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A way forward for diagnosis of patients with extremely rare genetic mutations

Single-gene mutations resulting in diseases with a prevalence of 1–30 patients with the same mutation worldwide are defined as nano-rare. Most nano-rare mutations occur de novo, and therefore patients are dispersed around the world, which creates unique challenges in diagnosis, identification and treatment of this virtually unserved patient population. With the advent of genomic sequencing, it is now apparent that the overall prevalence of nano-rare mutations is substantial and will grow as more human genomes are sequenced[1]. Recent progress reported by the Undiagnosed Disease Network (UDN) and the growing number of personalized medicine centers in tertiary care centers has begun to identify more patients with nano-rare mutations. Despite increased identification, the vast majority of patients with nano-rare mutations experience disease progression and succumb to their diseases without achieving a diagnosis. For the fraction of patients who are genetically and phenotypically diagnosed, the journey to diagnosis is long and perilous, replete with inappropriate referrals, misdiagnoses and either no or mistreatments[1–5]. To meet the therapeutic needs of many patients with nano-rare mutations, the non-profit foundation n-Lorem has taken advantage of the rapidity, efficiency and cost effectiveness of antisense technology to provide personalized experimental antisense oligonucleotide (ASO) treatments free for life to these patients[1,2].

The purpose of this Correspondence is to describe the challenges of achieving a diagnosis and treatment in the nano-rare population by presenting the experience of a patient with a particularly well-documented history. Patient 001 is a 13-year-old boy who experiences seizures that are unresponsive to standard antiepileptic agents; choreoathetotic and dystonic movement disorders that limit mobility and functionality; recurrent, often severe, gastrointestinal (GI) disorders; and developmental delays. As he has aged, he has become more anxious and hyper-responsive to various stimuli, and displays variable responses to pain. The patient suffers from severe developmental delays and is reliant on a caregiver to meet all basic needs.

Patient journey to diagnosis

Patient 001 is exemplary of most of our patients with one exception. His mother has assembled a detailed natural history that has been reviewed by experts. The patient was hospitalized at one month of age for severe GI symptoms and failure to thrive. He experienced his first seizure at 8 months of age, and his seizures rapidly increased and were refractory to treatment, resulting in the patient being placed in hospice care at age 2.

Patient 001 has been hospitalized 29 times in 9 different institutions. He has had 33 incorrect diagnoses and one correct diagnosis. As an infant and toddler, he was treated with as many as 12–15 drugs simultaneously. The frequency and severity of the seizures experienced by patient 001 have varied over time. He has had 20 EEGs, including several 24-hour and video EEGs. The patient is now 13 years of age and continues to have seizures, but they are a minor component of his current phenotype and typically occur while sleeping. However, he has severe motor disorders characterized by choreoathetotic movements, including uncontrolled motion of arms and legs and inability to coordinate motor activity. He has severe developmental delays and is on the autism spectrum. He continues to have severe GI distress and is incontinent, and these issues exacerbate his daily life and that of the family. Currently, patient 001 is untreated.

Patient 001 is a beneficiary of the broadened access to genomic sequencing and lower costs driven by advances in genomics technology. His early history reflects the lack of availability of genomic sequencing at the time. In 2014, when patient 001 was 4 years old, the family paid for one medical geneticist to perform exome sequencing and another investigator to determine the causative mutation from the results, which was determined to be a de novo mutation in the *SCN2A* gene. At the time, little was known about the function of *SCN2A* or the types of mutations present in this gene.

The sequencing results showed that patient 001 expresses a missense mutation, R853Q, that results in substitution of glutamine for arginine in the *SCN2A* gene, which encodes the α-subunit of a voltage-gated sodium channel, Nav1.2. Expression of Nav1.2 is limited primarily to the central nervous system (CNS)[6] and in the CNS, this protein is responsible for initiating the neuronal action potential, particularly in the axon initial segment and the nodes of Ranvier[6–8]. The R853Q mutation results in a complex loss- and gain-of-function phenotype with reduced sodium conductance impeding initiation of the axonal action potential and unregulated proton conductance. At present, 9 patients with the same mutation in the *SCN2A* gene have been reported, and they exhibit symptoms similar to those experienced by patient 001 (ref. 6).

As sequencing became more accessible, both the critical roles of Nav1.2 and the impacts of various mutations became known and of interest to the scientific community, and grants were established by the parents of children with *SCN2A* mutations to investigators at Indiana University in Indianapolis and the Flory Institute in Melbourne, Australia to define the molecular pathological causes of the disease and phenotype.

Discussion

The experience of patient 001 and his family, including a remarkably committed, diligent and organized parent, is similar to those of the more than 190 patients with nano-rare mutations who have applied to n-Lorem for treatment. This patient exemplifies the almost unimaginable burden placed on patients and families of patients with nano-rare diseases and the inability of current health care delivery approaches to meet the needs of these patients. It also highlights a number of urgently needed reforms.

