PREDICTING EXOME SEQUENCING OUTCOMES FROM PHENOTYPIC DATA IN AN AFRICAN PATIENT COHORT WITH DEVELOPMENTAL DELAY

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A research report (in the format of a “submissible” paper) submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Genomic Medicine).

Johannesburg

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26 November 2023

Re: Research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, by Ms. Anréé Bresler in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Genomic Medicine).

Dear Examiner,

Thank you for agreeing to examine the research report by Ms. Anréé Bresler (student number 303468) entitled "**Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay**". This research report is being submitted by the candidate in the format of a "submissible" paper as per the Style Guide for Thesis, Dissertation and Research Reports by the University of the Witwatersrand, Faculty of Health Sciences. The research report contributes 33% towards the final mark of the MSc (Med) Genomic Medicine degree for which the candidate is enrolled.

The candidate and her supervisors have chosen to submit the paper to the American Journal of Medical Genetics (AJMG) – Part A. The AJMG– Part A is a peer-reviewed medical journal dealing with human genetics (inherited disorders and birth defects research) published by Wiley. The submissible paper attached is therefore written in accordance with the author guidelines of the stipulated journal. These guidelines have been attached for your reference in the appendices section of this document. The only deviation from these guidelines is that the manuscript has been presented as one complete document (including all tables and figures) for ease of marking, rather than being submitted separately.

The research protocol has also been attached as an appendix for the purpose of providing an extended literature review and further contextualising this study. The protocol has already been assessed and approved by the Faculty of Health Sciences Post-Graduate Assessors committee and is therefore not for examination.

Please refer to the Table on Contents for a complete list of the contents provided in this research report.

Yours sincerely,

Anréé Bresler (Candidate)

Mr. Barry Shingwenyana (Supervisor 1)

Prof. Amanda Krause (Supervisor 2)

Declaration

I, Anréé Bresler, declare that this Research Report (in the format of a “submissible” paper) is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted previously for any degree or examination at any other University.

Anréé Bresler
Student number: 303468

26th day of February in 2024, Johannesburg

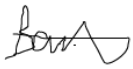
Contribution of the candidate to the paper

Declaration: Student's contribution to articles(s) and agreement of co-author(s)

I, Anréé Bresler, student number 303468, declare that this Research Report, entitled "Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay", is my own work and that I contributed significantly towards research findings presented in the paper intended for publication below.

Signature of Student: 

Date: 26/02/2024



Signature of Supervisor 1:

Mr. Barry Shingwenyana

Date: 26/02/2024

Signature of Supervisor 2:

Prof. Amanda Krause

Date: 26/02/2024

Agreement of co-authors:

By signing this declaration, the co-authors listed below agree to the use of the research findings intended for a published article by the student as part of their Research Report.

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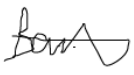
2nd author	Barry Shingwenyana		26/02/2024
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Dedication

This research report is dedicated to my husband, Driaan Bresler, and two daughters, Mia and Emma.

Acknowledgements

I would like to give my deepest thanks to my supervisors for the continued guidance and mentorship throughout this year. To Prof. Amanda Krause, it has been an absolute privilege to learn from your wealth of knowledge and experience. I thank you for the time, guidance and support you have given me during this period. To Barry Shingwenyana, I thank you for all the behind-the-scene administrative tasks and your continued encouragement. At times you truly went out of your way to assist me when needed and I am truly grateful.

To Nadja Louw, thank you for your contribution towards the study design, protocol and data sharing. You were always willing to address my questions and provide additional insight when needed. To Monica Araujo, you have played an invaluable role as a course supervisor and your words of encouragement were always helpful. Thank you to Prof. Zane Lombard for the continuous administrative assistance and guidance.

Lex Dingemans, thank you for the assistance with the PredWES data upload. You have been willing to assist me from the get-go and I always received feedback from you timeously. Nicole Fleischer, I thank you for the technical support with Face2Gene and the detailed data you have provided me with. The assistance I have received from you both have really simplified the data handling and analysis process and for this I am very grateful.

Lastly, I want to give my deepest appreciation to my husband, Driaan Bresler, who have made this year possible and provided me with unwavering support.

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List of Abbreviations

ACMG: American College of Medical Genetics and Genomics

AS: Angelman Syndrome

AMP: Association of Molecular Pathology

CMA: Chromosomal microarray

CLS: Coffin-Lowry Syndrome

CNV: Copy number variants

DECIPHER: Database of Genomic Variation and Phenotype in Humans using Ensembl Resources

DDD-Africa: Deciphering Developmental Disorders in Africa

DDD-UK: Deciphering Developmental Disorders-United Kingdom

DD: Developmental delay

DDG2P: Developmental Disorders Genotype-to-Phenotype database

DS: Down Syndrome

ES: Exome sequencing

F2G: Face2Gene

GDD: Global developmental delay

HREC: Human Research Ethics Committee

HPO: Human Phenotype Ontology

ID: Intellectual disability

MLPT: Machine learning predictive tools

MLPA: Multiplex ligation-dependant probe amplification

MS-MLPA: Methylation sensitive multiplex ligation-dependant probe amplification

NGS: Next generation sequencing

OMIM: Online Mendelian Inheritance in Man

REDCap: Research Electronic Data Capture

SNV: Single nucleotide variants

SD: Standard deviation

VUS: Variant of uncertain significance

Research report in the format of a “submissible” paper

To be submitted to the American Journal of Medical Genetics - Part A

Title page of the manuscript:

Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay.

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Data availability statement

The data that support these findings are available from the 1st author upon reasonable request.

Conflict of interest statement

The authors declare no conflict of interest.

Ethics approval statement.

The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) with ethics clearance number M230686 and is a sub-study of the DDD-Africa study with ethics clearance number M180678.

Patient consent statement.

Participants provided informed consent for the use of their data in a public domain. Participants used for facial photograph analysis provided specific consent for the use of photographs.

Abstract

Global developmental delay (GDD) affects 1 – 3% of the worldwide population with up to 40% of cases due to underlying genetic factors. Exome sequencing (ES) is now recommended as the first-line genetic test for developmental delay (DD), however despite the advances of ES, the diagnostic yield remains limited between 30 – 40%. The use of machine learning predictive tools (MLPT) for phenotyping can potentially play a role in pre-selecting patients for ES and/or improve the phenotype guided analysis of ES data ultimately increasing the ES diagnostic yield.

A data subset of 94 participants from the Deciphering Developmental Disorders (DDD)-Africa study was used to assess the performance of two MLPT, PredWES and Face2Gene (F2G). The study investigated if these MLPT can successfully predict the probability of a positive ES result and/or a genetic diagnosis based on the patient phenotype in an African cohort of participants with DD. This was done by investigating whether there is a correlation between; PredWES scores and ES outcomes, and F2G D-Scores and ES outcomes. In addition, F2G geneticist view was investigated and the list of 30 suggested syndromes with their corresponding gestalt scores, feature scores, and combined scores compared to the ES outcome of participants.

For PredWES, using Human Phenotype Ontology (HPO) data to predict exome positivity, the diagnostic yield for the top 10% of PredWES scores was 44%, lower than the diagnostic yield of 46% seen without any PredWES prioritisation. The F2G D-Score has shown to be a poor predictor of ES outcome; however, the D-Score classification did correctly predict the need for further genetic testing for 77 – 80% of the ES positive group. F2G identified the correct genetic syndrome in 46% of the ES positive participants with a top ten accuracy of 35%. F2G provided syndrome suggestions with high scores for 39% of the ES negative participants.

Overall, PredWES was shown to be ineffective in pre-selecting participants suitable for ES. The F2G performance was promising but still poorer than studies conducted on mainly European cohorts. The study provided valuable insight into the use of PredWES and F2G in an African setting and highlights the need for more diverse MLPT algorithm training and continued DD research in an African context.

Key words: Developmental Delay (DD), Exome Sequencing (ES), Face2Gene, Machine learning predictive tools (MLPT), PredWES

1. Introduction

Global developmental delay (GDD) or intellectual disability (ID) refers to a condition where children do not achieve their developmental milestones in an age-appropriate manner (Fieggen et al., 2019). Children with GDD experience delay in at least two or more functional domains – gross motor development, fine motor development, cognitive development, social and emotional development or speech and language development. Global developmental delay is estimated to affect 1 – 3% of the worldwide population (Mithyantha et al., 2017) with the prevalence in South-Africa poorly documented (Fieggen et al., 2019).

Up to 40% of developmental delay (DD) cases are due to underlying genetic factors with monogenic causes contributing up to 10% of cases and chromosomal abnormalities contributing up to 30% of cases (Miclea et al., 2015). Genetic testing for the diagnosis of DD has advanced over the years progressing from G-banded karyotyping to chromosomal microarray (CMA) and next generation sequencing (NGS) gene panels as diagnostic tools. The diagnostic yield of testing has been increasing as these new technologies are adopted and their resolution improves (Miller et al., 2010).

Despite these advances, many patients still remain undiagnosed and find themselves facing a diagnostic odyssey. This can largely be attributed to the phenotypic and genetic heterogeneity of DD, as reported in the Deciphering Developmental Disorders-United Kingdom (DDD-UK) study. The DDD-UK addressed this by utilising exome sequencing (ES), an approach which tests beyond the restricted genomic range of targeted testing strategies (Wright et al., 2015).

Exome sequencing (ES) covers a much broader genomic range by analysing all protein coding regions within the genome, consequently increasing the ability to identify causative genetic variants (Han & Lee, 2020). The current yield for ES ranges between 30 to 40% (Slavotinek et al., 2023; Srivastava et al., 2019; Wright et al., 2023); therefore, ES is now recommended by the American College of Medical Genetics and Genomics (ACMG) as the first-line genetic test for DD and should

preferably also include copy number analysis (Manickam et al., 2021). However, ES still remains limited in detecting methylation abnormalities, deep intronic variants, cryptic splice site variants, low level mosaicism and repeat expansions (Burdick et al., 2020; Corominas et al., 2022; Sanders et al., 2020). In addition, the diagnostic odyssey is further exacerbated by the limited resources in low- and middle-income countries (Porras et al., 2021) as well as the challenge of variant interpretation and classification within an African setting. A challenge which is attributed to the lack of African genomic data in public databases and the large complexity and variation within the African genome (Baine-Savanhu et al., 2023; Lumaka et al., 2022).

The DDD-UK study performed ES on 13 449 previously undiagnosed probands with the aim to advance clinical genetics practice for children with DD. The study played an integral role in DD gene discovery and led to the development of a comprehensive database, Developmental Disorders Genotype-to-Phenotype database (DDG2P), with over 1400 published genes recognised to be associated with DD (Wright et al., 2015, 2023). The success of DDD-UK justified the start of a local ongoing study, Deciphering Developmental Disorders in Africa (DDD-Africa), which aims to explore the genetic aetiology of DD in an African population therefore addressing the lack of phenotypic and genomic data and the associated challenges of diagnosis (https://h3africa.org/index.php/ddd-africa/).

The analysis of dysmorphology and phenotypic variation plays an integral role in the identification of variants in genomic data and should complement the process of genetic testing by ensuring genotype-phenotype correlation (Köhler et al., 2014). The advent of the Human Phenotype Ontology (HPO), a standardised vocabulary of phenotypic abnormalities (Robinson et al., 2008), enabled the development of machine learning predictive tools (MLPT) such as PredWES and Face2Gene (F2G), used in this study. Such MLPT can be valuable for assisting diagnosis at a point-of-care level as well as with the phenotype guided analysis of ES data (Gurovich et al., 2019; James et al., 2020).

PredWES is a free online predictive tool that estimates the probability of a positive ES outcome based on the patients' clinical phenotype (https://humandiseasegenes.nl/predwes). The developers of PredWES report a significant increase in diagnostic yield when only testing patients with high PredWES scores (Dingemans et al., 2022). Thus, Dingemans et al. (2022) suggests PredWES could be used to pre-select patients suitable for ES, therefore reducing unnecessary genetic testing and increasing the ES diagnostic yield.

