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Mini Review

Does RNA and Whole Genome Sequencing Help in Identification of Ultra Rare Degenerative Diseases in Children?

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Abstract

Neurodevelopmental disorders [NDDs] occur due to errors in the biological processes in the maturation and development of the nervous system [1]. NDDs include autism spectrum disorder [ASD], intellectual disability, attention deficit hyperactivity disorder [ADHD], schizophrenia, and bipolar disorder [2]. The medical global estimate prevalence of autism, intellectual disability and schizophrenia are: 62/10,000, 10.37/1000, and 4/1000 respectively [3].

NDDs are frequently due to genetic mutations and can be detected with whole exome sequencing [WES]. In Almana General Hospitals Saudi Arabia, we are frequently confronted with seriously disabling cases of NDDs in children. Even with extensive use of methods of genetic diagnostic tools we fail to reach a molecular genetics diagnosis.

Novel causal mutations can be identified using an approach such as WES [4]. Multiple genetic defects, like GRM7, STX1A, CCAR2, EEF1D, GALNT2, SLC44A1, LRRIQ3, AMZ2, CLMN, SEC23IP, INIP, NARG2, FAM234B, and TRAP1, were identified using WES method [5]. Continuum model gradient hypothesis showed that schizophrenia shares origin of disturbance of brain cells as seen in intellectual disability, ASD, and ADHD.

We propose here a prospective cohort experimental study by a mechanism by collecting blood and punch of the skin biopsy for detection and identification of causal mutations of NDDs. RNA sequencing [RNA Seq] followed by whole genome sequencing [WGS] will be used to develop models that will improve the utility of test in enhancing our ability to diagnose each family mutations. The goals will be:

1. This will lead to a determination of the mutation diagnostic pattern in the index patient and his/her family.
2. It will also allow screening of future pregnancies, through a chorionic villous sampling at 11 - 14 weeks age of pregnancy [6] or amniocentesis at 15 - 20 weeks of pregnancy [7], enabling the family to decide whether to continue or not with the pregnancy if ever the fetus is affected.

The study population will include consanguineous families who have child/children diagnosed with a neurodegenerative disease. Skin biopsies and blood samples will be collected from these individuals affected by the disorder and their family members. DNA obtained from blood samples will be used to assess changes in DNA sequences, or to perform tests to look at differences in proteins, enzymes, and cell types in blood. Skin cells will be used to create Induced Pluripotent Stem Cells [iPSCs], Inducible Differentiated Reprogrammed Muscle [iDRM] cells, fibroblast cells, that can be induced to create different cell lines. For example, for muscular dystrophy, these cells will be induced to create muscle-like cells. The obtained cells will be stored in a repository (bank) and made available for use in future research.

We expect to identify the genetic cause of rare NDDs among children seen in the Eastern province of Saudi Arabia. If successful the identification of the genetic mutation seen in diagnosing each family condition will lead to the creation of possible treatments like gene therapy in order to solve their underlying genetic disease.

Keywords: *Induced Pluripotent Stem Cells [iPSCs]; Inducible Differentiated Reprogrammed Muscle [iDRM]; Neurodevelopmental Disorders [NDDs]; Autism Spectrum Disorder [ASD]; Attention Deficit Hyperactivity Disorder [ADHD]*

Rationale/Background/Significance

Whole Exome Sequencing [WES] can be used to detect developmental disorders due to genetic mutations. In Almana General Hospitals in Saudi Arabia we frequently are confronted with seriously disabling cases of neurodevelopmental and neuromuscular disorders in children. Even with extensive use of conventional methods of genetic diagnostic tools we fail to reach the molecular genetics diagnosis [8]. We propose here to develop a clinical prospective cohort study by a mechanism by collecting blood and a punch skin biopsy for the detection and identification of the causal mutations of neurodevelopmental disorders [NDDs]. RNA sequencing [RNA-Seq] followed by Whole Genome Sequencing [WGS] will be used to develop models that will improve the utility of the tests in enhancing our ability to diagnose each family mutations which will help in future pregnancies. We will screen at first hand the index patients by exome sequencing, if successful we will examine other family members [parents and siblings] by specifically developed molecular test through our designated Disease Testing Center.

