

# Amended Informative Negative Whole Exome Sequencing Results

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## Abstract

**Background:** Whole exome sequencing (WES) is widely used as a first-tier diagnostic test. The diagnostic yield for WES is estimated at approximately 50%, leaving the requesting clinician with 50% ambiguous or negative results in cases where the diseases-causing variants could not be identified.

**Methods:** We retrospectively assessed the results of all WES tests requested from 2015 to 2018 at our institution. We included participants with a negative test result, irrespective of the phenotype. Complete data collection, medical-record extraction, and additional analysis were done for qualifying cases.

**Results:** The cohort included 151 participants with a negative WES result. Of the sample, 18% (n=27) were discharged from the genetic clinic, and only three cases required additional genetic testing prior to discharge. The decision to discharge a patient was based on several factors, including a mild phenotype (i.e. a phenotype that can be resolved and doesn't require surgical, rehabilitation or medical intervention), a confirmed multifactorial condition, or the patient is developing according to the milestones for his age group and has a negative family history. The discharge rate and the period of being followed at the clinic improved over time. In 2015, none was discharged, and in 2016, nearly 100% of the negative cases were discharged in 6-12 months. In 2017, 65% of the cases were discharged in less than six months, and all cases tested in 2018, were discharged from the clinic in less than three months.

**Conclusion:** Informative negative WES results lower the cost of additional testing, reduce the number of follow-up consultations, and result in the reassurance or discharge the patient. The informative negative WES results (18%) can be combined with the hit rate of the WES (50%) in consanguineous population, increasing the clinical utility of WES testing to 68%.

## Background

Next generation sequencing (NGS) caused a revolution in genetic research and the clinical diagnosis of human genetic disorders. NGS refers to the deep, high-throughput, in-parallel nucleic acid sequencing technologies that examines multiple genes at the same time (Rabbani, Tekin & Mahdieh 2014).

NGS technology is currently used in different assays, such as whole genome sequencing (WGS), whole exome sequencing (WES), and targeted gene panels. Of these tests, WES is preferred because it covers more than 85% of disease causing mutations (Rabbani, Tekin & Mahdieh 2014), is more cost-effective compared to WGS (Schwarze et al. 2018) and the diagnostic yield of WES ranges from 25 to nearly 49% (Alfares et al. 2017, Yang et al. 2013, Lee et al. 2014, Yang et al. 2014 ). Based on these factors, WES is recommended as the first tier testing for suspected genetic disorders (Clark et al. 2018, Stark et al. 2016). Approximately 25–50% of the cases will be identified after the initial genetic testing (i.e. WES), leaving the requesting clinicians with 50–75% ambiguous or negative results. Negative results are defined as the absence of disease causing variants (pathogenic (P) and/or likely pathogenic (LP)) that can fully explain the phenotype. When the variants cannot explain the patient's phenotype, the results are considered negative. The explanation is that the phenotype is not hereditary, or the genetic cause has not yet been revealed. Re-analysis of the raw data of inconclusive or negative results is recommended for cases that are highly suspicious of an underlying genetic cause before proceeding with further testing (Alfares et al. 2018, Ewans et al. 2018). Management based on a negative result, is completely case-dependent on the patient's medical and family history. Skinner et al. (2016) explained that the majority of the negative result interpretations were provisional, and could not rule out genetic diseases. Such results were called nuanced negative. The clinician should interpret the result based on the patient's phenotype and family data before excluding the possibility of an inherited disorder, as he/she may need to revisit the NGS raw data in future if indicated. However, the result of a negative WES supports reassurance in cases where the phenotype is not significantly linked to a single gene disorder, and with an unremarkable family history. In these cases, a negative result is indeed informative.

To add to the knowledge base, we reviewed 151 cases with a negative WES test result and we found that an early negative result optimizes the opportunity to influence the clinical management and discharge patients from the clinic, decreasing costs and the psychological burden on the family and community.