Face2Gene (FDNA Inc.), powered by an algorithm referred to as DeepGestalt, is a free online machine learning community-driven tool that assists in the phenotypic evaluation of patients with DD (https://www.face2gene.com/). It has a number of different tools. The F2G Paediatrician View utilises Facial D-Score technology which, based on a facial photograph, predicts the level of dysmorphism of paediatric patients to determine if further genetic assessment is required (Pantel et al., 2020). The F2G Geneticist View analyses facial photographs of paediatric and adult patients subsequently providing a list of 30 suggested possible diagnoses of genetic syndromes, each with a ranked gestalt score, predicted to be associated with the dysmorphism detected in the facial image. In addition, a feature score can also be obtained by entering patient HPO terms resulting in a combined prediction and score using gestalt and feature scores (Gurovich et al., 2019).

Although these MLPT have the potential to guide genetic testing and diagnosis, they do still have notable limitations, especially within an African context. Lumaka et al. (2017) found that in comparison to African clinicians, European clinicians are less likely to correctly identify dysmorphism in African patients, therefore demonstrating the subjective aspect of dysmorphology. As PredWES is a tool that relies on the ability of a clinician to correctly assign phenotypes, the subjective nature of the process and the impact of patient and clinician ethnicity are likely limitations of PredWES. In addition, PredWES and F2G are both trained on mainly European cohorts (Dingemans et al., 2022; Gurovich et al., 2019) bringing into question the current value of these tools when applied to African patients (Lumaka et al., 2017, 2022). This limitation of F2G has been demonstrated by Lumaka et al. (2017) where F2G correctly identified Down

Syndrome (DS) in only 36.8% of African DS participants in comparison to 80% of Flemish DS participants.

The aim of this study was to investigate whether MLPT can be used to assist in successfully predicting the probability of a positive exome sequencing result and/or a genetic diagnosis solely based on the patient phenotype in an African cohort of participants with DD from the DDD-Africa cohort. This was done by determining if PredWES and F2G D-Score can correctly predict a positive ES result for participants with DD. In addition, the predictive value of the F2G gestalt score, feature score and combined score was determined when compared to the ES molecular diagnosis in participants with DD.

2. Methods

2.1. Editorial policies and ethical considerations

The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) with ethics clearance number M230686. The study is a sub-study of the DDD-Africa study with ethics clearance number M180678. As participants were children with developmental delay, parental consent was given and assent obtained where possible. The consent included the use of photographs for medical record keeping, teaching, publication or neither. In addition, consent for the use the collected data in public domains was also accounted for. All participants included in this study provided informed consent for the use of their data in a public domain. Only participant with consent for the use of photograph for teaching or publication were used for photograph analysis. Parental consent was provided for photo publication of participant D3S.0016.01 seen in Appendix A and B.

2.2. Study sample:

The current study is a sub-study of the DDD-Africa study. The DDD-Africa cohort consists of 350 probands recruited with at least one of four inclusion criteria A) ID (moderate to profound), B) multiple malformation in 2 or more body systems, C) one major malformation and 3/more well documented minor anomalies or D) mild to

moderate ID with dysmorphic features. Individuals were excluded from the DDD-Africa study if they had a known condition with no strong evidence for a monogenic cause, suspicion of an acquired brain lesion or suspected multifactorial/environmental cause. The DDD-Africa study is ongoing and at the time of initiation of this sub-study, clinical phenotype data and ES results were available for 130 probands.

For the current study, only DDD-Africa probands with DD/ID were included (categories A and D as described above). Those with structural abnormalities only and no DD/ID were excluded (B and C as described above). In addition, only participants that provided consent for the use of their data in a public domain were included. As a result, there was a reduction from the initial 130 probands. Ninety-four participants were included in the PredWES analysis. Of these, 26 did not provide photo consent resulting in the inclusion of only 68 participants in the F2G analysis. F2G digitises the face into vectors (Gurovich et al., 2019), therefore no participant photos were stored in the F2G database.

2.3. Data handling and analysis:

The data subset consisted of 3 components as described below:

1) Participant HPO terms as documented in DDD-Africa research files and patient files created on Research Electronic Data Capture (REDCap) hosted at the University of the Witwatersrand, a freely available secure electronic data capture tool (Harris et al., 2019).

All participants in the DDD-Africa study underwent clinical phenotyping by various clinicians and the phenotyping information, as summarised in Table 1, was obtained from both the REDCap DDD-Africa research file and the REDCap patient file for each participant.

Table 1: Core systems and phenotypes obtained from the REDCap DDD-Africa research files and REDCap patient files respectively.

REDCap DDD-Africa research files	REDCap patient files
Level of ID	Cardiac system
Presence of motor delay	Skeletal system
Presence of hypertonia/hypotonia	Urogenital system
Presence of seizures	Brain and central nervous system
Delayed or absent speech,	Renal system
Presence of microcephaly/macrocephaly (±3 SD)	Chest and respiratory system
Presence of underweight/overweight (±3 SD)	Abdominal and gastrointestinal tracts
Presence of tall/short stature (±3 SD)	Ectodermal
Presence of undergrowth/overgrowth (±3 SD)	Head and neck
Presence of a hearing impairment	Cleft palate
Presence of a vision impairment	

Abbreviations: SD, standard deviation.

Both minor and major abnormalities were included as the exclusion of minor abnormalities might have resulted in the loss of important phenotypic data needed for accurate analysis by the MLPT. This approach was deemed appropriate as PredWES reportedly determines which features are the most pertinent for analysis, therefore reducing the need to distinguish between minor and major abnormalities. HPO terms and corresponding HPO codes were already assigned in the REDCap DDD-Africa research files. For the REDCap patient files, clinical phenotype information was assigned the most exact HPO term and corresponding HPO code by the researcher. A Medical Geneticist was consulted in a few cases where the researcher was unsure of the most accurate HPO description. The phenotypic information was collated using a Microsoft Excel spreadsheet.

2) Participant facial photographs in JPEG files stored in a secure DDD-Africa Google Drive.

Participant photographs were taken at the time of recruitment and the researcher subsequently identified the best quality frontal facial photograph for analysis in this study. A photo was considered of sufficient quality if it included the entire frontal face and was well focused.

3) ES genomic summary data for prioritised variants as documented in a secure DDD-Africa Google Drive.

For the DDD-Africa cohort, ES analysis has been done for single nucleotide variants (SNV) and copy number variants (CNV). Variants have been classified according to the American College of Medical Genetics and Genomics and Association of Molecular Pathology (ACMG/AMP) guidelines (Richards et al., 2015) by a multi-disciplinary team reviewing the clinical and molecular data. For the current study, a participant was considered ES positive if a pathogenic or likely pathogenic variant was found that was consistent with the clinical phenotype and ES negative if no variant or a benign, likely benign or variant of uncertain significance (VUS) was found.

2.3.1. PredWES analysis

The developers of PredWES at the Radboud University Medical Center (Dingemans et al., 2022) were approached and an agreement was made to process the participants' phenotype information in bulk, rather than the researcher having to submit each patient as an individual request. An anonymised Microsoft Excel spreadsheet with the DDD-Africa participant identifier code and corresponding HPO codes for the 94 participants was sent to the PredWES developers. The PredWES developers returned a PredWES score (probability of a positive ES result) ranging from 0 - 1 for each participant using the Microsoft Excel spreadsheet. The PredWES ES predictive ability, defined by the strength of association between the PredWES score and the ES outcome (positive/negative) was determined using a Point-Biserial correlation coefficient. In addition, patients were ranked according to their PredWES scores permitting a comparison between the highest- and lowest 10% of scores as well as the diagnostic yield associated with the top 10% of scores. Lastly, a Point-Biserial correlation coefficient was used to determine the correlation between the PredWES score and microcephaly or macrocephaly respectively as well as the PredWES score and hypotonia or hypertonia respectively, allowing for comparisons in line with Dingemans et al. (2022).

2.3.2. Face2Gene analysis

A total of 68 participant facial photographs and HPO terms were uploaded onto F2G generating a F2G case number for each participant. As the goal was to investigate the ability of F2G to detect facial dysmorphism of African patients using a facial photograph, HPO terms for head and neck abnormalities were not uploaded. This was done to eliminate any bias introduced by including these in the phenotypic descriptions. Sixty-seven of the participants were paediatric and therefore uploaded under the F2G paediatric view allowing the calculation of a D-Score, gestalt score, feature score and combined score. One participant was an adult aged 52 and therefore uploaded under the F2G geneticist view allowing the calculation of a gestalt score, feature score and combined score. Prior to upload, the developers of F2G, FDNA™, were approached and agreed to provide bulk feedback for the participants using Microsoft Excel spreadsheets. The feedback was based on the latest F2G algorithm version at the time, DeepGestalt version 22.3.0. The Microsoft Excel spreadsheet contained the F2G case number with corresponding D-Score (score of 0 - 1) for the 67 paediatric participants. The D-Score classification (low, unknown and high) was not included in the bulk data and was therefore manually obtained from the F2G website (https://app.face2gene.com). Refer to Appendix A for an example of the D-Score categorical classification. In addition, the Microsoft Excel spreadsheet contained the top 30 suggested rare syndromes for all 68 participants. Each suggested syndrome had a corresponding gestalt score with ranking, feature score, and combined score with ranking. Refer to Appendix B for an example of the F2G geneticist view output as displayed on the F2G website (https://app.face2gene.com). The Ultra-Rare function, which deals with ultra-rare conditions not currently trained by F2G, was not investigated in this study. The Ultra-Rare function allows patients to be matched to other patients based on their syndromic similarities, ultimately suggesting possible molecular diagnosis despite the syndrome not being previously trained by F2G (Hsieh et al., 2022).

The F2G D-score ES predictive ability, defined by the strength of association between the D-Score (0 – 1) and the ES outcome (positive/negative) was determined using a Point-Biserial correlation coefficient. Furthermore, the D-Score classification

(low/unknown significance/high) was compared between the ES positive and ES negative group using a contingency table and Chi-square analysis.

The genetic syndrome diagnosis of ES positive participants was compared to the top 30 F2G suggested syndromes. For participants where F2G identified the correct syndrome, the corresponding gestalt score with ranking, feature score, and combined score with ranking was documented and studied. For the participants where F2G did not identify the correct genetic syndrome, the syndrome in question was compared with a list of trained syndromes (provided by F2G, FDNA™) to determine if DeepGestalt (version 22.3.0) was trained for this syndrome. Lastly, ES negative participants with gestalt, feature or combined scores above 0.66 were documented and studied.

Statistical analysis was performed using Microsoft Excel and statistical significance accepted at a *p-value* of 0.05. The researcher remained blinded to the genomic summary data and ES outcome till after data from PredWES and F2G was received. This was to avoid any biases that might be introduced in the process of data handling.

3. Results

The data subset represented a total of 94 participants of which 43 (46%) were ES positive participants and 51 (54%) ES negative participants. The study population mostly consisted of paediatric participants with a median age of 10 (5-18) years and one adult participant aged 52 years. Overall, a median of 13 HPO terms were documented for each participant with a median of 14 for ES positive participants and 12 for ES negative participants. As illustrated in Figure 1, there was marked phenotypic heterogeneity observed across the study population with each participant having a sub-set of different features, but all having DD/ID.

