Reducing diagnostic turnaround times of exome sequencing for families requiring timely diagnoses

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ABSTRACT

Background and objective: Whole-exome sequencing (WES) has now entered medical practice with powerful applications in the diagnosis of rare Mendelian disorders. Although the usefulness and cost-effectiveness of WES have been widely demonstrated, it is essential to reduce the diagnostic turnaround time to make WES a first-line procedure. Since 2011, the automation of laboratory procedures and advances in sequencing chemistry have made it possible to carry out diagnostic whole genome sequencing from the blood sample to molecular diagnosis of suspected genetic disorders within 50 h. Taking advantage of these advances, the main objective of the study was to improve turnaround times for sequencing results.
Methods: WES was proposed to 29 patients with severe undiagnosed disorders with developmental abnormalities and faced with medical situations requiring rapid diagnosis. Each family gave consent. The extracted DNA was sequenced on a NextSeq500 (Illumina) instrument. Data were analyzed following standard procedures. Variants were interpreted using in-house software. Each rare variant affecting protein sequences with clinical relevance was tested for familial segregation.
Results: The diagnostic rate was 45% (13/29), with a mean turnaround time of 40 days from reception of the specimen to delivery of results to the referring physician. Besides permitting genetic counseling, the rapid diagnosis for positive families led to two pre-natal diagnoses and two inclusions in clinical trials.

Conclusions: This pilot study demonstrated the feasibility of rapid diagnostic WES in our primary genetics center. It reduced the diagnostic odyssey and helped provide support to families.

1. Introduction

Rare diseases are defined as those that affect a limited number of individuals (no more than one in 2000 individuals in the European Union and no more than about one in 1250 in the USA) and comprise >5000 disorders according to the WHO. For most rare diseases, no epidemiological data are available. They remain an important public-health issue and a diagnostic challenge for the physicians as they include a very heterogeneous group of disorders that can affect any system of the body (Schieppati et al., 2008). Concerning the diagnosis of rare diseases, the etiological identification of developmental disorders often engenders a lengthy and costly differential diagnostic search with no guarantee of a definitive diagnosis (Soden et al., 2014). A European survey on eight rare disorders showed an inaccurate initial diagnosis in 40% of subjects. Approximately 25% of cases waited up to 30 years for an etiological diagnosis, and the diagnosis remained uncertain for the majority (Faurisson and Kole, 2009). Indeed, such patients with multiple unresolved congenital abnormalities traditionally underwent long, sometimes invasive, and always multiple evaluations that included history-taking and clinical examinations, metabolic testing, chromosomal microarray, targeted single gene or gene panel testing, and occasionally tissue pathology. In the spectrum of intellectual deficiency (ID), if clinical expertise does not allow the study of targeted genes, the various aforementioned tools currently lead to a diagnosis in only 20% of patients on average (higher percentages if syndromic ID) (Willemsen and Kleefstra, 2013). Indeed, although previous strategies may lead to an etiological diagnosis in up to 50% of affected individuals when combined with careful clinical delineation, none of the conventional screening tests individually reach a diagnostic yield over 10% (Michelson et al., 2011). In this context, whole-exome sequencing (WES), which is the targeted sequencing of the subset of the human genome that codes for proteins, recently emerged as a relevant clinical tool to help resolve undiagnosed genetic conditions. This approach improves the diagnostic yield and the management of patients. The diagnostic rate of proband WES in a series of more than 2000 cases with suspected Mendelian disorders was 25% (Yang et al., 2014). Reaching a diagnosis improved the global and specific care in these patients, with significant implications for genetic counseling, reproductive planning and prenatal testing, prognosis, treatment, prevention, and quality of life, together with the healing touch of having a long-awaited answer with a name for the disorder (Schieppati et al., 2008). In cases of undiagnosed conditions, if prescribed early in the work-up screening, after normal array-CGH and Fragile X screening, if appropriate, WES may considerably shorten the diagnostic odyssey.

Currently, diagnostic WES is still considered a highly complex and expensive test that requires a chain of significant informatics infrastructure for data storage and management, specialized expertise in bioinformatics and medical genomics, all of which could lead to difficulty in achieving a short turn-around time in routine practice at smaller genetics centers. The standard turn-around times in several laboratories vary from 11 to 21 weeks, with an average of 18 weeks (Atwal et al., 2014). In the leader, the Baylor College of Medicine, the standard turnaround time for results is 15 weeks (Yang et al., 2013). However, there is a critical need to reduce turnaround times to make WES applicable to several particular diagnostic situations in everyday practice, and in particular for neonates and children hospitalized in intensive care units, where the high infant mortality rate indicates a substantial need for rapid genomic diagnoses, or for urgent requests for genetic counseling in cases of pregnancies with a family history of children with an undiagnosed probably genetic disorder. The automation of laboratory procedures and advances in sequencing chemistry since 2011 have made it possible to go from diagnostic whole genome sequencing (WGS) from the blood sample to the molecular diagnosis of suspected genetic disorders in around 50 h, even if this time frame is not compatible with a "real-life" molecular genetics lab (Saunders et al., 2012).

The aim of our pilot study was to demonstrate the feasibility and identity pitfalls of reducing the turnaround times for diagnostic WES in a primary genetics center for patients requiring rapid diagnostic orientation for their care or genetic counseling for a family member.

2. Material and methods

2.1. Enrolled patients

We performed WES in 29 unrelated patients recruited at five French genetics centers (Dijon, Nancy, Strasbourg, Paris, and Montpellier). The patients were included after an expert consultation with a clinical geneticist from a reference centre for congenital anomalies. The inclusion criteria were the association of an undiagnosed developmental disorder and i) an on-going pregnancy of at-risk relatives requesting genetic counselling; ii) hospitalization in an intensive care unit with a diagnostic request for guiding care. Written informed consent was obtained from all subjects (or their legal representatives) and their parents before enrolment in the study. The clinical and genetic data from all patients in the cohort was recorded in Phenome Central (See URLs), an online international anonymous data-sharing server. The consequences of a diagnosis following positive WES were assessed at the individual level in terms of prenatal diagnosis, specific treatment prospects and the reorientation of monitoring.

2.2. Exome sequencing

Genomic DNA was extracted from peripheral-blood samples from the proband and both parents via standard procedures using the GentraPuregene tissue kit (Qiagen). In several cases, genomic DNA extracted from fetal frozen tissue after termination of pregnancy was used. Whole-exome capture and sequencing were performed in the proband only at the Integragen platform (Integragen SA, Evry, Fance) on 1.5 μg of genomic DNA per individual using the SureSelect Human All Exon V5 kit (Agilent) for 22 samples and the SureSelect XT Clinical Research Exome kit (Agilent) for 7 samples. The resulting libraries were sequenced on a NextSeq 500 (Illumina) according to the manufacturer's recommendations for paired-end 75 bp reads.

2.3. Analysis and validation of variants

Reads were aligned to the human genome reference sequence