Review Article

Neurological Disorders, Genetic Correlations, and the Role of Exome Sequencing

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Abstract

Genomic information access and utilization by researchers and clinicians have barely begun the journey for fulfillment of their full potential in the research and clinical arenas. Exciting is the potential depth and breadth of research, clinical applications, and more personalized medicine, that remain on the horizon. Exome sequencing has clarified the responsibilities of over 130 genes, greatly expanding the medical genetics database and enabling the development of orphan disease-based pharmaceuticals. The focus of our research efforts was to review several literature sources related to rare genomic disease research and exome sequencing, as well as to review the new research and diagnostic strategies that were utilized. Using a systems approach, under discussion are neurology, neuropathy, and the central nervous and musculoskeletal systems. Also discussed will be the topics of inborn errors of metabolism, and the genetic targets related to developmental delay. Recommendations for future research will also be discussed.

Keywords: genomics, exome sequencing, rare disease, genetics, genome, diagnosis, personalized medicine

Exome Sequencing

A Review of New Strategies for Rare Genomic Disease Research

This literature review will examine several publications in which the authors demonstrate the success of whole exome sequencing (WES) in circumstances where traditional methods have presented ambiguous interpretations or failed altogether, for instance, single-subject populations, large candidate counts and reduced reproductive fitness.[1] By supporting exome sequencing, we will show that there is an increasing need for polymorphism databases and assay strategies that do not depend on positional information, if genomic medicine is to advance its research and clinical utility. Within our review, we will discuss the research related to neurology, neuropathy, the central nervous system, the musculoskeletal system, inborn errors of metabolism, and the genetic targets related to developmental delay. Recommendations for future research will also be discussed.

Disease Survey

Neurology

Positional cloning techniques have thus far unveiled 19 contributory genes in the study of 31 genetic variations of autosomal dominant spinocerebellar ataxia.[2] The method has yet to overcome the challenges that presented, such as phenotypic and biological manifestations which do not correlate well to molecular mutations due to the exceedingly complex nature of the brain. Exome sequencing has, however, shed additional light on causal mutations for sensory motor neuropathy with ataxia[3], which presents with cerebellar dysfunction, Spinocerebellar Ataxia with Psychomotor Retardation[4], and Spastic Ataxia-Neuropathy syndrome that manifested with remarkable variety between two brothers[5] due to different mutations in the same protease subunits.

A United States incidence of 1 in 12,500 live births makes neuronal ceroid lipofuscinoses the most common group of inherited neurological degenerative disorders.[6] Whether examining the mitochondrial defect implicated in prenatal ventriculomegaly,[7] the often early-stage atypical presentation of neurocanthocytosis,[8] or the fatal amyotrophic lateral sclerosis,[9] scientists recognize that exome sequencing "potentially widens the clinical spectrum associated with Amyotrophic Lateral Sclerosis (ALS) to include bone dysfunction and myopathy, and provides further insight into the importance of cellular protein degradation pathways…."[10]

Unable to identify a contributory mutation by using traditional polymerase chain reaction (PCR)-sequencing in their study of the brain demyelinating disorder called X-linked leucoencephalopathy, a group of researchers subsequently applied exome sequencing to only one lane of material and obtained enlightening results.[11] The disorder affects myelin—the material responsible for brain matter being designated as white matter instead of grey. Although the lack of nerve myelination normally leads to central and peripheral nervous system disturbances, such as motor deterioration, it is notable that another research group used various techniques in conjunction with exome sequencing to identify the variant -- hypomyelinating leukoencephalopathy in three individuals who could walk without support.[12]

The cortex of the human brain is composed of a complicated landscape of convolutions, the biological formation of which is controversial. Exome sequencing was used to "present conclusive genetic evidence that recessive *LAMC3* mutations cause human occipital cortical malformations characterized by complex gyration abnormalities and challenge the concept that cortical smoothening needs to be caused by disruption of early events in cortical neuronal migration exclusively."[13] A Yale study that also used exome sequencing to study the same subject concluded that the attained results "demonstrate that this technology will be particularly valuable for gene discovery in those conditions in which mapping has been confounded by locus heterogeneity and uncertainty regarding the boundaries of diagnostic classification, pointing to a very bright future for its broad application to medicine."[14]