## Methods

We performed a retrospective cohort study of all WES tests conducted from 2015 to 2018 at the genetic clinic at King Abdulaziz Medical City, Riyadh, Saudi Arabia. Only patients with a negative result were included in the study. For the sample, irrespective of their phenotype, complete data were collected and the medical records retrieved, and additional analyses were performed.

All WES tests were done in CLIA licensed, CAP certified clinical diagnostic commercial laboratories. The sequencing systems and types of kit are crucial for bioinformatics-pipeline configuration. The generated library is sequenced on Illumina NextSeq or Illumina HiSeq with ~ 100x average depth of coverage, and the minimum coverage for any variant to be considered is 10x.

The exclusion criteria were 1) the genetic diagnosis was not obtained by WES but with other tests such as fragile X or methylation analysis, 2) though negative, but with a suspected variant under investigation, 3) patients who declined testing, 4) families where there is a high suspicion of an underlying genetic disorder and the patient is being followed up in the genetic clinic, despite all the negative results.

The discharged cases were confirmed by the patient's geneticist, and the reasons for being discharged were extracted from the electronic health records. Discharge usually occurs when no underlying genetic explanation is identified, or the possible explanation of the phenotype is not likely linked to genetics or the patient's phenotype improved overtime.

All participants gave informed consent for inclusion in the study. The study protocol was approved by the Ethics Committee of the Institutional Research Board of King Abdullah International Medical Research Center (KAIMRC) with a study reference number RC19/315.

## Results

A total of 151 participants with a negative WES result were included. The demographic information of the cohort, as well as detailed clinical phenotype, in the human phenotype ontology (HPO) format, the age at testing, and the NGS results for informative negative cases (n = 27) is available in Table 1.

Table 1  
cohort demographics

Patient No.	Gender	Test Type	S, D, T, P	Date of Testing	Phenotype	Age at Testing	Results	Consanguinity	First Cousin	Family History
1	Male	WES	Solo	7-Oct-16	Intellectual disability HP:0001249	12.7	Negative	No	No	Yes
2	Male	WES	Trio	7-Oct-16	Delayed speech and language development HP:0000750, flexion contracture of finger HP:0012785	3.5	Negative	No	No	No
3	Male	WES	Solo	2-May-17	short stature HP:0004322, Growth hormone deficiency HP:0000824	13.8	Negative	Yes	Yes	Yes
4	Male	WES	Plus	11-Aug-17	Obesity HP:0001513, Enuresis nocturna HP:0010677	9.7	Negative	Yes	Yes	Yes
5	Female	WES	Plus	7-Jun-18	ulcerative colitis HP:0100279, constipation HP:0002019, bloody diarrhea HP:0025085	8.6	Negative	Yes	Yes	Yes
6	Male	WES	Trio	16-Mar-16	Oligohydramnios HP:0001562, Thrombocytopenia HP:0001873, Abnormality of the coagulation cascade HP:0003256, Hydronephrosis HP:0000126, Hypermethioninemia HP:0003235, Hypertyrosinemia HP:0003231, Hypoalbuminemia HP:0003073, Intrahepatic cholestasis with episodic jaundice HP:0006575, Elevated transferrin saturation HP:0012463, Decreased liver function HP:0001410	0.4	Negative	No	No	No
7	Female	WES	Trio	4-Apr-16	Obesity HP:0001513, Hepatomegaly HP:0002240, Elevated hepatic transaminase HP:0002910	13.1	Negative	No	No	Yes
8	Male	WES	Trio	28-Jun-16	Cleft palate HP:0000175, feeding difficulties HP:0011968, cough HP:0012735, Abnormality of the ear HP:0000598, Hyperostosis HP:0100774	1.6	Negative	No	No	Yes