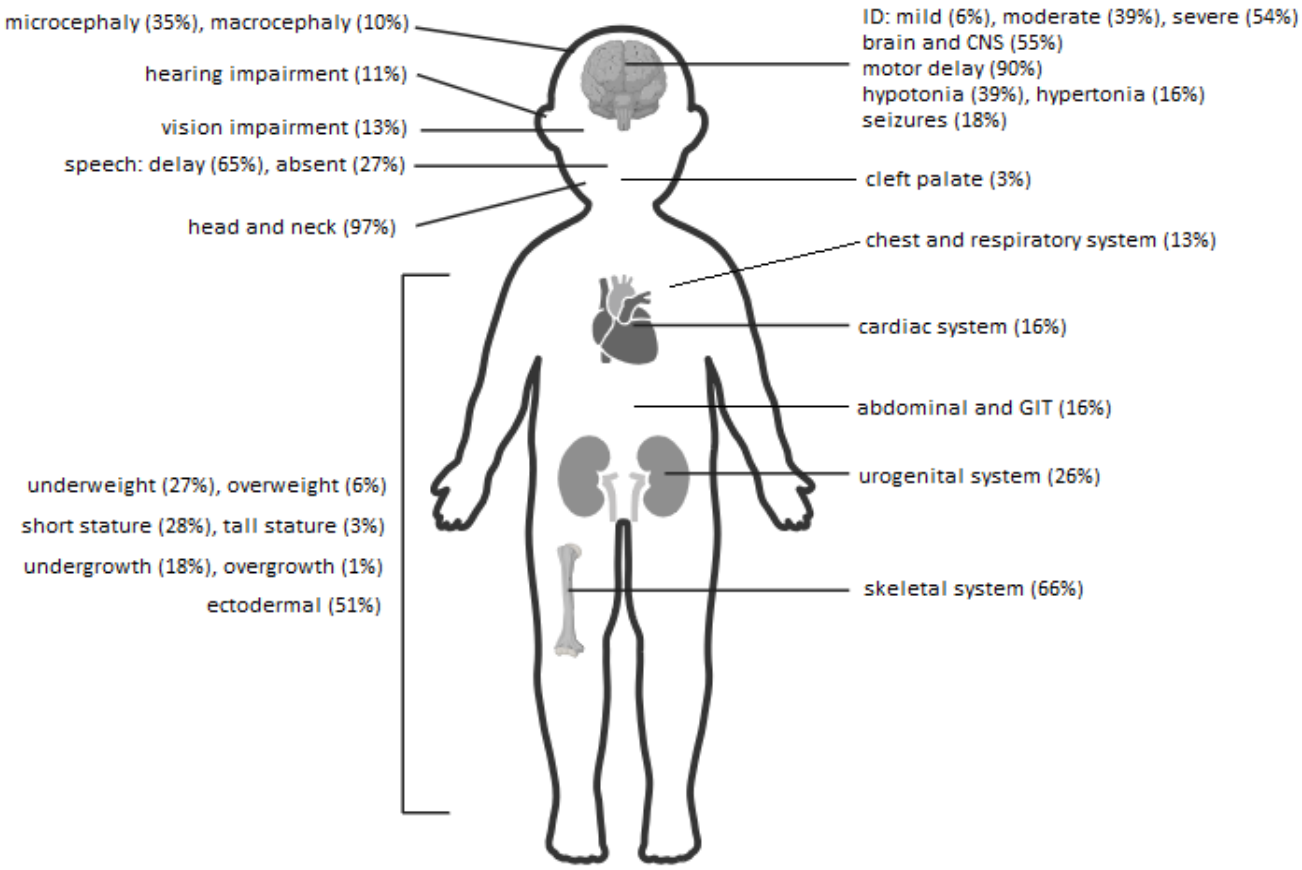

Figure 1: Phenotypic heterogeneity in the study population. Participants presented with a wide range of phenotypic anomalies (both minor and major abnormalities) across various body systems. Percentage (%) refers to the proportion of participants within the study population that has the particular phenotypic anomaly.

Supplement A summarises the phenotypic heterogeneity observed when comparing the ES positive and ES negative group. Of particular interest was the unexpected

observation of microcephaly being lower in the ES positive group (23%) compared to the ES negative group (43%). The analysis was however limited by a small sample size and none of the differences has shown to be statistically significant.

3.1. PredWES performance

All 94 participants were included in the PredWES analysis. The overall median PredWES score was 0.31 with the score for the ES positive group (0.30) similar to that of the ES negative group (0.32) (Figure 2a). This finding was noteworthy as PredWES indicates the probability of a positive ES result; therefore, one would expect the score for the ES positive group to be higher in comparison to the ES negative group. There was also no significant correlation between the number of HPO terms and PredWES score (Figure 2b).

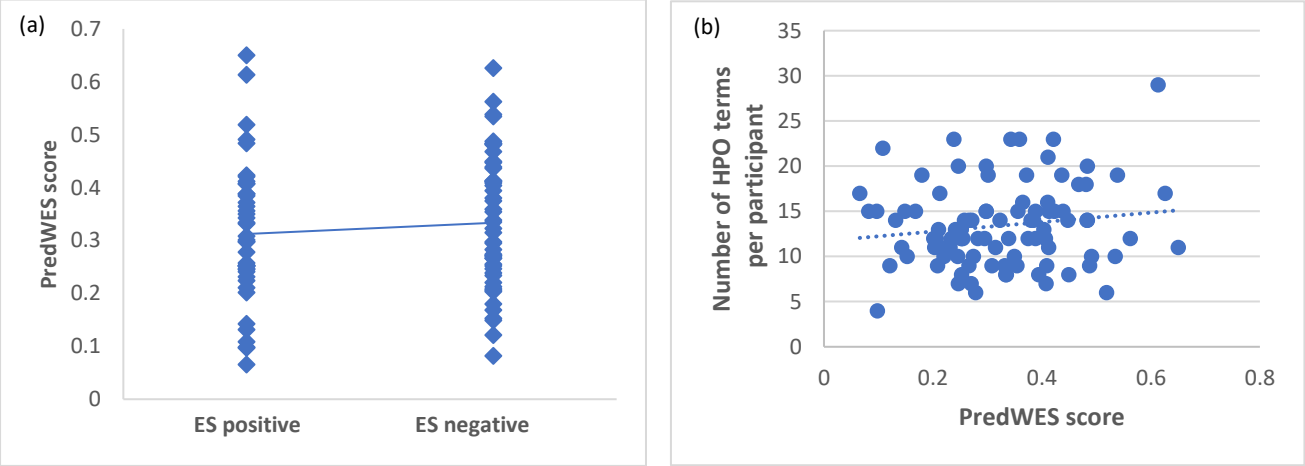

Figure 2: (a) Point-Biserial correlation between PredWES score and ES outcome. No significant correlation was observed with r = 0.083 and p > 0.05. **(b) Correlation between number of HPO terms and PredWES score.** No significant correlation was observed with r = 0.146 and p > 0.05.

For the top 10% of PredWES scores (range: 0.487 - 0.650, median: 0.54), 4 of the 9 participants had a positive ES result which equates to a diagnostic yield of 44%. For the bottom 10% of PredWES scores (range: 0.148 - 0.065, median: 0.11), 3 of the 9 participants had a negative ES result indicating no notable difference in number of ES positive participants when comparing the top and bottom 10% of PredWES scores.

Participants with microcephaly had a median PredWES score of 0.43 and individuals with a normal head circumference a median of 0.25. Participants with microcephaly

were statistically more likely to receive a higher PredWES score compared to those with a normal head circumference ($r = 0.62$, $p < 0.05$). Although this finding is consistent with that of Dingemans et al. (2022), it is unexpected in this study population as the ES negative group had a higher number of participants with microcephaly compared to the ES positive group (Supplement A). For hypertonia, analysis showed a statistically significant difference in PredWES scores between participants with hypertonia (0.36) and participants without hypertonia (0.30), the correlation was however weak ($r = 0.21$, $p < 0.05$). PredWES scores did not correlate with the presence/absence of macrocephaly ($r = 0.07$, $p > 0.05$) or the presence/absence of hypotonia ($r = 0.06$, $p > 0.05$).

3.2. Face2Gene Performance

Sixty-eight participants were included in the F2G assessment as they had consented to photo usage from the parent study. Of these, 35 (51%) had an ES positive result and 33 (49%) had an ES negative result. One frontal facial photograph was uploaded per participant with a median of 12 HPO terms per participant.

3.2.1. D-Score analysis

For each of the 67 paediatric participants, the F2G D-Score (based only on the facial photograph) was obtained as a score range (0 – 1) as well as a categorical classification (low, unknown significance and high). There was no significant difference in the D-Score when comparing the ES positive group D-Score (median = 0.92) and the ES negative group D-Score (median 0.88) ($r = 0.18$, $p > 0.05$). There was a small difference when assessing the categorical classifications with 77% of the ES positive group classified as high in comparison to only 59% of the ES negative group (Table 2). Chi-square analysis however showed no statistical significance when comparing the D-Score classifications of the ES positive and ES negative group ($x^2 = 3.32$, $p > 0.05$).

Table 2: Comparison of D-Score classifications between the ES positive group and ES negative group.

Group as per exome sequencing outcome	Number of participants classified under the respective D-Score categories		
	Low (D-Score: 0 - 0.33)	**Unknown Significance** (D-Score: 0.34 – 0.66)	**High** (D-Score: 0.67 – 1)
ES positive n=35	7 (20%)	1 (3%)	27 (77%)
ES negative n=32	9 (28%)	4 (13%)	19 (59%)

3.2.2. Geneticist view analysis for ES positive group

For 26 of the ES positive participants, the top-30 F2G suggested syndromes were compared to the ES genetic diagnosis of the respective participants. F2G identified the correct syndrome in 12 of the 26 participants (46%) as summarised in Table 3. Based on the gestalt position, in 9 out of the 26 participants (35%) the correct diagnosis was identified within the top 10 listed syndromes with all of these ranked in position 1 to 5. Although the remaining 3 participants had the correct syndrome identified, the scores were very low and it is unlikely a clinician would consider suggestions with such low rankings in practice. For these 3 participants, the top 10 incorrect suggestions that ranked above the correct syndrome can perhaps be considered false positive results.

Table 3: Summary of Face2Gene D-Score, as well as gestalt, feature and combined results for Exome Sequence positive participants with a syndrome correctly identified by Face2Gene.

	Exome sequencing outcome			D-Score	Gestalt, feature and combined results						
¥Participant	Gene	SNV/CNV	Confirmed Genetic Diagnosis		†Gestalt ranking	†Gestalt score	†Gestalt score classification	‡Feature score	‡Feature score classification	§ Combined ranking	§Combined score
D3S.0117.01.1	*NSD1*	SNV	Sotos Syndrome (OMIM 117550)	HIGH	1	0.83	HIGH	0.64	MED	1	1
D3S.0016.01.1	*PGAP3*	SNV	Hypophosphatasia with Intellectual Disability Disorder Syndrome 4 (HPMRS) (OMIM 615716)	HIGH	1	0.84	HIGH	0.43	MED	1	0.51
D3S.0051.01.1	*CHD7*	SNV	*CHD7* Disorder (CHARGE Syndrome) (OMIM 214800)	HIGH	1	0.20	LOW	0.45	MED	1	0.51
D3S.0118.01.1	*FLNA*	SNV	*FLNA*-Related Disorders (OPD1) (OMIM 311300)	HIGH	1	0.46	MED	0.42	MED	1	0.51
D3S.0119.01.1	*RPS6KA3*	SNV	*RPS6KA3*-Related Intellectual Disability (Coffin-Lowry Syndrome) (OMIM 303600)	HIGH	1	0.99	HIGH	0.37	MED	2	0.5
D3S.0014.01.1	*SETBP1*	SNV	Schinzel-Giedion Midface Retraction Syndrome (OMIM 269150)	HIGH	2	0.29	LOW	0.39	MED	3	0.26
D3S.0111.01.1	*PTEN*	SNV	*PTEN* Hamartoma Tumour Syndrome (Cowden Syndrome) (OMIM 615107)	HIGH	2	0.19	LOW	0.43	MED	4	0.25
D3S.0077.01.1	*SYNGAP1*	SNV	MRD5 (Intellectual developmental disorder, autosomal dominant, 5) (OMIM 612621)	HIGH	5	0.23	LOW	0.37	MED	9	0.1
D3S.0071.01.1	*EHMT1*	SNV	Kleefstra Syndrome 1 (OMIM 610253)	HIGH	5	0.13	LOW	0.54	MED	6	0.17
D3S.0050.01.1	*CHD7*	SNV	*CHD7* Disorder (CHARGE Syndrome) (OMIM 214800)	LOW	14	0.09	LOW	0.32	MED	27	0.04
D3S.0064 .01.1	*SMAD4*	SNV	Myhre Syndrome (OMIM 139210)	HIGH	25	0.16	LOW	0.55	MED	12	0.09
D3S.0047.01.1	*ARID1B*	SNV	*ARID1B*-Related Disorder (Coffin-Siris Syndrome) (OMIM 135900)	HIGH	25	0.07	LOW	0.58	MED	5	0.19

¥Participant ranking based on combined score. † Gestalt is based on the facial photograph only. ‡ Feature is based on the HPO terms only. §Combined refers to a combination of gestalt and feature. Abbreviations: CNV, copy number variants; OMIM, Online Mendelian Inheritance in Man; SNV, single nucleotide variants.