A successful exome sequencing study of the prenatally-fatal neurological condition known as Fowler syndrome prompted the investigators to announce their opinion that "this genetic approach is a much simpler and faster approach than traditional homozygosity mapping that makes full use of emerging technologies and is capable of identifying rare or common monogenic, recessive disorders."[15] This may be especially important in the attempt to diagnose brain formation disorders such as polymicrogyria, which is currently diagnosed by cranial magnetic resonance imaging,[16] spastic paraparesis, a presumed ion channelopathy identified via loss of function predictions,[17] and the most common paroxysmal movement disorder, paroxysmal kinesigenic which is often clinically misdiagnosed as epilepsy.[18]

Exome sequencing was also successfully used to study migraine headaches in which there was no aura (MWO), the most common type of migraine headache among the afflicted migraine-suffering population (n=4), and during which there were six [(EDA2R (G170A), UBE2NL (T266G), GBP2 (A907G), EMR1 (C264G), CLCNKB (A1225G), & ARHGAP28 (C413G)]novel rare non-synonymous mutations identified.[19] These researchers focused on a very small control group of four unrelated people who were not migraine sufferers, the results of which were studied in comparison to the four-member sole family migraine group in which each participant had the MWO affliction. Angiogenesis, cortical spreading depression, and trigeminovascular reflex theories each were supported by, or consistent with, MWO susceptibility under the currently supported theory of multi-gene predisposition. Additionally, the X-chromosome location of the EDA2R and UBE2NL mutations were reported to support gender difference predisposition.[20]

The chromosome 14 PRIMA1 gene novel splice site mutation (c.93+2T>C) was demonstrated in a mini-gene analysis of two siblings as having led to "skipping of the first coding exon of the PRIMA1 RNA...". [21] Research findings in the 300 nocturnal frontal lobe epilepsy subjects who were also studied, did not reveal further PRIMA1 mutations. The authors concluded that the likely causative factor in severe nocturnal frontal lobe epilepsy was an intensification of cholinergic responses.

Neuropathy

Researchers investigating the peripheral neuropathy of Charcot-Marie-Tooth (CMT) disease, the most common inherited muscular disorder [22], were faced with a population of 40 candidate genes and asserted that "linkage analysis followed by candidate gene Sanger sequencing, utilized in other studies would…have required the sequencing of at least the 78 exons."[23] Utilizing exome sequencing, the scientists were able to rapidly identify the disorder in a previously undiagnosed family and consequently take the position that their accomplishments "support the notion that genomic approaches in the form of targeted resequencing or whole-genome sequencing will replace traditional medical genetic testing in the near future."[24] Recently, whole exome sequencing was utilized to newly identify the p.I43N mutation in CMT disease in a family whose members exhibited autosomal dominant demyelinating CMT neuropathy.[25] Having performed subsequent research on transgenic mice, these authors concluded that there might be an implication by their data findings that "overexpression of wild type as well as mutant PMP2 also causes the CMT1 phenotype, which has been documented in the PMP22." [26] Kennerson et al. studied 67 families for whom there already had been a probable suspicion of X-linked CMT.[27] In the latter study, the researchers found clinically, electrophysiologically, and via neuroimagery, that "the p.R158H PDK3 mutation [findings from a second CMTX6 family] were similar to the axonal neuropathy reported in the [original] Australian family," even though the phenotype and genotype results indicated that the two families had mutations of a different haplotype.[28] Whole-exome sequencing research expanded HSPB8 mutations knowledge, which had previously only associated with either CMT type 2L or distal hereditary motor neuronopathy type IIa.[29] These researchers were also able to demonstrate that the eventual outcomes, when early neurogenic effects of HSPB8 mutations were present, were no longer limited to the aforementioned two diseases.[30]

