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EDITORIAL

MASSIVELY PARALLEL DNA SEQUENCING AND THE NEW APPROACH TO MUTATION DETECTION: A STEP TOWARDS A LYMPHEDEMA FINE PANEL

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In this issue of the journal, Michelini et al (1) present their search for mutations in the tyrosine kinase domain of FLT4 and in FOXC2. Since the authors used traditional sequencing techniques, this was a large amount of effort. Arguments can be made that the effort would have been reduced by using the flow chart of Connell et al (2) to select focused subgroups of patients for sequencing only one or the other of the two genes in each patient. However, the current availability of inexpensive exome only sequencing (all the amino acid coding sequences, i.e., the exons) changes the need to select one or a few genes for study.

We are rapidly approaching the "One thousand dollar" human genome sequence which has been a target for some time but, unfortunately, it currently requires the "Ten thousand dollar" analysis. Commercial laboratories are offering 30-fold coverage (any 1 sequence has been replicated an average of 30 times) of the exome for under $700 U.S. Since current commercial diagnostic laboratories only sequence the exons and splice donor and acceptor sites (usually at $1,000 or more per gene – depending on its size and complexity), there is an obvious disconnect in the information available from the former compared to the latter at comparable prices. The missing element is the analysis of the exome sequences for mutations in particular genes. Current software that comes with sequencing machines supports mutational analysis of specific genes, but not sets of genes.

Rather than analyze the coding sequences of all 26,000 or so genes, it should soon be possible to have programs that will analyze several dozen, or more, genes relevant to a particular phenotype at much reduced cost. Thus, to take lymphedema as an example, the ten to fifteen genes currently implicated to some degree as causative of lymphatic dysfunction could be analyzed for possible causative mutations at a fraction of the cost of analyzing all of them. One would order the "lymphedema" panel from a list of panels that would include others, for example the "neurodegeneration" or the "mental retardation" panel. This approach will not detect copy number variations (CNVs), duplications or deletions in one of the 2 copies of DNA, which are now more frequently implicated in the causation of disease (3). However, runs of homozygosity,

i.e., seeing only 1 base pair at every position instead of 2 bases at some of the positions due to single nucleotide polymorphisms (SNPs) in the gene (heterozygosity for common SNPs is mostly found at 1 in 1,000-2,000 base pairs) can suggest a deletion. In other words, one is only seeing one of the 2 copies of the gene(s) at that location. The cost of doing the "lymphedema panel," with a fair profit for the commercial unit, should only be about $2,000 U.S.

In the very near future, the total genomic sequence will be available for under a thousand dollars. This vastly larger amount of information (roughly 30-fold) will greatly increase the cost of the analyses but will detect regulatory mutations (still being sorted out) as well as the coding mutations found in the exome-only sequences. The programs targeting particular sets of genes for various phenotypes will even more greatly decrease the cost of analysis for the total genomic sequence.

Such targeted analyses also prevent finding mutations in genes with implications for health which one would (usually) rather not know, e.g., Huntingtons disease and in many genes where loss of function has unknown meaning (4) or might be due to somatic mosaicism (5). On the other hand, one could add analyses for mutations in genes for which preventive measures can be taken-"medically actionable" gene alterations. An example of this would be the genes in which mutations predispose to cardiac arrhythmias and sudden cardiac death for which pacemakers are preventive.

In summary, the fantastic advances in massively parallel DNA sequencing are rapidly bringing a new era of inexpensive mutation detections for genes causing Mendelian (single gene) disorders.

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