Specific Aim

We are planning to collect samples from index cases and their family members over a period of 2 years [9]. The sample size and power of the study will be calculated using Stata. Based on the exome-sequencing and integrated with RNA sequencing we will be able to detect genome wide, small indols, and single nucleotide variants [SNVs]. By doing this we will be able to identify individuals with genetic diseases and can also identify *de novo* mutations which are non genetic variants. As there are multiple *de novo* mutations in each genome which will have potential mutations for rare autosomal recessive diseases [10]. Here we're proposing to use RNA-Seq from non-neural tissues, [DNA and skin biopsy] to delineate selection scheme open reading frame [ORF] defects and intersect it with whole genome DNA sequencing. We intend to have a higher rate of patient diagnosis at a molecular level that will give better understanding of the disease pathogenesis. In this study we will obtain consent and collect samples of blood and skin punch biopsy to gather cells. The cells isolated from the blood sample will be used to get DNA and RNA. The skin punch will yield fibroblast that will be used to grow new cells. It will be induced and differentiated to Pluripotent Stem Cells [iPSCs] and then transferred into neural and muscular phenotypes. We are not aiming to treatment but our study may lead to new therapies [10-12].

Research Design and Methodology

We plan to study these disorders by obtaining skin biopsies and blood samples [RNA] from individuals affected by the disorder together with their family members. The blood cells might be used to make DNA, which will be used to access changes in DNA sequences, or to perform tests to look at differences in proteins, enzymes, and cell types, which could be easily used to create a blood cell line. Studied in the laboratory some of the skin cells will be used to create cells called Induced Pluripotent Stem Cells [iPSCs] by treatment with genes from DNA, the skin cells treated with genes will be changed into cell types that can be induced to become many other cell types of the body. Example, for muscular dystrophy, we will induce the skin cells to become iPSCs. The iPSCs will be induced to become muscle-like cells. For Autism, we will induce the cells to become neurons. The same treatment with genes is applied to other cells called Inducible Differentiated Reprogrammed Muscle Cells [iDRMCs], which will be changed to muscle cells which will grow in culture forever. The cells and the resulting products, like iPSCs will be deposited in a repository (bank) for many years and made available for future researches. Thus, we will study the iPSCs in the laboratory in the hope of pursuing the goals of regenerative medicine. The goals of regenerative medicine include helping us understand how the body replaces damaged cells, how it try to create cell-based therapies to treat diseases, and how it try to repair or replace damaged tissues and organs. Part of the blood sample may also be used to grow a long-term cell line. This is called a lymphoblastoid cell lines [LCLs] and will be available for future research. These samples will be prohibited for use in cloning or to be used to grow artificial organs or organisms. Samples will not be sold to other researchers. There is a possibility that the samples and obtained information may lead to the development of new medical tests or treatment that could be sold. There is no plan to compensate anybody and/or parents from future commercial developments [14,15].

Inclusion and exclusion criteria's

Volunteers that are selected for this study include:

1. Index patients with dysmorphic features.
2. Patients with multiple anomalies.
3. Patients with unexplained neurocognitive impairment.
4. Family history suggestive of hereditary diseases.
5. Concrete genetic family history including:
    - Age and sex of affected or died patients.
    - Ethnic background.
    - Consanguinity.
6. Index patients age up to 18 years.
7. Siblings age less than 18 years.
8. Parents of affected or suspected subjects with NDDs.

Individuals excluded from this study include:

1. Patients with neurocognitive disorder with a specific diagnosis.
2. Patients without particular ethnic background.
3. Patients without history of consanguinity.
4. Syndromic patients with NDDs.
5. Patients above 18 years of age

After signing the informed consent form volunteers participating in this study, Index patients and their families would be ask to do all of the following things:

1. Have blood drawn for purifying DNA from the white blood cells, and to look at the genetic relationship to disease.
2. Have a skin biopsy from the forearm for culturing skin cells. The blood extraction and skin biopsy collection will be performed by one of the participating study physicians in Al Mana Group of Hospitals or physicians using accepted clinical practices. If the cells obtained from the blood drawing and/or skin biopsy does not grow, volunteers may be asked to return for a repeat blood extraction and/or skin biopsy collection. The amount of blood drawn may vary from 2.5 cc to 3 cc and will be taken from the veins using a needle. The blood sample will be used to take DNA from the cells. Skin biopsy will involve cutting a small piece of skin (no