Patient No.	Gender	Test Type	S, D, T, P	Date of Testing	Phenotype	Age at Testing	Results	Consanguinity	First Cousin	Family History
9	Male	WES	Solo	12-Oct-17	Perimembranous ventricular septal defect HP:0011682, Tracheoesophageal fistula HP:0002575, Ectopic kidney HP:0000086	1.5	Negative	Yes	Unknown	No
10	Female	WES	Solo	10-Nov-17	Intellectual disability HP:0001249, obesity HP:0001513, Hyperactivity HP:0000752, Sleep disturbance HP:0002360, Myoclonic seizure HP:0032794	13.3	Negative	Yes	No	No
11	Female	WES	Duo	23-Nov-17	short stature HP:0004322, Global developmental delay HP:0001263, spasticity HP:0001257, toe walking HP:0040083	14.0	Negative	Yes	Unknown	No
12	Female	WES	Solo	25-Aug-17	Intellectual disability-mild HP:0001256, failure to thrive HP:0001508, short stature HP:0004322, Abnormal facial shape HP:0001999	14.5	Negative	Yes	Yes	No
13	Male	WES	Plus	8-Sep-17	Global developmental delay HP:0001263, Delayed speech and language development HP:0000750, Autistic behavior HP:0000729, Attention deficit hyperactivity disorder HP:0007018, Socially inappropriate behavior HP:0030220	7.5	Negative	Yes	Yes	Yes
14	Male	WES	Plus	24-Apr-18	Atrial septal defect HP:0001631, Delayed speech and language development HP:0000750, Developmental regression HP:0002376, Dysphagia HP:0002015, Global developmental delay HP:0001263, Seizures HP:0001250, Stereotypy HP:0000733	10.2	Negative	Yes	Unknown	Yes

Patient No.	Gender	Test Type	S, D, T, P	Date of Testing	Phenotype	Age at Testing	Results	Consanguinity	First Cousin	Family History
15	Male	WES	Trio	19-Feb-18	Aggressive behavior HP:0000718, Attention Deficit-hyperactivity Disorder OMIM:143465, behavioral abnormality HP:0000708, Delayed speech and language development HP:0000750, jaundice HP:0000952, lactic aciduria HP:0003648, talipes equinovarus HP:0001762	8.0	Negative	Yes	Yes	No
16	Male	WES	Trio	21-Jul-18	Abnormality of brainstem morphology HP:0002363, Abnormality of the cerebral ventricles HP:0002118, Abnormality of the corpus callosum HP:0001273, Absent septum pellucidum HP:0001331, Agenesis of corpus callosum HP:0001274, Congenital onset HP:0003577, Global developmental delay HP:0001263	0.9	Negative	Yes	Unknown	No
17	Male	WES	Solo	11-Jun-18	Delayed speech and language development HP:0000750, short thumb HP:0009778, functional abnormality of the middle ear HP:0011452, abnormal facial shape HP:0001999	5.2	Negative	Yes	Yes	Yes
18	Male	WES	Trio	7-Jun-18	Autism HP:0000717, Autistic behavior HP:0000729, Delayed speech and language development HP:0000750, Motor delay HP:0001270	6.3	Negative	Yes	Yes	No
19	Male	WES	Solo	12-Oct-17	Polycystic kidney dysplasia HP:0000113	15.2	Negative	Yes	Yes	Yes

Patient No.	Gender	Test Type	S, D, T, P	Date of Testing	Phenotype	Age at Testing	Results	Consanguinity	First Cousin	Family History
20	Male	WES	Trio	22-Aug-17	intellectual disability HP:0001249, global developmental delay HP:0001263, motor delay HP:0001270, generalized hypotonia HP:0001290, Joint hypermobility HP:0001382, Abnormal facial shape HP:0001999, Low-set ears HP:0000369, Strabismus HP:0000486, Abnormality of mouth shape HP:0011338, micropenis HP:0000054	10.2	Negative	Yes	Unknown	Yes
21	Male	WES	Trio	28-Jun-17	Autism HP:0000717, Attention deficit hyperactivity disorder HP:0007018, Macrocephaly HP:0000256, Intellectual disability HP:0001249, Scoliosis HP:0002650	7.3	Negative	Yes	Yes	Yes
22	Male	WES	Plus	5-Dec-17	Cafe-au-lait spot HP:0000957, Prolonged neonatal jaundice HP:0006579, Abnormal facial shape HP:0001999, Low-set ears HP:0000369, hypertelorism HP:0000316, Aplasia cutis congenita HP:0001057, Thoracic hypoplasia HP:0005257	1.5	Negative	No	No	No

