

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☒	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Not applicable
Data analysis	Softwares used sequencing and base calling: MinKnow distribution (version 22.05.7) Guppy (version 6.1.5) Software for processing: Aligner - minimap2 (version 2.22-r1101). Variant/Methylation caller - Epi2Me workflow wf-human-variation (v1.2.0), CuteSV(v2.0.3) Annotation - AnnotSV(v3.2.3), ClassifyCNV(1.1.1), SpliceAI (1.3.1) Filtering and Selection – Custom scripts (https://github.com/Shruti-BioCode/AJCH_ONT_Diagnostic_Utility)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Provide your data availability statement here.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	The study aims to identify clinically relevant variants in the patients for the purpose of diagnosis without confining to a particular sex group; as such it comprises of approximately equal sex representation - 44% female and 46% male.
Reporting on race, ethnicity, or other socially relevant groupings	Cohort consisted mostly of Arab and Middle Eastern pediatric patients.
Population characteristics	The study focused on pediatric patients aged 9 months to 17 years (mean age = 8 years), with an equal sex distribution, all of whom underwent whole exome genetic testing. The cohort was divided into two groups: Positive (N = 17) and Negative (N = 51). The Positive cohort included patients with confirmed genetic diagnoses, encompassing a diverse array of genomic and epigenomic pathogenic variants, which were used to optimize the workflows. The Negative cohort consisted of patients who did not receive a confirmed diagnosis by exome sequencing, and was used to assess the utility of the optimized workflow.
Recruitment	Parents/ Guardians of patients were approached and consented to participate in the study.
Ethics oversight	his study was reviewed and approved by the Dubai Scientific Research Ethics Committee, Dubai Health Authority (approvals no. DSREC-SR-03/2023_08 and DSREC-08/2024_09).All patients were consented for clinical genetic testing and under an approved de-identified research protocol. A group of patients (N=46) were further consented for additional investigations and for data sharing. T

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The study aims to assess the clinical utility of long-read sequencing in a heterogeneous group of 51 patients with highly suspected monogenic disorders who previously underwent non-diagnostic short-read whole exome sequencing. The study does not involve group comparisons or comparisons with population-level data. Additionally, a cohort of 17 diagnosed patients (as well as an independent dataset from 57 patients with known methylation profiles), with a diverse range of variant types, was used to optimize the sequencing workflows and achieve 100% sensitivity, ensuring the removal of technical issues. Given these objectives, the sample size of 51 patients (negative cohort) is deemed sufficient to meet the goals of the study.
Data exclusions	No data generated in the study was excluded from the analysis
Replication	Not applicable
Randomization	Patients with negative exome sequencing were randomly selected (gender distribution was randomly equal).
Blinding	Not applicable

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involvement in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA