWGS does not fully exclude genetic etiology as there still remains a lot of limitation to WGS. Thus, instead of saying "confirming an non-genetic etiology", the author could say "ruling out a list of suspected or implicated genes and thus confirming a diagnosis of a non-genetic transient neonatal hyperinsulinemia"	We have made this change. Thank you for the suggestion.
	In lines 438-440, the sentence "Project Baby Bear,33 the NSIGHT Consortium, 17,34,35 the Australian Acute Care Genomics Program,36,37 NHS England's national rWGS service, 38 GEMINI Study,39 and NICUSeq Study.40" is incomplete.	Thank you. This was most likely due to issues with converting the file to PDF in the system. I think it should be fixed now.
	One big limitation for the wide implementation of WGS besides the manpower and technical limitations is perceived high cost. Would it be possible for the authors show the contrary by calculating the cost saved (cost effectiveness) of implementing rWGS from early diagnosis in this cohort against the standard of care testing in the retrospective cohort?	As we now mention in results and discussion, our study was intentionally designed to focus on feasibility, clinical utility, and capacity building rather than formal cost-effectiveness analysis. A major historical barrier to implementation of rWGS in critical care has been the perceived high cost of sequencing. However, we contend that sequencing cost is no longer a primary limitation in this context. As sequencing and analytical technologies continue to advance, costs also continue to decline substantially. Earlier studies reported a median rWGS cost of $9,239 per proband (rang: $6,300-$16,063) and a median cost per diagnosis of $23,602 (range: $14,072-

		$37,480)[1]. In contrast, the current cost of rWGS in our setting–benchmarked against commercial laboratories in the United States–is approximately $3,500 per case, resulting in a cost per diagnosis of $6,603 in our cohort. This is markedly lower than prior reports even though those earlier studies (reviewed in Ref. 1 below) still demonstrated overall cost savings per patient despite significantly higher sequencing expenses. Preliminary analysis of our cohort further suggests that rWGS is economically favourable when broader healthcare expenditures are considered. Furthermore, economic modelling using published cost estimates suggests that accelerated diagnosis through rWGS generate considerable savings by reducing the financial burden of diagnostic delay. Beyond direct care savings, rWGS may also provide long term economic benefits through reproductive counselling and prevention given the nature of recessive inheritance, and the associated 25% recurrence risk, in most diagnoses in our population. We therefore posit that rWGS is no longer prohibitively expensive and may, in fact, offer substantial clinical and economic value through reduced hospitalization costs, faster diagnosis, and downstream prevention benefits. [1]Kingsmore, S.F., Nofsinger, R. & Ellsworth, K. Rapid genomic sequencing for genetic disease diagnosis and therapy in intensive care units: a review. *npj Genom.*

		Med. **9**, 17 (2024). https://doi.org/10.1038/s41525-024-00404-0.
Reviewer #3	My main critique relates to novelty and how the findings from this study advance the field of implementation of rGS in the critical care setting. The authors state that the novelty of this study is implementation in the Middle East, and the presentation of a framework for city-wide implementation that can be adapted elsewhere. However, the details of such a framework are lacking. In particular, the authors purport that this study demonstrated feasibility of rGS incorporation into the health system, although it's not clear what the measure of feasibility is. Based on the wealth of literature that exists on use of rGS in critical care settings (as cited in the Discussion), insight into a new implementation would be very useful. However, the authors actually provide very little detail in to the implementation process. For example, who was responsible for the cost of this sequencing and analysis? What did it take to shift from the prior testing approach to this one? Key stakeholder perspectives? Otherwise, it seems that the diagnostic yield and genomic landscape is similar to other prior, large studies of genomic sequencing in Middle Eastern populations (e.g. PMID: 37344829). The impact on clinical care in critical care units has also been well-established.	Thank you. We agree with the reviewer about adding more details on implementation. We have expanded the first Results section, "Implementation of Patient Referral and Rapid Whole Genome Sequencing Workflow", to add information on implementation aspects of this approach, including: - referral network, - multidisciplinary clinical team, - selection criteria (**with more details in Methods and Figure 1**), - patient uptake and time from admission to rWGS referral (**new figure S1**), - genomic capacity (analysis, facility, team), - funding model. - This is in addition to other implementation aspects such as turnaround time, diagnostic yield, and impact on care trajectories in this setting, which are all detailed in other Results sections. With respect to key stakeholders, we work very closely with the healthcare regulator in Dubai (DHA – Dubai Health Authority) to ensure the implementation of this program. The program was initially co-funded by Dubai Health and Illumina and was intentionally designed to focus on feasibility, clinical utility and capacity building. Thereafter, with continuous reduction in sequencing costs, the program is transitioning to a sustainable