Patient No.	Gender	Test Type	S, D, T, P	Date of Testing	Phenotype	Age at Testing	Results	Consanguinity	First Cousin	Family History
23	Male	WES	Trio	20-Apr-17	Intrauterine growth retardation HP:0001511, microtia HP:0008551, Abnormal facial shape HP:0001999, Low anterior hairline HP:0000294, low set ears HP:0000369, depressed nasal bridge HP:0005280, Abnormal eye morphology HP:0012372, retrognathia HP:0000278, flat occiput HP:0005469, short neck HP:0000470, brachycephaly HP:0000248	0.8	Negative	Yes	Yes	No
24	Male	WES	Trio	26-Apr-17	Global developmental delay HP:0001263, Seizures HP:0001250, Spastic diplegia HP:0001264, ventriculomegaly HP:0002119, polyhydramnios HP:0001561, polymicrogyria HP:0002126, cerebellar hypoplasia HP:0001321, Abnormality of the femoral head HP:0003368	8.5	Negative	Yes	No	No
25	Male	WES	Solo	1-Aug-17	Developmental regression/speech HP:0002376, Intellectual disability HP:0001249	10.8	Negative	No	No	No
26	Female	WES	Duo	12-Mar-18	Morbid obesity HP:0001513, Short toe HP:0001831, waddling gait HP:0002515	12.6	Negative	Yes	Yes	Yes
27	Male	WES	Trio	31-May-18	Arthralgia HP:0002829, Corneal dystrophy HP:0001131, Keratoconus HP:0000563, Multiple joint contractures HP:0002828, Scarring HP:0100699	11.7	Negative	Yes	Unknown	Yes

The median age of the patients at the time of testing was 6.5 (1 month – 32 years) years. A paediatric patient is defined as a person younger than 14 years. There were only two adult cases.

A clinical characteristics analysis was performed on the sample (n = 151). As shown in the supplementary document, a total of 278 phenotypic anomalies, defined by HPO (The Human Phenotype Ontology), were observed in the patients, with global developmental delay

the most recurrent feature in 34 of the 151 cases. Five abnormalities, including motor delay, delayed speech and language development, global developmental delay, abnormal facial shape, and hypotonia, were present in 10 patients.

Nearly 18% (n = 27) of the cohort has been discharged, based on the negative result of the NGS test (Fig. 1). The negative cases were unevenly distributed between the paediatric and adult patients, as 26 of the cases were paediatric patients.

The most frequent reason for not reaching a genetic diagnosis, was a mild phenotype (Table 2). Only four cases had a confirmed multifactorial condition, defined as diseases caused by more than one factor interacting for instance genetic and environmental factors. These cases have been investigated extensively through the Online Mendelian Inheritance in Man (OMIM), and multifactorial was assigned. For two cases, the patient's development was according to the milestones expected for his age and there was a negative family history. Of the negative cases, only three cases required additional testing, for example WGS, CMA and MGS prior to discharge. In terms of the WES test type, 12 cases had trio analysis, 8 cases solo analysis, 6 cases trio plus analysis, and 2 cases duo analysis.

Table 2  
Reason for discharged cases.

Reasons for discharged	Count	Percentage
1. Multifactorial	7	28%
2. Normal development	3	11%
3. Mild phenotype	10	36%
4. All genetic work up is negative	7	25%
Total Nuanced WES	27	100%

A quarter of the cases (n = 7, 26%) was discharged within a month of releasing the result, and the majority (n = 20, 74%) was discharged within a year of the time of the report. The reason for requiring more time was due to extended molecular testing.

In 2015, none of the tested patients were discharged, at the date of this study. All the discharged cases were tested in 2016 to 2018. All the cases (n = 5, 100%) tested in 2016 were discharged within 6 months to one year of testing. In 2017, 14 cases (n = 14) were tested, and three cases (22%) were discharged within one month of testing, six cases (43%) within six months, and five cases (35%) discharged after a year. The last group were patients had a wide range of symptoms affecting different body systems. Interestingly, all cases (n = 8, 100%) tested in 2018, were discharged from the clinic in less than three months.