In contrast, F2G was unable to identify the correct diagnosis for 14 (54%) of the 26 participants (Table 4). F2G (DeepGestalt algorithm version 22.3.0) was however not trained for 5 of the 14 genetic syndromes (36%) listed in Table 4 which explains why F2G did not correctly identify these particular syndromes.

In addition, it seems F2G was less likely to detect the correct syndrome if the facial D-Score was low. Within the ES positive group, 5 participants had a low D-Score classification. F2G was not able to detect the correct genetic syndrome for 4 of the 5 participants (D3S.0024.01.1, D3S.0052.01.1, D3S.0056.01.1 and D3S.0086.01.1) (Table 4). For one participant (D3S.0050.01.1), F2G did detect the correct syndrome, but with a very low gestalt score and ranking (Table 3).

Two ES positive participants (D3S.0085.01.1, D3S.0101.01.1) had CNVs without well-defined syndromes or corresponding Online Mendelian Inheritance in Man (OMIM) numbers and were therefore analysed separately (Table 5). The gene regions affected by the CNV were investigated on DECIPHER in order to identify the OMIM genes most likely associated with disease. The list of OMIM genes were then compared to the top ten listed genes on F2G. For both of the participants, there was no overlap between the OMIM genes and the genes suggested by F2G.

3.2.3. Geneticist view analysis for ES negative group

For the ES negative group, 13 out of 33 participants (39%) had one or more syndrome/s suggested with either a high gestalt, feature or combined score above 0.66 (Table 6). Three participants (9%) received a high gestalt score, 9 participants (27%) received a high feature score and 1 participant (3%) received a high combined score.

Table 4: Exome sequence positive participants with a genetic diagnosis which was not identified by Face2Gene.

Participant	D-Score	Confirmed Genetic Diagnosis	Alternative title or synonym name[†]	Gene Region	SNV/ CNV	Genetic Syndrome trained on F2G
D3S.0013.01.1	UNKNOWN SIGNIFICANCE	Smith-Kingsmore Syndrome (OMIM 616638)	*NA*	*MTOR*	SNV	Yes Smith-Kingsmore Syndrome (OMIM 616638)
D3S.0024.01.1	LOW	Tatton-Brown Rahman Syndrome (OMIM 615879)	*NA*	*DNMT3A*	SNV	Yes Tatton-Brown Rahman Syndrome (OMIM 615879)
D3S.0034.01.1	HIGH	*CHD7* Disorder (CHARGE Syndrome) (OMIM 214800)	*NA*	*CHD7*	SNV	Yes CHARGE Syndrome (OMIM 214800)
D3S.0043.01.1	HIGH	Syndromic Neurodevelopmental Disorder with Corpus Callosum, Axon, Cardiac, Ocular and Genital Defects (OMIM 618929)	Agenesis of corpus callosum, cardiac, ocular and genital Syndrome (ACOGS) (OMIM 618929)	*CDH2*	SNV	No[‡]
D3S.0052.01.1	LOW	*MECP2*-Related Disorder	Rett Syndrome; RTT (OMIM 312750)	*MECP2*	SNV	Yes Rett Syndrome; RTT (OMIM 312750)
D3S.0053.01.1	HIGH	*AUTS2* Syndrome (OMIM 607270)	Intellectual developmental disorder, autosomal dominant 26*/MRD26* (OMIM 616521)	*AUTS2*	SNV	No[‡]
D3S.0056.01.1	LOW	PLP1 disorder (Pelizaeus-Merzbacher disease (OMIM 312080) to spastic paraplegia 2 (OMIM 312920)		*PLP1*	SNV	Yes Pelizaeus-Merzbacher Disease; PMD (OMIM 312080)
D3S.0058.01.1	HIGH	Neuronal Ceroid Lipofuscinosis 7/CNL7 (OMIM 610951)		*MFSD8*	SNV	No[‡]

Table 4 (continue)

D3S.0070.01.1	HIGH	Wilms Tumour, Aniridia, Genital Anomalies and Mental Retardation (WAGR) Syndrome (OMIM 194072)	Chromosome 11q13 deletion Syndrome (OMIM 194072)	67 genes (8.78 Mb deletion)	CNV	No‡
D3S.0082.01.1	HIGH	Zhu-Tokita-Takenouchi-Kim Syndrome/ZTTK (OMIM 617140)		*SON*	SNV	Yes ZTTK Syndrome; ZTTKS (OMIM 617140)
D3S.0086.01.1	LOW	*STXBP1*-Related Disorder (OMIM 602929)	STXBP1 Encephalopathy with Epilepsy Epileptic Encephalopathy, Early Infantile/EIEE4 (OMIM 612164)	*STXBP1*	SNV	Yes Epileptic Encephalopathy, Early Infantile (OMIM 612164)
D3S.0094.01.1	HIGH	*DDX3X*-Related Neurodevelopmental Disorder (OMIM 300958)	Snijders Blok type, MRXSSB (OMIM 300958)	*DDX3X*	SNV	Yes Intellectual developmental disorder, X-linked Syndrome, Snijders Blok type, MRXSSB (OMIM 300958)
D3S.0107.01.1	HIGH	Intellectual Disability with Pigmentary Mosaicism	Intellectual developmental disorder, x-linked, syndromic, with pigmentary mosaicism and coarse facies (MRXSPF) (OMIM 301066)	*TFE3*	SNV	No‡
D3S.0124.01.1	HIGH	*PACS1* Neurodevelopmental Disorder (OMIM 615009)	Schuurs-Hoeijmakers Syndrome (OMIM 615009)	*PACS1*	SNV	Yes Schuurs-Hoeijmakers Syndrome; SHMS (OMIM 615009)

†Alternative titles and synonym syndrome names were searched for on OMIM and GeneReviews as Face2Gene might have the syndrome trained under a different title or synonym name. ‡ The genetic syndrome, alternative title or synonym name has not been trained on DeepGestalt version 22.3.0. Abbreviations: CNV, copy number variants; F2G, face2Gene; OMIM, Online Mendelian Inheritance in Man; SNV, single nucleotide variants.

Table 5: Summary analysis of participants with copy number variants without well-defined syndromes.

Participant	D-Score	Molecular diagnosis	Gene region	OMIM genes within the region	F2G top genes suggested
D3S.0085.01.1	LOW	[GRCh38/hg38] 12p13.33p13.31(1262908_5647947)x1	61 genes (4.39Mb deletion)	*CACNA2D4, CACNA1C, TULP3, CCND2, FGF23, C12orf4, AKAP3, NDUFA9, KCNA1, KCNA5*	*COMT, FMR1, ACTA2, H19, APP, CAT, MLH1, ELN, ERCC4, GABRD, GABRB3, GP1BB, GH2, MSX1, CCLS, NFIX, SKI, INS, CD4, TERT*
D3S.0101.01.1	HIGH	[GRCh38/hg38] 17q22q23.1(53822906_58756569)x1	64 genes (4.39 Mb deletion) *NOG* likely causative gene	*COX11, NOG, DGKE, MRPS23, VEZF1, SRSF1, EPX, MKS1, MPO, TSOAP1, RNF43, TEX14, RAD51C*	*KANSL1, COMT, CALCA, MLH1, ERCC4, GABRB3, GP1BB, INS, ARTX, CDKL5, SLC9A6, ARX, PORCN, HUWE1, SMARCA2, NRXN1, RNU4ATAC, TRIO, TCF4*

Abbreviations: F2G, Face2Gene; OMIM, Online Mendelian Inheritance in Man.

Table 6: Summary of syndrome/s suggested by Face2Gene based on high gestalt, feature or combined scores for exome sequence negative participants.

Participant	Syndrome/s suggested based on a high †gestalt score (>0.66)	†Gestalt score	†Gestalt ranking	Syndrome/s suggested based on a high ‡feature score (>0.66)	‡Feature score	Syndrome/s suggested based on a high §combined score (>0.66)	§Combined score	§Combined ranking
D3S.0019.01.1	None			Phelan-McDermid Syndrome (OMIM 606232)	0.68	None		
D3S.0027.01.1	None			Phelan-McDermid Syndrome (OMIM 606232)	0.74	None		
D3S.0030.01.1	Angelman Syndrome (OMIM 105830)	0.72	1	None		None		

Table 6 (continue)

D3S.0061.01.1	None			Phelan-McDermid Syndrome (OMIM 606232) Rubinstein-Taybi Syndrome (OMIM 180849)	0.72 0.69	None		
D3S.0069.01.1	None			Phelan-McDermid Syndrome (OMIM 606232) Saethre-Chotzen Syndrome (OMIM 101400) Rubinstein-Taybi Syndrome (OMIM 180849)	0.82 0.78 0.76	None		
D3S.0083.01.1	None			None		Angelman Syndrome (OMIM 105830)	1	1
D3S.0088.01.1	Velocardiofacial Syndrome (OMIM 192430)	0.91	1	None		None		
D3S.0091.01.1	None			Witteveen-Kolk Syndrome (OMIM 613406) Rubinstein-Taybi Syndrome (OMIM 180849)	0.83 0.73	None		
D3S.0096.01.1	None			Phelan-McDermid Syndrome (OMIM 606232)	0.8	None		
D3S.0113.01.1	None			Microphthalmia isolated with coloboma (OMIM 615145)	0.69	None		
D3S.0115.01.1	None			Seckel Syndrome (OMIM not specified by F2G)	0.75	None		
D3S.0126.01.1	Coffin-Lowry Syndrome (OMIM 3030600)	0.97	1	None		None		
D3S.0127.01.1	None			Phelan-McDermid syndrome (OMIM 606232) Hermansky-Pudlak Syndrome (OMIM not specified by F2G)	0.71 0.69	None		

† Gestalt is based on the facial photograph only. ‡ Feature is based on the HPO terms only. §Combined refers to a combination of gestalt and feature. Abbreviations: OMIM, Online Mendelian Inheritance in Man.

4. Discussion

The use of machine learning predictive tools (MLPT) such as PredWES and F2G show great potential to guide genetic testing and diagnosis (Dingemans et al., 2022; Gurovich et al., 2019; James et al., 2020). There are however few studies available investigating the utility of these tools within an African setting. In this study, the performance of PredWES and F2G were investigated in a cohort of African participants with DD where phenotypic as well as ES data were available.

PredWES is designed to predict the probability of a positive ES outcome for patients with neurodevelopmental disorders based on their phenotypic presentation. Dingemans et al. (2022) proposes PredWES scores can be used to prioritise patients suitable for ES, consequently increasing the diagnostic yield and reducing the burden of unnecessary genetic testing. They reported a doubling in diagnostic yield (from 26% to 56%) when testing only the patients within the top 10% of PredWES scores. The PredWES score range for the top 10% is however relative to the cohort and was not reported by Dingemans et al. (2022), making a direct comparison with the current study challenging.

