17_CONCORSO PUBBLICO, PER TITOLI ED ESAMI, PER LA COPERTURA A TEMPO DETERMINATO, DELLA DURATA DI CINQUE ANNI PER N. 1 POSTO DI RICERCATORE SANITARIO, CAT. D, LIVELLO D SUPER DA ASSEGNARE ALLA UOC NEUROLOGIA 3 – NEUROALGOLOGIA

PROVA NON ESTRATTA

PROVA 1

Erika Solvi 05/05/2022

1. Potenzialità e limiti del Whole Exome Sequencing

2. Sono sistemi operativi:
   a. Windows e Linux
   b. Word ed Excel
   c. Windows e PowerPoint

3. Leggere e tradurre il testo sul retro

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D'Auria

frontiers in Genetics

ORIGINAL RESEARCH
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Characterization of *FMR1* Repeat Expansion and Intragenic Variants by Indirect Sequence Capture

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Traditional methods for the analysis of repeat expansions, which underlie genetic disorders, such as fragile X syndrome (FXS), lack single-nucleotide resolution in repeat analysis and the ability to characterize causative variants outside the repeat array. These drawbacks can be overcome by long-read and short-read sequencing, respectively. However, the routine application of next-generation sequencing in the clinic requires target enrichment, and none of the available methods allows parallel analysis of long-DNA fragments using both sequencing technologies. In this study, we investigated the use of indirect sequence capture (Xdrop technology) coupled to Nanopore and Illumina sequencing to characterize *FMR1*, the gene responsible of FXS. We achieved the efficient enrichment (> 200×) of large target DNA fragments (~60–80 kbp) encompassing the entire *FMR1* gene. The analysis of Xdrop-enriched samples by Nanopore long-read sequencing allowed the complete characterization of repeat lengths in samples with normal, pre-mutation, and full mutation status (> 1 kbp), and correctly identified repeat interruptions relevant for disease prognosis and transmission. Single-nucleotide variants (SNVs) and small insertions/deletions (indels) could be detected in the same samples by Illumina short-read sequencing, completing the mutational testing through the identification of pathogenic variants within the *FMR1* gene, when no typical CGG repeat expansion is detected. The study successfully demonstrated the parallel analysis of repeat expansions and SNVs/indels in the *FMR1* gene at single-nucleotide resolution by combining Xdrop enrichment with two next-generation sequencing approaches. With the appropriate optimization necessary for the clinical settings, the system could facilitate both the study of genotype–phenotype correlation in FXS and enable a more efficient diagnosis and genetic counseling for patients and their relatives.

Keywords: long fragment enrichment, indirect sequence capture, repeat expansion, single nucleotide variants, *FMR1*

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PROVA NON ESTRATTA Erika Solci 05/05/2022

PROVA 2

1. Utilizzo dell'RNA sequencing in ambito di diagnostica genetica

2. A cosa serve il programma Microsoft Excel?
   a. A realizzare fogli elettronici per analisi di dati
   b. A realizzare presentazioni
   c. A gestire spool di stampa

3. Leggere e tradurre il testo sul retro

Oggetti: 15 31

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Review

Technological Improvements in the Genetic Diagnosis of Rett Syndrome Spectrum Disorders

Clara Xiol [1,2], Maria Heredia [1,2], Ainhoa Pascual-Alonso [1,2], Alfonso Oyarzabal [1,2,3] and Judith Armstrong [2,3,4,*]

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Abstract: Rett syndrome (RTT) is a severe neurodevelopmental disorder that constitutes the second most common cause of intellectual disability in females worldwide. In the past few years, the advancements in genetic diagnosis brought by next generation sequencing (NGS), have made it possible to identify more than 90 causative genes for RTT and significantly overlapping phenotypes (RTT spectrum disorders). Therefore, the clinical entity known as RTT is evolving towards a spectrum of overlapping phenotypes with great genetic heterogeneity. Hence, simultaneous multiple gene testing and thorough phenotypic characterization are mandatory to achieve a fast and accurate genetic diagnosis. In this review, we revise the evolution of the diagnostic process of RTT spectrum disorders in the past decades, and we discuss the effectiveness of state-of-the-art genetic testing options, such as clinical exome sequencing and whole exome sequencing. Moreover, we introduce recent technological advancements that will very soon contribute to the increase in diagnostic yield in patients with RTT spectrum disorders. Techniques such as whole genome sequencing, integration of data from several "omics", and mosaicism assessment will provide the tools for the detection and interpretation of genomic variants that will not only increase the diagnostic yield but also widen knowledge about the pathophysiology of these disorders.

Keywords: Rett syndrome; Rett-like; NGS; WES; WGS; RNAseq; genetics; MECP2

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1. Rett Syndrome Spectrum Disorders: Clinical Picture

1.1. Rett Syndrome

Rett syndrome (RTT, OMIM #312750) is a severe neurodevelopmental disorder characterized by a regression of acquired skills, including purposeful hand use and language, after a normal psychomotor development in the first months of life [1]. RTT has an incidence of approximately 1:10,000–20,000 live female births and is the second most common cause of severe intellectual disability in females [2,3]. RTT was first reported in 1966 by the Austrian doctor Andreas Rett, and in 1983, Bengt Hagberg further described the syndrome in a larger cohort of patients [3].

Although belonging to the same clinical entity, patients with RTT show heterogeneous phenotypes, with varying symptoms and severity. In the classic form, patients display a regression in psychomotor development, partial or complete loss of acquired purposeful hand skills and spoken language, gait abnormalities and stereotypic hand movements, which are the required features to diagnose typical RTT. These symptoms are frequently accompanied by breathing disturbances, bruxism, impaired sleep patterns, abnormal muscle tone, and scoliosis, which constitute supportive criteria [4,5]. It is also common that patients with RTT present with acquired microcephaly and epilepsy [3–5].

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PROVA 3

PROVA ESTRATTA Erika Sdei 05/05/2022

1. Short-reads vs. long-reads: vantaggi e svantaggi dei due approcci per l'analisi del DNA

2. Con il termine "Base di dati" si intende:

   a. una collezione di dati, inerenti una specifica attività, opportunamente strutturati e accessibili tramite un software di gestione

   b. un linguaggio di programmazione

   c. un insieme di dati distribuiti sulla rete e accessibili solo tramite un browser

3. leggere e tradurre il testo sul retro

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ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

Transcriptome analysis of collagen VI-related muscular dystrophy muscle biopsies

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Abstract

Objective: To define the transcriptomic changes responsible for the histologic alterations in skeletal muscle and their progression in collagen VI-related muscular dystrophy (COL6-RD). **Methods**: COL6-RD patient muscle biopsies were stratified into three groups based on the overall level of pathologic severity considering degrees of fibrosis, muscle fiber atrophy, and fatty replacement of muscle tissue. Using microarray and RNA-Seq, we then performed global gene expression profiling on the same muscle biopsies and compared their transcriptome with age- and sex-matched controls. **Results**: COL6-RD muscle biopsy transcriptomes as a group revealed prominent upregulation of muscle extracellular matrix component genes and the downregulation of skeletal muscle and mitochondrion-specific genes. Upregulation of the TGFβ pathway was the most conspicuous change across all biopsies and was fully evident even in the mildest/earliest histological group. There was no difference in the overall transcriptional signature between the different histologic groups but polyserial analysis identified relative changes along with COL6-RD histological severity. **Interpretation**: Overall, our study establishes the prominent dysregulation of extracellular matrix genes, TGFβ signaling, and its downstream cellular pathways at the transcriptomic level in COL6-RD muscle.