		funding model, with government coverage for UAE nationals and support for non-nationals provided through insurance or philanthropic organizations, including Al Jalila Foundation within Dubai Health. All this information is added in results and discussion sections of the manuscript.
	A more minor detail - there is a part of the discussion that seems to be out of place, and I wonder if some of the text was inadvertently deleted (line 303 jumps to line 438).	Thank you. This was most likely due to issues with converting the file to PDF in the system. I think it should be fixed now.
Reviewer #4	A major weakness of this study is a failure to examine changes in care cost as resulted by rWGS.	Thank you for this important comment. As we now mention in results and discussion, our study was intentionally designed to focus on feasibility, clinical utility, and capacity building rather than formal cost-effectiveness analysis. A major historical barrier to implementation of rWGS in critical care has been the perceived high cost of sequencing. However, we contend that sequencing cost is no longer a primary limitation in this context. As sequencing and analytical technologies continue to advance, costs also continue to decline substantially. Earlier studies reported a median rWGS cost of $9,239 per proband (rang: $6,300-$16,063) and a median cost per diagnosis of $23,602 (range: $14,072-$37,480)[1]. In contrast, the current cost of rWGS in our setting–benchmarked against commercial laboratories in the United States–is approximately $3,500 per case, resulting in a cost per diagnosis of $6,603 in our cohort. This is markedly lower than prior reports even though those earlier studies (reviewed in ref. 1 below) still

		demonstrated overall cost savings per patient despite significantly higher sequencing expenses. Preliminary analysis of our cohort further suggests that rWGS is economically favourable when broader healthcare expenditures are considered. Furthermore, economic modelling using published cost estimates suggests that accelerated diagnosis through rWGS generate considerable savings by reducing the financial burden of diagnostic delay. Beyond direct care savings, rWGS may also provide long term economic benefits through reproductive counselling and prevention given the nature of recessive inheritance, and the associated 25% recurrence risk, in most diagnoses in our population. We therefore posit that rWGS is no longer prohibitively expensive and may, in fact, offer substantial clinical and economic value through reduced hospitalization costs, faster diagnosis, and downstream prevention benefits. [1]Kingsmore, S.F., Nofsinger, R. & Ellsworth, K. Rapid genomic sequencing for genetic disease diagnosis and therapy in intensive care units: a review. *npj Genom. Med.* **9**, 17 (2024). https://doi.org/10.1038/s41525-024-00404-0.
	In this population, especially one where metabolic disease is so prevalent and readily (and inexpensively comparted to rWGS) testable do the incurred cost of	In this context and while many metabolic disorders may appear readily diagnosable biochemically in retrospect, critically ill neonates often present with non-specific features—such as encephalopathy, acidosis, or sepsis-

	sequencing outweigh the cost savings (if any) by changes in management?	like illness—encompassing a broad and heterogeneous differential diagnosis. In this context, biochemical testing, although essential for initial stratification, frequently does not provide rapid diagnostic certainty or precise disease classification, particularly when testing is sequential or requires external processing. In contrast, rWGS enables early, comprehensive molecular diagnosis within clinically actionable timeframes and has been shown to significantly influence management in critically ill pediatric populations. Furthermore, rWGS extends beyond the scope of conventional biochemical approaches by identifying conditions that may not be detectable or clearly distinguishable through metabolic testing alone, including mitochondrial disorders and dual diagnoses. In our cohort, genomic sequencing enabled earlier diagnosis of lysosomal disorders compared to enzyme testing, including Wolman disease (*LIPA*), where treatment was initiated approximately two weeks prior to enzyme confirmation. Additionally, a dual diagnosis (*GLB1* and *TRAPPC12*) was identified, highlighting the ability of rWGS to resolve complex or overlapping phenotypes
	Because this is a unique population, rates of de novo, homozygous, recessive vs. dominant disease rates should be compared to European and/or north American rates. It would also be interesting to note	This is a great point. We surveyed inheritance patterns in published rWGS cohorts and compared to those in our cohort. Expectedly, we see an enrichment of autosomal dominant and *de novo* variants in published cohorts (50-85% of all pathogenic variants) which are mainly from