According to Dingemans et al. (2022), the PredWES probability is a very accurate estimate of the actual diagnostic yield. For the top 10% of PredWES scores, the median score of 0.54 in this study should therefore translate to a diagnostic yield of 54%. The actual diagnostic yield for the top 10% was however 44%, bringing into question the reported association between PredWES score and diagnostic yield. In addition, the diagnostic yield for the top 10% (44%) was lower than the diagnostic yield without any PredWES prioritisation (46%). Therefore, the improved diagnostic yield reported by Dingemans et al. (2022) was not replicated in this study. In addition, there was no notable difference in PredWES scores between the ES positive and ES negative groups indicating that PredWES could not differentiate between these two groups.

The poor performance of PredWES could be due to bias within the PredWES algorithm. Dingemans et al. (2022) reported microcephaly and abnormal muscle tone

to be the only features showing a clear positive correlation with a positive ES result and consequently (due to algorithm training) correlated with a higher PredWES score. This explains why participants with microcephaly in this study were statistically more likely to receive a higher PredWES score. This correlation however contradicts the fact that microcephaly was more prevalent in the ES negative group compared to the ES positive group. Identifying the reason for this high prevalence of microcephaly in the ES negative group is however beyond the scope of this study. That said, the high prevalence of microcephaly might have introduced an inherent bias towards higher PredWES scores in the ES negative group. As a result, there was little difference in PredWES scores when comparing the ES positive and ES negative group.

The absence of correlation between PredWES scores and ES outcome may also have been due to a cohort bias as the DDD-Africa participants were preselected for the clinical likelihood of an ES positive outcome. The sample size under investigation was however very small and further investigation will be needed to confirm these hypotheses.

Lastly, the subjective nature of dysmorphology and the ability of a clinician correctly assign HPO descriptions (Lumaka et al., 2017) can perhaps be considered a limitation of PredWES. In addition, HPO terms describe phenotypes in a hierarchical manner and MLPT algorithms use the hierarchical classification to weight findings during the analysis process (Köhler et al., 2019). Therefore, the incorrect use of HPO terms can result in reduced performance of the MLPT. It is also suspected that the participant phenotyping process was not always done to a similar level by the Medical Geneticists and therefore may not have been of consistent quality. The poor predictive ability of PredWES might be a function of the accuracy with which HPO descriptions are assigned, rather than the performance of the PredWES algorithm. This illustrates the importance of thorough phenotyping in a clinical setting as well as consistent data collection when conducting research.

The F2G D-Score tool was designed with the purpose of identifying the level of facial dysmorphism in paediatric patients ultimately suggesting to clinicians whether further

genetic assessment and investigations are needed (Pantel et al., 2020). In this study however, the D-Score was investigated to determine if it has value as a predictor of ES outcome (similar to the purpose of PredWES).

For the F2G paediatric study population the average D-Score was high across both ES positive and ES negative participants. This finding was anticipated as facial dysmorphism is expected in patients with DD and 98.5% of F2G participants were clinically assessed to have some form of facial dysmorphism with no difference in prevalence between the ES positive and ES negative group (Supplement A). Therefore, in this cohort, F2G D-Score was not an effective predictor of ES outcome. F2G D-Score was however effective in its intended purpose as the tool correctly predicted the need for further genetic assessment in 77% of the ES positive group. An additional 3% received an unknown significance classification indicating the algorithm is unsure if the level of dysmorphism warrants further investigation. The interpretation of the unknown significance classification can perhaps be considered quite subjective and a clinician's decision (based on the D-score) to conduct further testing may possibly be influenced by the resources available in the clinical setting.

Fifty-nine percent of the ES negative participants received a high D-Score classification which is lower than the 77% for the ES positive group. Although there was no statistically significant difference found between the ES positive and ES negative group, reinvestigation with a larger sample size is warranted as the trend suggests that the D-Score classification might be able to differentiate between ES positive and ES negative participants.

The purpose of F2G geneticist view is to assist a clinician in the diagnostic process either as a point-of-care diagnostic tool or as a tool to guide ES variant interpretation and classification (Gurovich et al., 2019). In this study, the performance of the F2G geneticist view was assessed in two ways. Firstly, investigating the alignment of the overall F2G output for ES positive participants. Secondly, investigating the ES negative group to analyse the F2G output of suggested syndromes.

For a group of ES positive participants, F2G identified the correct diagnosis in 46% of the participants. Based on the gestalt score, F2G had a top 10 accuracy of 35% which is significantly less than the 91% top 10 accuracy reported by Gurovich et al. (2019), a study designed specifically to reflect a clinical setting. The clinical test set used by Gurovich et al. (2019) only included 2 participants of African ancestry (<1% of the test set), therefore the marked difference in top 10 accuracy could suggest F2G has reduced accuracy in African patients. This is consistent with the fact that F2G has mainly been trained on European populations (Gurovich et al., 2019).

An interesting finding was that the gestalt score performed better than the feature score with all these participants receiving a medium feature classification. Therefore, despite the overall reduced performance in African participants, the analysis of a facial photograph still performed better than the analysis of clinician assigned features. This echoes the finding that the F2G DeepGestalt algorithm is more effective than experienced clinicians in identifying syndrome specific dysmorphism (Gurovich et al., 2019). As with PredWES, the performance of the feature score might also be a function of subjective nature of dysmorphology and the accuracy with which HPO descriptions are assigned. Investigation of this is however beyond the scope of the study. Lastly, since the combined score incorporates both gestalt and feature scores, it was slightly less effective when compared to the gestalt score only. It is therefore recommended to mainly refer to the gestalt ranking (and perhaps the combined ranking) when using F2G. F2G does not provide feature score rankings and, based on the results, the feature score should not be interpreted as a stand-alone predictor as it might result in false negative results.

In contrast, F2G was unable to detect the correct syndrome in 54% of participants. This was partly explained by the fact that F2G was not trained for 36% of the syndromes in this group and could therefore not correctly suggest these syndromes. Upon further investigation it was also found that F2G did not report any high gestalt or combined scores for these participants. Therefore, although F2G was unable to detect the correct syndrome, F2G also did not make any false positive suggestions. Future investigation can be done to determine if the F2G Ultra-Rare tool will be valuable for

these particular participants. The high percentage of missed diagnoses nevertheless highlights the need for additional algorithm training, especially on African patients.

Three participants in the F2G ES positive group had CNV molecular diagnoses and for these participants, F2G was unable to correctly identify the syndrome (D3S.0070.01.1) or any of the OMIM genes within the affected gene regions (D3S.0085.01.1, D3S.0101.01.1). Although F2G has been trained for some CNV, the training is limited and F2G was not trained for WAGR syndrome, the molecular diagnosis for participant D3S.0070.01.1. These finding are however to be expected as CNV span across numerous genes and therefore the resulting phenotype is often highly variable (Park et al., 2019). It is plausible that the detection of syndromes associated with CNV is a limitation of F2G and future studies investigating the performance of F2G with CNV molecular diagnoses will be very valuable.

High gestalt, feature and/or combined scores were received for 39% of participants in the ES negative group. Of particular interest was 3 participants (D3S.0030.01.1, D3S.0088.01.1, D3S.0126.01.1) that received high gestalt scores with gestalt rankings at position 1, perhaps warranting a reinvestigation of the sequencing data. Participant D3S.0030.01.1, who received a very high gestalt score for Angelman Syndrome (AS), has undergone previous karyotyping and methylation sensitive (MS)-MLPA (Probemix p245 and p036) testing prior to the ES negative outcome. Several different aetiologies are known to contribute to the development of AS; these are chromosome 15q11q13 deletion (70% of cases), paternal uniparental disomy (2% of cases) and point mutations (10% of cases). It is however estimated that 10% of cases that present with an AS phenotype remain without a molecular diagnosis despite undergoing extensive testing. This can be due to the clinical diagnosis being incorrect or perhaps an AS genetic aetiology which is currently unknow (Wheeler et al., 2017). The combination of testing done on this participant would however have covered all the known AS aetiologies and perhaps this participant falls within the 10% of cases that remain undiagnosed despite testing. Participant D3S.0088.01.1, with a high gestalt score for Velocardiofacial Syndrome (22q11.2 deletion syndrome), has undergone previous karyotyping. Of note, the participant did not undergo CMA which is currently

considered the first-line test for Velocardiofacial Syndrome (Szczawińska-Popłonyk et al., 2023). That said, the ES analysis did include CNV analysis which should have detected a pathogenic 22q11.2 deletion if there was one present. Participant D3S.0126.01.1 has a high gestalt score for Coffin-Lowry Syndrome (CLS) which supports the CLS clinical diagnosis made by a Medical Geneticist. Prior to the negative ES outcome, the participant also received negative results for MS-MLPA (Probemix p245 and p036) and Fragile-X syndrome testing. It can however be difficult to analyse the *RPS6KA*3 gene for CLS as it is a large gene with many exons and a wide range of mutations has been documented. In addition, mutations within the intronic regions have also been documented (Hanauer, 2002). Since phenotype-driven variant prioritization is associated with an improved diagnostic yield (James et al., 2020), the high gestalt score for these three participants warrants more robust reinvestigation of the sequencing data to rule out the possibility of missed variants in the gene regions corresponding with the suggested syndromes (Burdick et al., 2020; Gurovich et al., 2019). If these participants remain ES negative after such reinvestigation; disease aetiologies beyond the scope of previous testing, novel disease aetiologies or differential diagnosis should be considered.

Several ES negative participants received high feature scores, especially for Phelan-McDermid Syndrome. Closer investigation of these participants showed no definite overlap in shared phenotypes, therefore the reason for this result is unclear. Also, as discussed earlier, feature scores should not be used as stand-alone predictors as such interpretation can lead to false positive results. There was a single ES negative participant with Angelman Syndrome scoring a very high combined score of 1, despite scoring low for both gestalt (0.24) and feature (0.6) scores. The reason for this is unclear and could perhaps be an algorithm artefact as the same anomaly was not seen for any other participants.

The study provides valuable insight in the use of PredWES and F2G in an African setting, however; the study does have notable limitations. Firstly, the differences in phenotyping observed between participants could in part be an indication of a flawed phenotyping process rather than actual differences in levels of dysmorphism. This inconsistency is also true for the quality of the facial photographs used for the F2G

analysis and an evaluation of the photo quality is justified. Secondly, clinical genetics is a rapidly advancing field and future reanalysis of the sequencing data might lead to additional molecular diagnosis of participants currently in the ES negative group (Schobers et al., 2022). In addition, ES cannot detect all disease-causing variants or mechanisms such as methylation abnormalities, deep intronic variants, cryptic splice site variants, low level mosaicism and repeat expansions (Burdick et al., 2020; Corominas et al., 2022; Sanders et al., 2020). Furthermore, the ES variant outcome data was provided to the researcher from a third party and it is suspected that some of the ES positive classifications were in fact VUSs and should have been excluded from the ES positive group. Lastly, although the study produced some useful insights, it is based on a small sample size with limited statistical power. Further investigation with a larger number of participants will be required to confirm these results.

In conclusion, selecting participants suitable for ES based on high PredWES scores has not been shown to effectively increase the diagnostic yield in the DDD-Africa cohort. Further investigation is required to determine if this is due to poor PredWES algorithm performance or due to the limitations of this study. F2G D-Score was not a good predictor of ES outcome which is understandable as it is not the purpose of the tool. The D-Score classifications were however valuable to identify paediatric participants that will benefit from additional genetic investigation as there seems to be a trend between a high D-Score classification and positive ES outcome. F2G geneticist view did show some promising results, especially when analysis was based on the facial photograph only. The range of results however highlights that tools such as F2G should be used as a complementary to the diagnostic process and cannot replace the role of the clinician. Overall, the F2G performance was promising but much lower in comparison to studies conducted on mainly European cohorts; therefore, demonstrating to need for more extensive training of phenotypic MLPT in the context of DD in an African setting. Future studies can be extremely valuable as much is still to be learned. These studies can include PredWES performance when comparing a control set of African participants without DD to a set of African participants with DD, the performance of F2G before and after training of African patients, a comparison of F2G performance for SNV and CNV, larger scale analysis of F2G gestalt score versus combined score performance, the effect of photo quality on the performance of F2G

and the performance of the F2G Ultra-rare tool for participants with a diagnosis untrained by F2G.