Central Nervous System

Nervous system disorders continue to challenge the medical genomics of rare Mendelian mutation identification. Two examples in which exome sequencing has proven successful are the new neuroectodermal CHIME (*C*oloboma, *H*eart defects, *I*chthyosiform dermatosis, *M*ental retardation, *E*ar anomalies with hearing loss) syndrome that is "presumed to be an autosomal recessive disorder because of recurrence in sibs"[31], and the *Joubert* syndrome, a disorder in which, for the majority of subjects, "the causal mutations have not yet been identified…"[32]. The recent 2015 French Canadian population study that utilized a combined targeted and exome sequencing approach successfully identified two non-French Canadian subjects with the CEP104 mutations of *Joubert* syndrome and demonstrated a 94% (n=43) prevalence of CEP104 disruption as conclusive evidence of causation "despite substantial locus and allelic [population] heterogeneity."[33]

Kohlschutter-Tonz syndrome is another example in which researchers are using exome sequencing to shed light on disorders for which "the molecular basis has not yet been elucidated."[34] Such is also the case regarding what researchers described as "a new lethal autonomic sensory neuropathy" [35] and the incompletely understood brain malformation disorder Baraitser-Winter syndrome. [36]

We find an ambitious application of exome sequencing in the molecular research area dedicated to mental retardation. A letter published by *Nature Genetics* focused on researchers' assertion that "*de novo* mutations may compensate for allele loss due to severely reduced fecundity in common neurodevelopmental and psychiatric diseases, explaining a major paradox in evolutionary genetic theory." [37] Based on exome sequencing-based application of their theory, the investigators further claimed that "together with de novo copy number variation, de novo point mutations of large effect could explain the majority of all mental retardation cases in the population." [38]

Early infantile epileptic encephalopathy was studied (n=48) utilizing a SureSelectXT custom multiple encephalopathy/developmental delay gene panel, the results of which were determined to have identified GABRB3 mutations as the causative factor(s). Identified during this research study was c.860C>T (p.Thr287Ile), described as a novel *de novo* GABRB3 heterozygous missense mutation and implicated as an emerging causative factor in early-onset epilepsy.[39]

Defective selenocysteine biosynthesis was the conclusion in a study whose design was to potentially identify a genetic molecular basis for progressive encephalopathies with CSF or serum lactate elevation. [40] These researchers were successful in identifying new phenotypes for both a missense (p.Thr325Ser) and a nonsense (p.Tyr429*) mutation in their study that of three families, each of whom had a neuropathological presentation of laminar necrosis, severe myelin loss, neuron loss, and astrogliosis. These mutations had been associated with progressive cerebellocerebral atrophy, which resulted in reduced, but still measurable, selenoprotein levels, as well as quantifiable brain oxidative protein damage in the study subjects.

Musculoskeletal

Musculoskeletal disorders (MS) affect up to 33% of adults in the United States.[41] The tendency of these disorders to worsen with age, combined with the significant number of citizens approaching geriatric age, indicates that we can reasonably expect a statistical escalation in the near future. By exposing previously unidentified therapeutic targets in MS disorders such as Osteogenesis imperfecta[42], Skeletal Dysplasia[43], and Primary Hypertrophic Osteoarthropathy[44], deciphering of the exome sequencing has a promising role in the development of new treatment modalities. Researchers asserted the utility of exome sequencing in "the diagnostic algorithm for infants presenting with congenital dislocations, chondrodysplasia, and short stature".[45] Exome sequencing may prove equally valuable in early screening for heritable, but poorly understood, conditions such as metachondromatosis[46], Hajdu-Cheney[47], Early Onset Generalized Dystonia[48], Distal Arthrogryposis Type 1[49], and Nager syndrome[50].