larger than a 1/8th inch by 1/4th inch wide and 1/16th inch deep). A local pain reliever (an EMLA patch) will be placed over the area to be biopsied one hour before the procedure in order to reduce pain. Alternatively, a local anesthetic Lidocaine injection will be administered to lessen the pain prior to the biopsy. After the biopsy no pain medicines are needed. We will perform skin biopsy using an instrument to remove a piece of skin (approximately a 3/8th inch in diameter, 1/16th inch deep) usually from the forearm (called a "skin punch biopsy"). The total time to participate in this study is limited to the duration of the above procedures, almost less than 2 hours. As transportation may be a problem to some patients, if individuals are unable to travel to the Al Mana Group of Hospitals, we will attempt to arrange Al Mana Hospital physician's office/clinic close by the volunteers homes where a skin punch biopsy and venous blood sample, can be performed safely. If no site can be identified sufficiently close to the volunteer's home, the participation will be terminated. About 0 - 10% of people who have a skin biopsy experience significant local irritation that may persist for several days. This typically requires no treatment and resolves by itself. The risk of infection requiring topical or oral antibiotics is less than 1%. Pain medications, such as EMLA patch or Lidocaine, may have possible risk for side effects in the form of restlessness, anxiety, dizziness, blurred vision, tremors, and in very rare cases, an allergic reaction that might make breathing difficult. The risk of more serious complications, including fainting, temporary clotting of the vein, bleeding under the skin, infection of the surrounding tissues, or significant blood loss, is much less than 1/1,000.

Study protocol and power calculation

The sample size and power of the study will be calculated using Stata with considerations of the following:

1. Power
2. Alpha level [p-value]
3. Difference between the groups (hypothesized difference and variability in the data)
4. Specified standard deviation
5. We will use Z-test.

In our study patients are collected from 4 different cities. Chi square test will be used in our study for changes in clinical observations; the result will be compared to previous mutations from literature. We will design two randomized arms in our population and the controls, we will use Logistic Regression to assess our patients with mutations and compare it to controls. We are required to share the genetic information in controlled access databases of the chosen study center; we will also share clinical and research information in research databases and with researchers not associated with the primary research center. We will also share clinical and research samples with researchers not associated with our selected test center. We may want to use samples and information for research beyond the present disease and its characteristics in the future, for example, the samples created from skin biopsies may be sent to experts outside of the center for research projects not related to the present disease. However, these samples will not be used for cloning or to grow artificial organs or organisms. The samples will not be sold to other researchers. Although it is possible that the samples and information may lead to the development of new medical tests or treatment that could be sold, there is no plan to compensate anybody from future commercial developments and/or patents.

Anticipated Results

The information which will become available about these disorders may be important to the diagnosis and development of new treatments. This could be anticipated as benefits to society**.** It should be emphasized that this is not treatment research; volunteers will not

be paid for participation in the research, therefore, an alternative is not to participate. As we are planning to conduct this research in one of the university research centers, all tissue and/or fluid samples are owned by the University chosen for research, or by a third party designated by the University (such as another university or a private company). The study will observe rules of confidentiality the only people who will know that volunteers are a research subject are members of the research team, and if appropriate, their physicians, and nurses. The information provided by them during the research will not be disclosed to others without their written permission, except, if necessary to protect their rights or welfare (for example, if they are injured and need emergency care); or if required by law. When the results of the research are published or discussed at conferences, no information will be included that would reveal their identity. The biological materials and related medical information will be coded. The link between their code and their identity will be stored securely in the primary invitatory office at the designated University. During the course of the study, volunteers will be informed of any significant new findings. If new information is provided to the volunteer, their consent to continue participating in this study will be re-obtained. The volunteer may withdraw their consent at any time and discontinue participation without penalty.

In this study involving patients and their families with suspected genetic conditions like NDDs, whole genome sequencing and RNA sequencing will show higher diagnostic yield compared to traditional diagnostic molecular methods. Additional studies designated to validate, explore the clinical and economic outcomes of these findings are warranted.

Potential Study Limitation and Solutions

In our research, samples are non-randomized, with potential confounders; to overcome this we will repeat the study in other locations. We aim to increase external validity and increase sample size. The cost is an issue to our study as RNA and whole genome sequencing are relatively expensive. The issue of expenditures will be discussed with the potential funding group. Sensitivity might not be able to detect all mutation types. Technical difficulties arise in handling the storage of samples, especially RNA, to overcome this difficulty of RNA denaturation, we will shorten the time between collection and processing of samples. As we are investigating ultra rare diseases, the designated period of two years could not be an enough period to complete the study, increasing the sample size and power for this study might overcome this problem.

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