5. Declarations

Author contributions

Anréé Bresler designed the study, collected data (from the parent study dataset), reviewed and analysed the data and drafted the manuscript. Barry Shingwenyana and Amanda Krause provided valuable input in the study design, reviewed the data and provided critical revisions of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The data that support these findings are available from the 1st author upon reasonable request.

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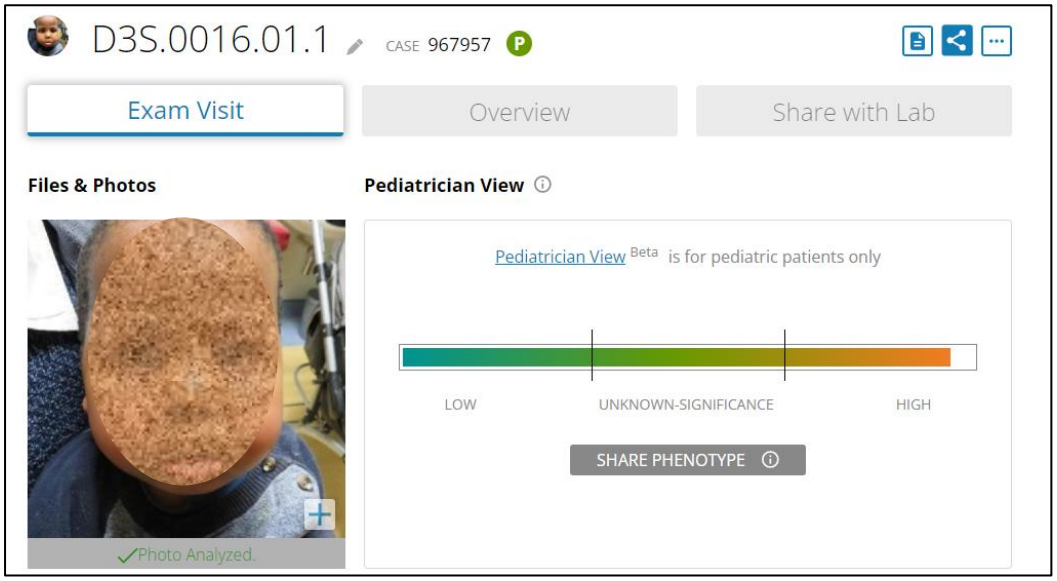
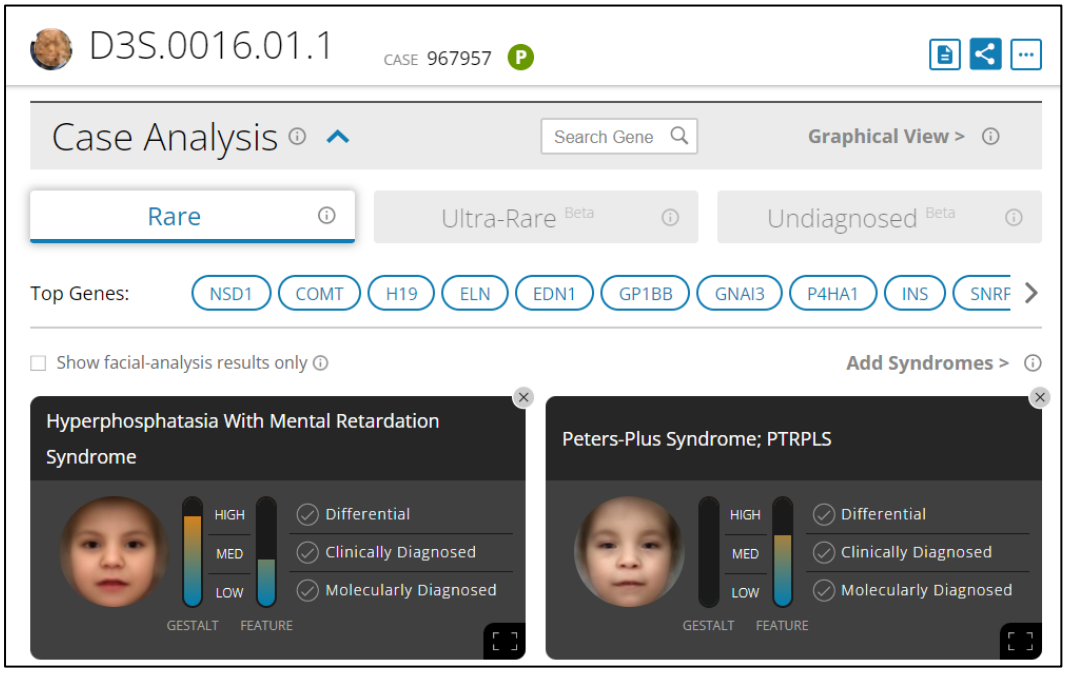
7. Appendices

Appendix A: D-Score classification



Appendix A: Face2Gene D-Score output for participant D3S.0016.01.1. The figure indicates the D-Score classification (low, unknown significance and high) with participant D3S.0016.01.1 scoring high for facial dysmorphism. (D-Score = 0.96 as provided with bulk feedback on Microsoft Excel spreadsheet from F2G developers).

Appendix B: Face2Gene Geneticist view



Appendix B: Face2Gene Geneticist view output for participant D3S.0016.01.1. Based on a combination of a high gestalt score (facial analysis) and a medium feature score (HPO analysis) HPMRS was correctly ranked at position 1 for participant D3S.0016.01.

8. Supporting information

Supplement A: Table indicating the [†]difference in prevalence of phenotypic features or affected body systems between the ES positive and ES negative group.

Phenotypic feature / Body system	**ES positive group (n = 43)** n (%)	**ES negative group (n = 51)** n (%)
Mild intellectual disability	1 (2)	5 (10)
Moderate intellectual disability	15 (35)	22 (43)
Severe intellectual disability	27 (63)	24 (47)
Motor delay	40 (90)	45 (88)
Hypertonia	6 (14)	9 (18)
Hypotonia	18 (42)	19 (37)
Seizures	9 (21)	8 (16)
Delayed speech	27 (63)	34 (67)
Absent speech	13 (30)	12 (24)
Microcephaly (±3 SD)	10 (23)	22 (43)
Macrocephaly (±3 SD)	4 (9)	5 (10)
Underweight (±3 SD)	10 (23)	15 (29)
Overweight (±3 SD)	4 (9)	2 (4)
Tall stature (±3 SD)	2 (5)	1 (2)
Short stature (±3 SD)	11 (26)	15 (29)
Undergrowth (±3 SD)	7 (16)	10 (20)
Overgrowth (±3 SD)	1 (2)	0
Hearing impairment	5 (12)	5 (10)
Visual impairment	6 (14)	6 (12)
Cardiac system	6 (14)	9 (18)
Skeletal system	28 (65)	34 (67)
Urogenital system	13 (30)	11 (22)
Brain and central nervous system	24 (56)	28 (55)
Chest and respiratory system	7 (16)	5 (10)
Abdominal and gastrointestinal tract	9 (21)	6 (12)
Ectodermal	20 (47)	28 (55)
Head and neck	42 (98)	49 (96)
Cleft palate	1 (2)	2 (4)

† Two proportion z-tests showed no significant differences when comparing prevalence (of each category) in ES positive and ES negative group.

Appendices

Appendix 1: Human Research Ethics Committee (Medical) Ethical Clearance Certificate

Appendix 2: Approved Research Protocol

Appendix 3: Plagiarism declaration and Turnitin Report

Appendix 4: Journal Author Guidelines for Submissible Format

Appendix 1: Human Research Ethics Committee (Medical) Ethical Clearance Certificate (M230686)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:	Ms A Bresler School of Pathology Division of Human Genetics Medical School University E-mail: 303468@students.wits.ac.za
CC:	Supervisor: Ms N Louw and Mr B Shingwenyana <Nadja.Louw@wits.ac.za> and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	2023/07/19
REF:	R14/49
PROTOCOL NO:	**M230686** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R49 Ms A Bresler

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230686

NAME: (Principal Investigator)	Ms A Bresler
DEPARTMENT:	School of Pathology Division of Human Genetics Medical School University
PROJECT TITLE:	*Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M18/06/78
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Ms N Louw and Mr B Shingwenyana
APPROVED BY:	Dr M Vorster, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/07/19 **EXPIRY DATE:** 2028/07/18

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **June** and therefore reports and re-certification will be due in the month of **June** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2023/07/20
Date

Human Research Ethics Committee (Medical) Ethical Clearance Certificate (M180678) for DDD-Africa

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Prof. A Krause; Drs N Carstens & Z Lombard; Ms N Louw

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180678

NAME: (Principal Investigator)	Prof. A Krause; Drs N Carstens & Z Lombard; Ms N Louw
DEPARTMENT:	School of Pathology Department of Human Genetics National Health Laboratory Service
PROJECT TITLE:	*Decyphering Development Disorders in Africa (DDD-Africa) - evaluating clinical exome sequencing in an African setting*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M160830 New investigator added 2021/09/06 NIH Study No. UO1MH115483
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Not applicable
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/07/2018

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Philip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **«Missing mail merge field»** and therefore reports and re-certification will be due in the month of **«Missing mail merge field»** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator Date

Appendix 2: Approved Research Protocol

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

07 July 2023
Person No: 303468
PAG

Mrs A Bresler
19 B Villagers lane
Irene
0157
South Africa

Dear Mrs Anree Bresler

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

100 1922 2022

Predicting exome sequencing outcomes from phenotypic data in an African patient cohort with developmental delay.

Anréé Bresler

303468

MSc (Med) Genomic Medicine

Supervisor 1:

Mr. Barry Shingwenyana

MSc (Med) Genetic Counselling (Wits)

Genetic Counsellor, Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand.

Supervisor 2:

Professor Amanda Krause

MBBCh (Wits), PhD

Medical Geneticist and Head of Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand.

1. Background literature, analysis and critique

1.1.1. Deciphering Developmental Disorders

Developmental disorders (DD) refer to a broad category of developmental delays where developmental milestones are not reached in an age-appropriate manner. It has been reported that up to 40% of DD cases can be attributed to underlying genetic factors (Miclea et al., 2015) which is often associated with monogenic causes (Wright et al., 2015). Due to the phenotypic and genetic heterogeneity of DD, there is often a significant delay in diagnosis. The scarcity of genetic resources in low- and middle-income countries further exacerbate this delay, which is referred to as the diagnostic odyssey (Porras et al., 2021).

Deciphering Developmental Disorders in Africa (DDD-Africa) is an ongoing study which aims to explore the genetic aetiology of DD in an African population. This study has utilised whole exome sequencing (WES) to identify disease-causing variants in a cohort of African patients with DD (Lombard et al., 2017). The WES data and variant classification is stored on the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER) (Firth et al., 2009). Phenotype information is stored on a secure web based clinical database, Research Electronic Data Capture (REDCap) (Patridge & Bardyn, 2018).

1.1.2. Whole exome sequencing

Whole exome sequencing allows the sequencing of all the protein coding regions within the genome. This protein coding sequence is compared to a normal reference sequence to identify variants (Han & Lee, 2020b) which are then classified according to the American College of Medical Genetics and Genomics and Association of Molecular Pathology (ACMG/AMP) guidelines. The guidelines facilitate sequence interpretation by classifying sequence variants into five categories (pathogenic, likely pathogenic, uncertain significance, likely benign and benign), therefore standardising variant reporting (Richards et al., 2015). Molecular diagnosis of DD using WES data is however challenging within an African context. The biggest contributing factor is the lack of African genomic data in reference databases making it difficult to identify and interpret variants within an African cohort (Lumaka et al., 2022). Therefore, the

use of additional tools, such as machine learning predictive tools, could be valuable in improving the diagnostic process.