	which families had a previously affected child and/or a family history of the same disease.	outbred populations, while diagnostic variants associated with autosomal recessive inheritance accounted for 70% of positive cases in our cohort. Most patients in our cohort did not have family history of the same disease. **As requested, we added this information in discussion**.
	This study is also complicated by the fact that it is a mxi of PICU and NICU populations. Statistics (dx rate, homozygous rate, etc etc etc) should be broken out for each care setting and presented as a supplement. Differences should be noted in the main text.	Thank you. As requested, we made a **new supplementary table S3** with the breakdown of NICU and PICU populations along with statistics you mention (gender, ethnicities, indications, dx rate, inheritance, utility, etc.). This information is also explained in the text where relevant.
	All identified variants should be listed in a supplementary table (genomic coordinate, p. and c.) as well as any ClinVar ID or MAF in gnomad or similar database. And whether the variant was considered P or LP. I would note in the text the proportion of novel vs. known variants identified.	We made **two supplementary tables (S5 and S6)** listing all reported P/LP variants (SNVs and CNVs) along with coordinates, ClinVar IDs, gnomAD, etc. We also write in text the proportion of variants which are novel or reported.
	Comparison of patient demographics of cohort to controls (line ~262) must all have p-values. Further, I suggest having all demographics (cohort, control, p-values) represented in a table.	This is a great point. We compared demographics and clinical indications as highlighted in the text as well as in a **new supplementary table S12**.
	Paper should include a description of how a result was determined to be diagnostic or not. (physician review, all P/LP?)	As we **now describe in results and methods sections**, variants were classified according to ACMG/AMP guidelines and pathogenic or likely pathogenic variants consistent with disease mechanism, mode of inheritance, and patient phenotype were considered diagnostic and were usually confirmed by clinical team. However, for complex or uncertain findings, ad hoc interdisciplinary discussions with referring clinicians

		were held during the analysis phase, to support consensus-driven variant interpretation and clinical correlation.
	What is the sensitivity of CNV calling? What is the smallest and/or largest CNV called?	Thank you for noting this. We validated our WGS-based CNV calling pipeline using 19 samples in which 21 CNVs (ranging 3.5Kb – 155Mb in size) have been previously detected by chromosomal microarrays, MLPA, or PCR gel electrophoresis. All CNVs were accurately detected for a 100% sensitivity. We added this information in Methods and also provided a **new supplementary table S13** listing all validation samples and detected CNVs.
	Supplemental figure 3 is redundant to figure 4D.	While they look similar, **figure 4D** displays the overall diagnostic yield stratified by relatedness (consanguineous versus non-consanguineous families), while the supplementary figure (**now Figure S5**) displays the proportion of diagnoses due to recessive inheritance in these groups.

Point-by-point response

Reviewer #1:
Remarks to the Author:
Thank you for the opportunity to review a revised version of this manuscript. The authors have satisfactorily addressed my previous comments and suggestions.

Thank you for taking the time to provide highly valuable feedback on our manuscript.

Reviewer #2:
Remarks to the Author:
We thank the authors for addressing all of our comments. The additions and clarifications have improved the manuscript further and all my comments were well-addressed. However, there remains minor small points that may be clarified further to avoid confusion if possible

1) In line 224 and Figure 4E, where did the 63 molecular diagnoses come from when cases with diagnostic results are 53? Even when we add those with dual diagnoses, the number is still 64? the list in S4-S6 are long with many pathogenic and likely pathogenic variant creating more confusion on the numbers.

We apologize for the confusion. We fixed this issue by only counting patients with primary molecular diagnoses (N=53) but since 5 of those patients had dual diagnoses, the total number of primary molecular diagnoses is 58. We have updated text and Figure 4E to clarify this.

2) Line 237 indicate that "70 diagnostic variants were identified" and line 240 states "monogenic diagnosis encompasses 64 variants'"); yet tables S5-6 list many more pathogenic and likely pathogenic variants. Can you kindly clarify to avoid confusion on the numbers?