Researchers from the Kennerson study on CMT type 6 confirmed that muscularly, MRI results revealed lower extremity distal muscle fatty infiltrates and that the majority of the infiltrates noted were in the soleus muscles, with lesser fatty deposition in the tibialis anterior muscles. The latter study's authors concluded that the finding "differs from demyelinating CMT1A patients and is similar to axonal CMT2A patients."[51] Autosomal dominant distal neuromuscular disease research on HSPB8 mutations as the causal factor that resulted in myofibrillar aggregates and rimmed vacuolar myopathy combined with neurogenic changes revealed by MRI that the lower limb diffuse tissue alterations in the early disease stages eventually progressed in later stages to myopathy with a typical fatty deposition.[52]

Genetic Targets Related to Developmental Delay

Successful identification of the Schinzel-Gideon syndrome allele underscores the idea that, while "Protein coding genes constitute only approximately 1% of the human genome but harbor 85% of the mutations"[53], they need not remain unidentified. Developmental delay-related genetic targets such as non-syndromic mental retardation[54], mental retardation[55] and sporadic autism spectrum disorders characterize novel candidates for identification by whole exome sequencing, even when complicated by consanguinity[56] and *de novo* occurrences[57]. It is not uncommon for diseases seemingly unrelated by signs, symptoms or phenotype to share a common molecular mechanism or pathway. Examples are the monogenic comorbidities of the neurodevelopmental disorder autism such as Rett Syndrome and Fragile X[58,59.] It was especially useful however, to find common genes connecting cancer and autism. Specifically, *TSC2, NF1*, and *TSC1* gene mutations intensify the cancer predisposition in autism patients. Moreover, because common genomic pathways suggest common treatment targets, exome sequencing empowers drug research with a multifaceted approach. Since exome sequencing, together with genome-wide-association studies and other modalities have recently uncovered the polygenic nature of autism[60], this leaves wide open the possibility of therapeutic advances in several diseases.

Whole exome sequencing has begun to gain utility as an affordable clinical biomarker. A study of 50 patients at the Radboud University Medical Centre Nijmegen concluded that WES "has a higher yield and lower costs to provide a diagnosis in child presumed to have a genetic origin of their disease".[61] The margin of savings was not trivial, reported cost for WES €3,600 compared to €12,241 for the non-WES care. A neurogenetics clinic went further by conducting a retrospective cohort study on patients with neurodevelopmental disabilities whose non-WES evaluations had yielded unactionable or inconclusive results. Presentations, new WES-informed diagnoses and subsequent changes in treatment plans were tracked for the 78 patients. The investigators concluded that "the high diagnostic yield of WES supports its use in pediatric neurology practices. It may also lead to earlier diagnosis, impacting medical management, prognostication, and family planning. WES therefore serves as a critical tool for the child neurologist."[62]

Cancer

The Cancer Genome Atlas (TCGA) consortium has used exome sequencing to evolve the decades-long strategy of analyzing melanoma somatic mutation at the at the single gene level. In general, melanomas are of neurologic concern and the related melanocytes arise neural crest cells, which also give rise to neural structures such as the iris, choroid and substantia nigra. The exome-based technique developed by TCGA has generated the largest database to date while elucidating the structure of 333 cutaneous melanomas.[63]

As mentioned above, one of the strengths of WES is that is can identify overlapping genes between multiple diseases. Conversely, and equally instructive, WES can highlight diseases that do not share common genes. Chronic lymphocytic leukemia and acute myeloid leukemia are two such examples.[64] Classification of disease subtypes is also useful as it can identify patient-specific genomic rearrangements such as those found in glioblastoma tumors.[65]

Inborn Errors of Metabolism

The textbook *Inborn Errors of Metabolism* (1923) is a 20th century publication written by Archibald Garrod, the British physician who also coined the like-worded phrase still in use today. Inborn errors of metabolism (IEM) typically involve a defective enzyme that was supposed to convert one substance into another.[66] Classical categories of the disorder include the deranged metabolism or modification of organic acids, carbohydrates, amino acids, or lysosomal storage components. Technological developments such as exome sequencing, however, have enabled the expansion of these categories.