1.1.3. Machine learning predictive tools

Machine learning predictive tools incorporate phenotypic data into machine learning algorithms to predict WES outcomes (Dingemans et al., 2022) and molecular diagnosis (Gurovich et al., 2019). A standardised clinical vocabulary, Human Phenotype Ontology (HPO), has been developed to standardise research in the field of computational deep phenotyping. HPO is used to describe the specific phenotypes and clinical abnormalities associated with human disease (Gao et al., 2019). This study will specifically focus on PredWES and Face2Gene (F2G) as machine learning tools that utilise HPO terms and/or facial dysmorphic features as part of their algorithm.

PredWES is an online tool developed by Dingemans et al. (2022) which is incorporated into the Human Disease Genes website (https://humandiseasegenes.nl/predwes) and is freely available for clinicians. PredWES uses HPO terms to help clinicians pre-select patients suitable for WES by estimating the probability of a positive diagnostic WES outcome. PredWES is based on a computational logistic regression model, the Finnish horseshoe, trained on a set of 1663 patients with neurodevelopmental disorders. PredWES uses the full phenotypic profile of a patient and claims to automatically include the most appropriate phenotypes to predict the likelihood of a positive WES outcome. Interestingly, the patients with a positive WES result were more likely to have microcephaly, facial dysmorphism, congenital abnormalities and severe intellectual disability. Dingemans et al. (2022) reported a WES diagnostic yield of 56% in the patients that scored within the top 10% of the highest PredWes probability. This is a significant improvement when compared to the initial 26% diagnostic yield seen without PredWES selection. Therefore, PredWES is reported to be a useful tool to prioritize which patients to test using WES.

Face2Gene (F2G) is a collection of online tools available to healthcare practitioners to assist with genetic evaluations. F2G uses DeepGestalt technology which is a

facial image analysis framework that combines deep learning algorithms and computer vision (Gurovich et al., 2019). An expansive dataset is used to develop syndrome gestalts which are classifiers for specific syndrome facial morphologies. When a healthcare practitioner uses F2G they upload a facial photograph of the patient and the DeepGestalt technology will convert this photo into facial descriptors. The learning algorithm then compares the patient's facial descriptors to the genetic syndrome gestalts available in the software to generate the output depending on the tool in use. In paediatric view, a facial D-score will be generated based on the patients' facial descriptors. In geneticist view, F2G will generate a list of suggested syndromes together with a gestalt score based on the photo only. Alternatively, HPO terms can be uploaded to obtain a feature score.

These tools have mainly been trained on European individuals (Gurovich et al., 2019; Dingemans et al., 2022) therefore, ethnicity remains a potentially concerning limitation in the use of these tools (Lumaka et al., 2017). Since Africa is a resource constrained setting with marked ethnic and racial diversity, it is essential to analyse the effectiveness of these machine learning tools within an African context.

2.1.1. Study aim

To investigate whether machine learning tools can be used to successfully predict the probability of a positive exome sequencing result and/or a genetic diagnosis solely based on the patient phenotype in an African cohort of patients with developmental delay.

2.1.2. Study objectives

1. Determine if a standardised phenotypic tool, PredWES, can correctly predict a positive WES result for participants with developmental delay.
2. Determine if a standardised dysmorphology tool, F2G D-score, can correctly predict a positive WES result for participants with developmental delay.
3. Determine the predictive value of a standardised dysmorphology tool, F2G gestalt score and feature score, when compared to the WES genetic diagnosis in participants with developmental delay.

3. Methods

3.1.1. Study sample

The study is a sub-study of the DDD-Africa project and will make use of a subset of the DDD-Africa dataset. The participants were recruited from one of the following sites: Charlotte Maxeke Johannesburg Academic Hospital, Rahima Moosa Mother and Child Hospital and Chris Hani Baragwanath Academic Hospital. Participants are children with developmental delay and are mainly of African ancestry.

The data subset will consist of DDD-Africa participants that met inclusion criteria A and D as listed below. This study will not include participants that met inclusion criteria B and/or C only, as these participants do not have developmental delay.

DDD-Africa participants were included if they fulfilled one of the following criteria:

A. Intellectual disability (moderate to profound)
B. 1. Multiple major malformations in 2 or more different organ systems >1month
   2. Multiple major malformations in 2 or more different organ systems <1month
C. One major malformation AND 3 or more well-documented minor anomalies
D. Mild to moderate intellectual disability AND dysmorphic features

Individuals were not recruited if they had a known condition with no strong evidence for a monogenic cause, suspicion of an acquired brain lesion, suspected multifactorial or environmental cause or in the case of mild intellectual disability only (with no additional dysmorphic features or structural abnormalities).

As participants were children with developmental delay, parental consent was given and assent provided where possible. A separate consent was signed for the use of photographs as well as for the use of data in a public domain.

The data subset includes WES data, HPO terms and facial photographs. It is a convenience sample as it is the first batch of available WES results from the DDD-Africa cohort. The subset represents 93 participants with consent for the use of their data in a public domain such as DECIPHER. For the PredWES analysis all 93 participants will be included as no photo consent is required. Of these participants,

35 have a pathogenic or likely pathogenic WES classification which is referred to as a positive WES outcome. This translates into a positive WES yield of 37.6%. For the F2G analysis, only the 69 participants that provided consent for the use of photographs will be included in this study. Of these participants, 29 have a pathogenic or likely pathogenic WES classification. Therefore, 42% of the F2G participants are known to have a positive WES result.

Genomic summary data for prioritised variants classified by ACMG/AMP guidelines will be obtained from DECIPHER (Firth et al., 2009). Patient HPO terms will be obtained from a REDCap (Patridge & Bardyn, 2018). The patient photographs in JPEG files will be obtained from their respective patient folders in a secure DDD-Africa Google drive. The DDD-Africa Google drive is protected and only staff and collaborators on the project have access to the stored information. For this study, the main researcher will only have access to de-identified participant information in the form of DDD participant IDs.

3.1.2. Study design and data processing

A correlational study design will be followed. HPO terms and/or photographs will be uploaded to two different predictive tools namely PredWES and F2G. Data will be captured in an Excel spreadsheet as listed: DDD participant ID, PredWES probability, Facial D Score, F2G top 10 listed syndromes with separate gestalt and feature scores, summary of genomic data, and positive or negative WES outcome. The investigator will be blinded to the WES outcome till after PredWES and F2G analysis has been conducted.

PredWES: PredWES is a new tool and follows a manual process where the clinician enters the HPO terms into the online tool and receives the PredWES probability score (a range of 0 – 1) via e-mail. After a discussion with the developers, they have agreed to assist in the process and will run all the participant HPO terms as a batch. An Excel spreadsheet with anonymised HPO terms will be sent to the developers and they will return a probability score for each participant using this Excel spreadsheet.

Face2Gene D-Score: A facial photo of the participant will be uploaded using the paediatric view and a facial D-score will be generated based on the participants facial descriptors. The D-Score is categorised as either low, unknown significance or high with the latter two suggesting the presence of dysmorphism. Although F2G D-Score does not predict WES outcome, for the purpose of this study, the D-Score will be compared to the WES result in order to investigate predictiveness.

Face2Gene Gestalt and Feature Score: The F2G geneticist view will be used to determine the specific syndromes F2G predicts to be associated with the participant's phenotype. The F2G tool does this by comparing the participant's facial descriptors to the genetic syndrome gestalts. For this study, the participant photo and HPO terms will be uploaded. F2G will generate a list of suggested syndromes together with a gestalt score (based on the photo) and feature score (based on HPO terms) of low, medium, or high.

As illustrated by Figure 1 below, F2G digitises the face into vectors, therefore no participant photos will be stored in the F2G database (Gurovich et al., 2019).

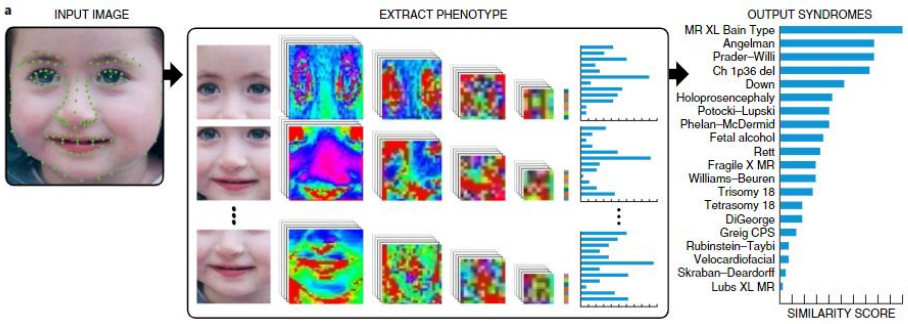


Figure 3: DeepGestalt: High-level flow and network architecture. The input image is pre-processed and cropped into facial regions after which it is fed into a deep convolutional neural network to obtain a softmax vector indicating correspondence to each syndrome in the model.

Genomic data and whole exome sequencing: Genomic summary data, WES outcome and genetic diagnosis will be obtained from DECIPHER. For the PredWES and F2G D-Score analysis, participants will be classified as negative WES participants if no pathogenic, likely pathogenic or variants of uncertain significance are identified. Participants will be classified as positive WES participants if pathogenic or likely pathogenic variants are identified.

4. Data analysis

Data analysis will be quantitative in nature using R statistical software and Excel for analysis.

1. a. The correlation between the PredWES probability score (range of 0 – 1) for each participant and the WES outcome (positive/negative) will be investigated using the Point-Biserial correlation coefficient allowing comparison between categorical and continuous data.
   b. Calculate the percentage of participants that tested positive with WES and also scored within the top 10% of the PredWES probability scores. Calculate the percentage of participants that tested negative with WES and also scored within the lowest 10% of the PredWES probability scores. This analysis is in line with the analysis done by (Dingemans et al., 2022).

2. The categorical data from the F2G Facial D-score (low/uncertain/high) and WES outcome (positive/negative) will be captured in a 3 x 2 contingency table. The strength of the association between the D-Score and WES outcome will be assessed using a Pearson's chi-square test and used as an indicator of the F2G D-score predictive ability.

3. The F2G top 10 listed syndromes for both the gestalt score and the feature score will be compared to the respective participant WES genetic diagnosis. The F2G listed syndromes will be grouped into 4 classes according to their ranking; ranked 1 – 3, ranked 4 – 6, ranked 7 – 10 and absent. The predictive value for each class will be determined and expressed in terms of test sensitivity and specificity for both the F2G gestalt score and the F2G feature score.

Where applicable, statistical power will be determined with a 95% confidence level and statistical significance accepted at a $p\text{-}value < 0.05$. If needed, statistical support will be enlisted from the University of the Witwatersrand statistical consultation service provided by the research office.

5. Ethics

Ethical clearance for this study has been applied for and is awaiting outcome pending protocol approval. The DDD-Africa project does have ethical clearance. Ethical clearance no: M180678 (renewal in process). All participants will remain anonymised, and the main researcher will only have access to the participant ID's. Participants for this study cohort did provide consent for the use of their information on a publicly available database. Please see appendix with consent forms.

6. Timing

	March	April	May	June	July	August	September	October	November
Literature review									
Preparing protocol									
Protocol assessment									
Ethics application									
Collecting data									
Data analysis									
Writing up research report									

7. Funding

Resource	Cost and funding
DECIPHER, REDCap, Google drive, R studio and Microsoft Excel	No associated cost*

*The software, data and databases are already available and free.

8. Possible problems and study limitations

1. Although physical examinations are done by geneticists, there might be some inconsistencies between clinicians in the use of HPO descriptions to describe the participant phenotype.
2. Some disease-causing variants might not be detected using WES. Therefore, a participant might be classified as a negative WES even though the participant does have an underlying monogenic cause.
3. Face2Gene has been trained on roughly 300 syndromes (Hsieh et al., 2022). Therefore, participants might have a rare DD not yet analysed and trained by

Face2Gene resulting in the incorrect prediction by Face2Gene. The F2G ultra-rare tool will not be considered in this study.