Again, we focused on the 58 primary molecular diagnoses which were caused by 63 variants (since 5 were compound heterozygous with two variants). We updated text to clarify, and we added a column in Tables S5 and S6 to highlight if the listed variant is related to the primary diagnosis or not (newborn screening, ACMG, secondary, etc.).

One minor suggestion: Figure 1C 47 should be "non-diagnostic or inconclusive" to match Figure 4A.

Thank you. We fixed this.

I have no other suggestions or edits. The author did well in addressing the comments from the

prior version.

Thank you for taking the time to provide highly valuable feedback on our manuscript.

Reviewer #3:
Remarks to the Author:
Thank you to the authors for providing this revision of their manuscript. They have done an admirable job describing the cohort and sequencing approach in addition to the genomic analysis. I think that these data describing the genomic aspects of their process will be a helpful benchmark for future centers looking to implement rapid genomic sequencing.

Thank you for taking the time to provide highly valuable feedback on our manuscript.

They did not address my concern about a measure of feasibility. The outcomes reported are generally effectiveness outcomes. It is difficult to conclude that their data demonstrate the feasibility of an rGS program in their health system without defining how they are measuring feasibility or defining success. When I think about feasibilty, I think about ways they could demonstrate that their approach is implementable. One way to do it would be to ask the clinicians or hospital administrators not only if the diagnoses were useful, but if they think the rGS system is feasible and why/why not. There are other outcomes measures that could indirectly reflect feasibility. For example: 100 probands were enrolled. What if this was only 10% of patients who met eligibility criteria? does that meet a threshold for implementation success? Please correct me if this information (reach of rGS) is reported and was overlooked by me.

Thank you. We measured patient enrolment/uptake and time to rWGS referral (since admission) over time and summarize this data in Extended Data Fig. 1. As we explain in manuscript text and now in discussion, we believe that our multidisciplinary approach which included genetic counselors helped to increase uptake and reduce time-to-referral leading to seamless integration and appropriate utilization of this tool. After recruitment of eligible patients, our data shows that rWGS is feasible in terms of return results within a short turnaround time and utilizing diagnostic findings to implement timely management plans. You will also notice that the discussion is now more focused on rWGS implementation, diagnostic resolution and clinical utility. I hope this addresses your question.

Relatedly, I think it's an oversimplification to state that the main barrier is cost of the sequencing (which was partially covered by Illumina for this study). Even if rGS was completely free, there were workforce considerations to implement it -- were new genetic counselors hired? How many hours per week of their time were required? What about the genetics MD workforce, any

considerations regarding the costs of their time? This is certainly not to say that I don't support this initiative; I actually do, but I feel that in order to realistically justify implementation across other health systems, a reflection of the whole "cost" of implementation is needed. This may be out-of-scope for this paper, but suggesting that reduced cost of GS alone means that other centers could feasibly do what was done here is a vast oversimplification. There are some very nice reports describing the layers of barriers (particularly for private payer healthcare systems) that are involved in this implementation context. Here's one example: https://pubmed.ncbi.nlm.nih.gov/40367948/

Another interesting consideration is how much having an 'internal' lab system contributed to the success of this program. Is it advised for future health systems to also build their own CLIA lab? Or, if another ICU system wanted instead to work with an external diagnostic lab for test ordering, would that impact the workflow described here? Maybe this is inconsequential and ties into the cost consideration (e.g. estimate of $3500 total cost) but it could be worth considering especially as related to turnaround time and lab processing when sending samples externally.

Thank you for your comments. Yes, we agree that there still needs to be upfront capital investment and workforce capacity building, but we hope that the long-term clinical and cost saving benefits (which we elaborate further in discussion) can be taken into consideration to justify this upfront investment. We modified the discussion section to address this.

Overall, I do think the authors mostly responded to the critiques. I still think that there could be more to learn about what was unique in this approach that led to their success so that other centers could learn from it. We have known about diagnostic yield, clinical utility, turnaround time for a while now and yet many high-level ICUs still face substantial barriers that I don't think entirely related to just the flat cost of GS itself.

Thank you for your time and valuable feedback on our manuscript.

Reviewer #4:
Remarks to the Author:
I appreciate the author's feedback and the inclusion of the new data tables, I feel it greatly enhances the value of the paper for the audience. This is an important piece of research and will contribute to the body of work in the field of rapid sequencing in critically ill infants/children.

Thank you for taking the time to provide highly valuable feedback on our manuscript.