We found an important example in the elucidation of what researchers describe as "a previously undescribed, severe IEM shedding light on a functional link between the methionine cycle and adenosine metabolism."[67] Combined Malonic and Methylmalonic Aciduria is a disorder of excess acid in the blood involved in failure to thrive, B-12 malabsorption, immunodeficiency, seizures, and possible death. The treatments for this disorder can vary, and is based on the specific offending amino acid mutation. Exome sequencing has contributed to the discovery of an increasing number of variants. [68, 69]

Future Research

Future research opportunities to explore exome sequencing are abundant. There is a dearth of clinical information for the endocrine, renal, reproductive, urinary, and respiratory systems, as well as research related to connective tissue. An exceptional opportunity exists for sentinel research and also for further exploration into the world of exome sequencing as it relates to rare genomic diseases. Alzheimer's disease is one of the most exciting areas of research focus that can be boosted by exome sequencing. The TOMMORROW Trial of AD-4833 for the Delay of Onset of Mild Cognitive Impairment Due to Alzheimer's Disease in Subjects Selected Using a Genetic-Based Biomarker Risk Assignment Algorithm TOMMORROW study is currently in clinical trials and based on the theory that a molecule called apolipoprotein E (ApoE) regulates both Amyloid-B deposition and ApoE repair mechanism for neurons under stress. Relatedly, the TOMM40 gene contains a non-coding stretch of DNA on chromosome 19 adjacent to APOET. The aim is to find a common thread while genotyping these two entities that may lead to a common treatment. For example, the study hypothesizes that the type 2 diabetes treatment pioglitazone may also be prescribed to those with an Alzheimer Disease predisposition in order to delay the onset of the disease.[70] Exome sequencing might also facilitate research around the biophotonic biomarkers of Alzheimer's disease by mitigating the enormous number of targets investigated by the modality.[71]

Several studies discussed had an extremely limited subject focus. We suggest that there needs to be significantly larger population participants in exome sequencing research, not only to further this area of medicine but also to be more applicable to a greater portion of the general population in which the same, or similar, mutations might exist. The mutations related to migraine without aura, for instance, offer significant opportunity for future research that should focus not only on a much larger population for both the MWO group, but also the control group. We suggest that, given the historical knowledge that females are overwhelmingly represented in the MWO population, near-future exome sequencing research should focus on gender differences as they relate to MWO predisposition. Early-onset (infantile) epilepsy is another area in which research focus should be specifically related to an age-based population, both for further case identification and for BRBR3-related genotype and phenotype delineation.[72] We suggest that early studies also lean toward identification of any gender predisposition.

Exome sequencing may also be useful in the differential diagnosis of neuropsychiatric conditions. For instance, nightmares, sleep walking and night terrors are currently distinguishable by sleep phase –the first occurring during rapid eye movement and the latter two during slow-wave delta sleep. Diagnostic sensitivity might be increased by research targeted at the common genetic predisposition sleepwalking and night terrors.[73] Finally, although we have only reviewed the utility

of exome sequencing in the so-called forward direction, we anticipate progress towards reverse phenotyping which would be of great value to the field of personalized medicine. Exome sequencing organizations have begun to multiply as the science matures (table 1), and will likely continue to do so for some time.

Summary

In the investigation of rare congenital diseases, exome sequencing overcomes several interpretation challenges presented to traditional study designs of Mendelian genetics and genomic medicine while contributing clinical utility as a molecular diagnostic tool that can expand disease phenotypes and therapeutic targets. Geneticists traditionally analyze hereditary pedigrees using decision trees established on the observed incidence of mutation for a minority of key subtypes. Consequently, the relative uniform phenotypes of many genetic diseases make it difficult to determine which genes are affected, for example, syndromes with apparent mutation-phenotype disharmony or multigenerational compound heterozygosity.

Declaration of Interest: The authors have no financial or other conflicts of interest.