Personal Research Paper

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Purpose

The general topic of the article I chose to examine was based on the detection of inherited mutations. This is interesting to me because I am planning on going into genetics research while looking at diseases/disorders including cancer. Since this article examines the different processes used for genetic sequencing using genes that, when mutated, increase the risk of cancer it is relevant to the work I will be doing in the future. The actual study was to test the new method of genetic sequencing paired with hybridization-based enrichment. This new method of sequencing is called New Generation Sequencing, NGS.

New Generation Sequencing has increased sensitivity and throughput which allows researchers to increase the amount of genetic information gathered in one single reaction/run compared to the previous, and widely accepted, method called Sanger Dideoxy Sequencing. Specifically, the genes that can increase the risk of cancer are large sequences, have no specific location for mutations, and can be influenced by more than one gene. This means that the whole gene, and even multiple genes, would need to be sequenced to identify relevant mutations. If you used Sanger Sequencing for this process then you would need to run multiple reactions in order to sequence the whole gene/many genes; whereas, NGS allows researchers to sequence a whole gene/many genes with only one reaction. (Guan et al., 2015).

Study

This study focused on testing the accuracy of the NGS process and comparing the results to: 2 patients with previous Sanger Sequencing results of *BRCA1/2* without any mutations, 4 candidates with known *BRCA1/2* copy number variations (CNV; changes in the gene that include insertions, deletions, and duplications (2018)), 85 individuals that have cancer with no previous genetic sequencing done, and as a control 89 individuals that are healthy with no previous genetic sequencing done.

Before being able to identify the CNV sequences of each sample, the adaptor region had to be removed to prevent contamination. The adaptor region is the sequence of the primer used to amplify a genetic sequence; therefore, this is not an accurate representation of the actual sequence.(2016). This and the rest of the bioinformatics process of the researchers to identify mutations in the gene is described in the workflow chart in figure 1. Figure 2 consists of a workflow chart of how the researchers interpreted the variants of sequences. This was done in multiple steps, but ultimately always concluded possible deleterious mutations leading to validation of the NGS process.

Table 1 directly shows the accuracy and sensitivity of their sequencing method based on two different criteria. Without getting too specific, mainly because I'm not 100% sure how to interpret it, measurements used in Table 1A were much stricter than those in Table 1B. Under the criteria for A the accuracy of NGS was calculated as 99.99% and sensitivity as 92.12%, and under criteria for B the accuracy of NGS was calculated as 99.97% and sensitivity as 93.66%. Based on the increased sensitivity of criteria B, it was chosen to be further analyzed.

Figure 3 shows the specific exons of the *BRCA 1* gene and the known CNV results after sequencing. This diagram is mainly a visual aid for the different types of mutations that can occur with entire exons leading to breast cancer. These were previously known before this study though.

Table 2 demonstrates the different types of mutations that were observed in specific genes after sequencing, whether they were previously known or not, and what type of cancer the individual had. The types of mutations observed include nonsense, frameshift, and splicing errors. While looking at the *BRCA 1/2* genes they received two nonsense mutations that were previously known and one that was new; two

of the nonsense mutations were isolated from individuals with breast cancer and one came from an individual with prostate cancer. All the frameshift mutations of the *BRCA 1*/*2* genes were novel meaning that they have not been previously identified, and all the individuals that had these frameshift mutations had breast cancer. Finally, all splicing errors observed in the *BRCA 1*/*2* genes were previously identified, and every individual screened in this study that demonstrated these errors had breast cancer.

This helps to show that the method of NGS is accurate to identify mutations in genes that can lead to an increased risk to cancer. Plus, using the hybridization-based enrichment process allowed researchers to evaluate multiple cancer-causing genes to identify any possible mutations that may enhance another mutation. This was seen in table 2 while looking at the MLH_1 gene. An individual with breast cancer, colorectal cancer patients, and uterine cancer was seen to have a nonsense mutation in the MLH_1 gene. Even though this patient developed breast cancer, the mutation was in the MLH_1 gene. By using hybridization-based enrichment you can compare mutations in multiple genes to have a better understanding of the individuals' risk.

Relevance

This study is relevant to cell biology because the *BRCA 1*/*2* genes are tumor suppressor genes. Mutations in these genes can lead to the necessary tumor suppressor proteins to not be produced or be non-functional. Tumor suppressor genes produce proteins that inhibit/slow the cell cycle, this includes checkpoints, repair enzymes, and proteins needed for apoptosis (Cooper, 2000). A relevant example of this would be p53 which is known to be an important factor in some leukemias if mutated. Since the mutations in *BRCA 1* and *2* increase the risk of cancer and is inheritable it is crucial to understand how the mutation is affecting the produced protein and how its' function has changed, whether the protein is made at all or if its structure has changed. By identifying a more accurate and sensitive process to identify mutations we have the possibility to develop personalized treatments for patients. The genes evaluated in this study cause heritable mutations leading to increased risk to cancer, if genetic testing is done and reveals that they contain a known mutation then they can better prepare themselves and future generations that may inherit the same mutation.

Reference

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