## Discussion

The study aimed to assess the clinical utility of WES testing to provide effective clinical care and reduce the burden on the healthcare system, and the impact of the negative result on the patient's management plan. An early and accurate genetic diagnosis could have a major impact on the clinical management, reduce cost and the psychological burden on the family and the community. In this cohort, 18% patients were discharged because their condition is not inherited or it is linked to a complex genetic disorder. This is the first study to assess the usefulness of a negative WES result for the patient and the community. Nigidula et al. (2018) conducted a survey to evaluate the actions of healthcare providers based on the result of WES and the effect on the medical management of 62 patients. Of the six patients who received a negative result, four were referred to another subspecialty, or discontinued a special diet and an inherited disorder was excluded. They demonstrated that even a negative WES result has an impact on the medical management (Nigidula et al. 2018).

Previously, we reported that the paediatric age group is more likely to receive a negative result, 46% compared to 31% in the adult group (Alfares et al. 2020). In this study, we report that the paediatric group is more likely to have an informative result, compared to adults and that early testing would assist in a large proportion of children with a negative result, to re-assure the family about the result.

Global developmental delay is a broad descriptive medical terminology term. The 2020 ICD-10-CM diagnosis code R62.50 for developmental delay is defined as an unspecified lack of expected normal physiological development in childhood, and the physician usually use this term for different phenotypes. In the current study, we showed that that the reason children with developmental delay have an informative negative result, was because they were healthy or it was unlikely that genetics were the underlying disorder.

A milder phenotype can sometimes be difficult to evaluate and would require a long clinical follow-up for reassurance. In this study, we showed that a milder phenotype is the most frequent reason why a patient is discharged from the genetic clinic after receiving a negative

result.

The negative WES result could lower the cost of additional testing, as of 27 patients with a negative result, only three patients required additional testing, such as WGS or aCGH. However, in 24 patients, the physician was satisfied to use the negative result, with no additional testing and reassure or discharge the patient. This not only lower the cost of testing, but reduce the number of patients being followed up, as almost 18% of the patients were discharged from the clinic due to the negative results. If we add the positive hit rate of the WES in our population ~ 50% (3) to the negative informative cases ~ 18%, the total clinical utility of the WES is ~ 68%. It is noteworthy that there is an increasing trend of physicians being more accepting of a negative result, which may be explained by an increased understanding and confidence in the technology, as well as an improvement in the genetic tools available to support analysis.

In conclusion, a negative WES result is an important informative result for patient care and management, and can be an effective tool to re-assure the treating physician and the patient about the possibility of an underlying inherited disorder or that the patient has a mild to no phenotype. Being able to discharge the patient with confidence, reduce the need to follow-up in the clinic or the need for extensive testing.

## Declarations

### AVAILABILITY OF DATA AND MATERIAL:

The authors declare that the data supporting the findings of this study is available within the paper and its supplementary information files.

### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

### AUTHORS CONTRIBUTIONS:

LA, TA, A.Farsi and A.Alfares designed the study, interpreted the clinical data, and wrote the article. LA, TA, A.Farsi and A.Asinan collected samples, genotyped the cases and helped in statistical analysis. M.A, FA, A.Alsward, A.Alothaim, and WE contributed in samples collection, clinical correlation and manuscript revision. R.Alniwaider and A.Almuitiri contributed to the WES sequencing pipeline. All authors have read and approved the final manuscripts. And to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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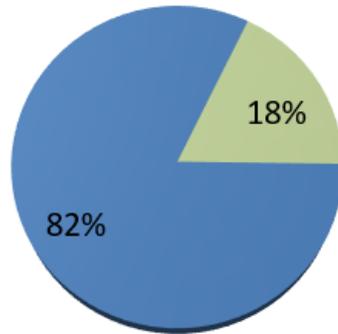
## Supplementary Data

No supplemental data was provided with this version

## Figures

# Negative WES

■ Informative WES   ■ uninformative WES



**Figure 1**

breakdown of negative cases.