4. Face2Gene might have limited utility in young infants as facial dysmorphism might not be sufficiently apparent. However, most children included were not young infants.
5. The study is a pilot study based on a convenience sample of a selected cohort. The study will be useful to investigate trends but might show to have limited statistical power.
6. For practical reasons, the F2G top 10 ranked diagnosis (gestalt and feature score) will be categorized into 4 classes. However, it limits the findings as it only considers the ranking and does not consider the respective scores of low, medium and high for each ranked diagnosis.

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10. Appendices

	**DDD-Africa** Deciphering Developmental Disorders in Africa
CONSENT FORM – PARENTAL CONSENT FOR CHILD PARTICIPANTS	

PARTICIPANT STUDY ID:

The purpose of this study, procedures to be followed, risks and benefits, have been explained to me and my child. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have questions, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this consent form and agree to allow my child to participate in this research study with the understanding that I may withdraw my child at any time. To the best of my ability, I have discussed the study with my child, who agrees to be in the study. I have explained to my child that he/she may approach the researchers to be re-consented when he/she turns 18 if he/she wishes and that he/she may withdraw from the study at any time. I have been told that I will be given a signed and dated copy of this consent form if I would like one.

	Please Initial	
I understand that the purpose of this consent form is for me to inform the study what they can or cannot do with my child/children's samples.		
I understand that all procedures/tests on the stored samples have been or will be approved by the Human Research Ethics Committee of the University of the Witwatersrand. I understand that every time a new study is done on my sample, permission will be obtained from this ethics committee for the study to make sure that it is used only for the purposes stated above.		
I understand that if I have any questions at any time, they will be answered.		
I understand that confidentiality will be maintained at all times through the use of a code and numbering system.		
I understand that my family and I may withdraw from the study at any time.		
I have discussed the study with my child.		
I am in agreement that my child's samples may be stored and used for the purposes described in the information sheet.		
I understand that I can choose to have my child's sample destroyed at any time.		
I understand that my child may not benefit directly from the research done on their sample and my family's samples.		
If this research does identify information that is relevant to my child's developmental delay, I would like to be contacted to receive the information.	Yes ☐	No ☐
If this research does identify genetic information that would be important or of health benefit to my family, or me, I would like to be contacted to receive the information.	Yes ☐	No ☐

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DDD-Africa

Deciphering Developmental Disorders in Africa

CONSENT FORM – PARENTAL CONSENT FOR CHILD PARTICIPANTS

PERMISSION FOR REUSE	Samples		Data	
I agree that my samples & data can be shared with other investigators for research related to developmental disorders once the ethics committee has approved the new study. My samples & data will be made available without any identifiers.	Yes ☐	No ☐	Yes ☐	No ☐
I agree that my samples & data can be shared with other investigators for research in any field once the ethics committee has approved the new study. My samples & data will be made available without any identifiers.	Yes ☐	No ☐	Yes ☐	No ☐

PERMISSION FOR STORAGE		
I agree to have my samples stored in the CLS H3Africa biobank, in Johannesburg without any identifiers.	Yes ☐	No ☐
I agree to have the data generated from my samples stored in a public domain database without any identifiers.	Yes ☐	No ☐

PERMISSION FOR TRANSFER		
I agree that a small bit of my DNA or RNA samples may be sent out of the country if the research cannot easily be done in South Africa.	Yes ☐	No ☐

PERMISSION FOR PHOTOGRAPHS		
I agree to the use of my/my child's photographs for medical records ONLY.	Yes ☐	No ☐
I agree for my/my child's photographs to be used for teaching purposes AND to be used for medical records but NOT for medical publication.	Yes ☐	No ☐
I agree for my/my child's photographs to be used in medical publications, including medical journals, textbooks and electronic publications AND to be used for teaching purposes AND to be used for my medical records. I understand that the images may be seen by members of the general public, in addition to scientists and medical researchers that use these publications in their professional education. Although these photographs will be used without identifying information, such as my/my child's name, I understand that it is possible that someone may recognize me/my child.	Yes ☐	No ☐ ☐ Understood

Researcher	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Parent Participant	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)

	**DDD-Africa** Deciphering Developmental Disorders in Africa
ASSENT FORM FOR CHILD PARTICIPANTS	

PARTICIPANT STUDY ID:

The purpose of this study, procedures to be followed, risks and benefits, have been explained to me. I have been allowed to ask the questions I have, and my questions have been answered to my satisfaction. I have been told whom to contact if I have questions, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this assent form (or had it read to me) and agree to participate in this research study with the understanding that I may withdraw at any time. I understand that that I may approach the researchers to be re-consented when I turn 18 if I want to and that I may withdraw from the study at any time. I have been told that I will be given a signed and dated copy of this consent form if I would like one.

Researcher	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Parent or Legal Guardian	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Child Participant	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Translator (If applicable)	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)

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Appendix 3: Plagiarism declaration and Turnitin Report

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Anree Bresler (Student number: 303468) am a student registered for the degree of MSc (Med) Genomic Medicine in the academic year 2023.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 24 November 2023

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turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Anree Bresler
Assignment title:	Research Report
Submission title:	A Bresler MSc Research Report Turnit in.pdf
File name:	A_Bresler_MSc_Research_Report_Turnit_in.pdf
File size:	597.72K
Page count:	27
Word count:	8,572
Character count:	46,149
Submission date:	28-Nov-2023 12:04PM (UTC+0200)
Submission ID:	2240708761


Research report in the format of a 'submissible' paper

Please refer to the link for the full report:
https://ev.turnitin.com/app/carta/en_us/?lang=en_us&student_user=1&o=2240708761&u=1144405183&s=1&ro=103

Appendix 4: Journal Author Guidelines for Submissible Format

Refer to the following link for the full author guidelines:

https://onlinelibrary.wiley.com/page/journal/15524833/homepage/forauthors.html?

American Psychological Association (APA) 7th edition referencing style has been used throughout.

The section below describes the Author Guidelines: Preparing the Submission

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4. PREPARING THE SUBMISSION

Free Format Submission

AJMGA now offers **Free Format submission** for a simplified and streamlined submission process.

Before you submit, you will need:

- Your manuscript: this should be an editable file including text, figures, and tables, or separate files – whichever you prefer. All required sections should be contained in your manuscript, including abstract (which does need to be correctly styled), introduction, methods, results, and conclusions. Figures and tables should have legends. Figures should be uploaded in the highest resolution possible. If the figures are not of sufficiently high quality your manuscript may be delayed. We also encourage you to include your figures within the main document to make it easier for editors and reviewers to read your manuscript. References may be submitted in any style or format, as long as it is consistent throughout the manuscript. Supporting information should be submitted in separate files. If the manuscript, figures or tables are difficult for you to read, they will also be difficult for the editors and reviewers, and the editorial office will send it back to you for revision. Your manuscript may also be sent back to you for revision if the quality of English language is poor.
- An ORCID ID, freely available at https://orcid.org. *(Why is this important? Your article, if accepted and published, will be attached to your ORCID profile. Institutions and funders are increasingly requiring authors to have ORCID IDs.)*
- The title page of the manuscript, including:
  - Your co-author details, including affiliation and email address. *(Why is this important? We need to keep all co-authors informed of the outcome of the peer review process.)*
  - Statements relating to our ethics and integrity policies, which may include any of the following *(Why are these important? We need to uphold rigorous ethical standards for the research we consider for publication)*:
    - data availability statement
    - funding statement
    - conflict of interest disclosure
    - ethics approval statement
    - patient consent statement
    - permission to reproduce material from other sources
    - clinical trial registration

To submit, login at **https://wiley.atyponrex.com/journal/AJMG** and create a new submission. Follow the submission steps as required and submit the manuscript.

Parts of the Manuscript

Manuscripts can be uploaded either as a single document (containing the main text, tables and figures), or with figures and tables provided as separate files. Should your manuscript reach revision stage, figures and tables must be provided as separate files. The main manuscript file can be submitted in Microsoft Word (.doc or .docx) format or LaTex (.tex) format.

If submitting your manuscript file in LaTex format via Research Exchange, select the file designation "Main Document – LaTeX .tex File" on upload. When submitting a Latex Main Document, you must also provide a PDF version of the manuscript for Peer Review. Please upload this file as "Main Document - LaTeX PDF." All supporting files that are referred to in the Latex Main Document should be uploaded as a "LaTeX Supplementary File."

Main Text File

Your main document file should include:

- A short informative title containing the major key words. The title should not contain abbreviations;
- The full names of the authors with institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
- Acknowledgments;
- Abstract;
- Up to seven keywords;
- Main body
- References;
- Tables (each table complete with title and footnotes);
- Figures: Figure legends must be added beneath each individual image during upload AND as a complete list in the text.
- Appendices (if relevant)

Authorship

Please refer to the journal's Authorship policy in the Editorial Policies and Ethical Considerations section for details on author listing eligibility.

Acknowledgements

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

Conflict of Interest Statement

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the Conflict of Interest section in the Editorial Policies and Ethical Considerations section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Author Contribution Statement

For original articles, a dedicated Author Contributions section must be included on the title page of the paper to provide information about individual author contributions to the work. For details on what to include in this section, see the General Instructions above.

Data Sharing and Data Accessibility

All accepted manuscripts are required to publish a data availability statement to confirm the presence or absence of shared data. If you have shared data, this statement will describe how the data can be accessed, and include a persistent identifier (e.g., a DOI for the data, or an accession number) from the repository where you shared the data. For details on what to include in this section, see the General Instructions above.

Abstract

Please provide an abstract of 200 words containing the major keywords summarizing the article.

Keywords

Please provide up to seven keywords.

Main body

The journal uses US spelling; however, authors may submit using either option, as spelling of accepted papers is converted during the production process.

References

The accuracy of references is the responsibility of the authors. Only published papers and those in press may be included in the reference list. Unpublished data and submitted manuscripts must be cited parenthetically within the text. Personal communications should also be cited within the text; permission in writing from the communicator is required.

This journal recommends that references be prepared according to the *Publication Manual of the American Psychological Association* (7th edition). The APA website includes a range of resources for authors learning to write in APA style, including an overview of the manual, free tutorials on APA Style basics, and an APA Style Blog. For more information about APA referencing style, please also refer to the APA FAQ. Please note that the editorial office will update the formatting into journal style when the manuscript is accepted for publication.

EndNote users can download the style here.

According to APA style, in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper.

Authors should note that the APA referencing style requires that a Digital Object Identifier (DOI) be provided for all references where available. Also, for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

Reference examples follow:

Journal article

Beers, S. R., & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *The American Journal of Psychiatry, 159,* 483–486. *doi*:10.1176/appi.ajp.159.3.483

Book

Bradley-Johnson, S. (1994). *Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school* (2nd ed.). Austin, TX: Pro-ed.

Internet Document

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from http://www.youtube.com/watch?v=Vja83KLQXZs

Footnotes

Footnotes should be placed as a list at the end of the paper only, not at the foot of each page. They should be kept to a minimum. Keep footnotes brief; they should contain only short comments tangential to the main argument of the paper and should not include references. They should be numbered in the list and referred to in the text with consecutive, superscript Arabic numerals.

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P- values. Statistical measures such as SD or SEM should be identified in the headings.

Figure Legends

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

Figures

Although authors are encouraged to send the highest quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted. Click here for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

Figures submitted in color will be published free of charge for every article. Please note, however, that it is preferable that line figures (e.g. graphs and charts) are supplied in black and white so that they are legible if printed by a reader in black and white.

Additional Files

Appendices

Appendices will be published after the references. For submission they should be supplied as separate files but referred to in the text.

Supporting Information

Supporting information is information that is not essential to the article, but provides greater depth and background. It is hosted online and appears without editing or typesetting. It may include tables, figures, videos, datasets, etc.

Click here for Wiley's FAQs on Supporting Information.

Note: if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

General Style Points

The following points provide general advice on formatting and style.

- **Abbreviations**: In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only.
- **Units of measurement**: Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website for more information about SI units.
- **Numbers**: numbers under 10 should be spelt out, except for: measurements with a unit (8 mmol/L); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).
- **Trade Names**: Chemical substances should be referred to by the generic name only. Trade names should not be used. Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.