As more patients have undergone genomic sequencing, the number of patients with disease-causing mutations in *SCN2A* has increased to approximately 300 (ref. 6) and the number and types of mutations and syndromes have expanded and become more complex. Today we understand that pathologic mutations include loss and gain of function, as well as mutations with mixed gain- and

loss-of-function characteristics. Just as the mutations vary widely, the syndromes vary, from infantile epileptic encephalopathy, in which seizure activity begins neonatally, to a syndrome with no apparent seizure activity but autistic features and emotional disorders. Though gain-of-function mutations are typically associated with onset of seizures within a few days after birth, loss-of-function mutations can result in what is referred to as "late onset," in which seizures usually present 6–12 months after birth. Other loss-of-function mutations are associated with seizures that do not manifest until 18–48 months after birth, with autism as a predominant manifestation, while patients with other loss-of-function mutations exhibit only autism features[6,9,10]. Although GI symptoms are common in patients with *SCN2A* mutations, they are poorly described in the literature[11]. The number of different mutations and the variation in pathophysiological consequences speak to the critical roles of Nav1.2 in neurological function and neurodevelopment, and it is likely that still other mutations will be identified. This also speaks to the need to look beyond genomics more thoroughly for factors such as post-translational modifications as well as compensatory responses that may affect the severity or features of the syndrome.

The experience with *SCN2A* reflects a general trend that is associated with expanded access to genomic sequencing for undiagnosed patients. Once a gene is sequenced and a pathologic variant thought to be nano-rare identified in a population, the scientific community responds by identifying and expanding the number of patients with syndromes associated with mutations in the newly sequenced gene. In a few years' time, the growing number of identified mutations and increasing number of types of pathogenic consequences resulting from these mutations mean that they are no longer considered nano-rare. Additionally, the range in frequency of mutations, including a relatively common mutation or two and many far less common mutations, is consistent across our portfolio. One can extrapolate from our experience and posit a future when genome sequencing to find nano-rare and frequent disease-causing mutations in newborns becomes universal.

Lessons can also be learned by looking at the evolution in phenotype (phenotype drift) displayed by patient 001. Initially, GI symptoms were most prominent and problematic, and then the severe seizure syndrome manifested and became life threatening, necessitating admission to hospice at 2 years of age. The decline in seizure activity as a function of age is not unexpected as this trend has been reported for patients with early-onset epilepsy[12]. However, the variability in seizure activity and sporadic surges in seizure frequency and severity are important to note. Also of note is that movement disorders, behavioral and emotional issues emerged and worsened with age, and these were compounded by severe developmental impairment. However, throughout the clinical course, GI symptoms were severe and limiting. Once again, phenotype drift is a common observation in patients who have applied for treatment to n-Lorem. In our view, the fact that these issues are poorly described and poorly understood in the literature reflects a somewhat myopic view of all of the problems that plague such patients and suggests that further research into a potential role in autonomic signaling of this and other ion channels is warranted. This is significant for others working in rare diseases as well.

It seems clear that reforms in the delivery of health care may be needed if these underserved patients are to be treated more effectively. The UDN estimates that, for the fortunate few patients with undiagnosed diseases who achieve diagnoses, the time to diagnosis is 8 years on average[4,5]. Our experience is too limited to draw a definitive conclusion about the time required to achieve a diagnosis for a nano-rare disease, but it is clearly years.

To fully appreciate the challenges and impact of the journey to diagnosis on the patient and family and the healthcare delivery system, it may be of value to divide the journey from symptom onset to diagnosis into specific steps. The first step is to identify the patient as having a serious health issue. For many patients, particularly newborns, months to years are often consumed before a gatekeeper physician concludes that the patient has a substantial health problem. During that time, patients are often referred to various specialists, and many migrate from physician to physician. Though the severity of the GI symptoms of patient 001 was identified and thoroughly clinically evaluated, the possibility that there might be a neurological component to the phenotype of patient 001 was not pursued until the first seizure, at 8 months of age.

The next step would be the achievement of a genetic diagnosis. For patient 001, this was complicated by the timing of his illness, as genomic sequencing was evolving and becoming broadly available at affordable costs during that time. That said, only the persistent efforts of the family and the willingness of the family to pay for this effort resulted in the determination that there was likely a pathological variant in the *SCN2A* gene.

Once a genetic diagnosis is in hand, it becomes necessary to understand the molecular pathological effects of the mutation. Despite the successful efforts to sequence the human genome supported by the National Institutes of Health (NIH) and the NIH-supported personalized medicine centers throughout the country, this process can still take years. In this case, it took 8 years to find scientists willing to characterize the nature of the mutation and to understand the molecular pathology, and the majority of this effort occurred in Australia. Once again, the required basic research was supported financially by the family and a company established by parents of two patients.

The final step, identifying a research physician at a tertiary care center willing and able to care for and potentially treat the patient with an experimental therapeutic, also can consume years. A surprising number of physicians and institutions declined to treat this unique patient and participate in testing a genetically directed experimental therapeutic, even though, in our view, this was an incredible opportunity. At n-Lorem we have encountered multiple patients like patient 001 who are genetically diagnosed and are ready to be treated with an experimental ASO, but neither they or we are able identify a research physician at a tertiary care facility willing to care for the patient. Based on a number of conversations, we surmise that several issues were of concern, including the lack of insurance coverage and potential risk associated with experimental treatments.

The journey to genotypic diagnosis and phenotypic characterization of patient 001 exemplifies many deficiencies in the delivery of integrated, genomically directed health care for patients with rare, single-gene-caused diseases. Today, the identification of these patients is cumbersome, uncoordinated and often unavailable, demanding the active involvement and financial support of parents and patients who should instead be focused on their care. Of equal concern are the challenges these patients face in identifying a physician to provide long-term care. Finally, the failure of the healthcare system to provide holistic care and support for the patient and the family affected is both a health and economic issue and is extremely costly to the healthcare system and to the productivity of affected families.

Yet the plight of patients and families affected by rare single-gene-caused diseases points to an extraordinary opportunity to establish an integrated approach to the delivery of care for such patients. This approach would establish genetically based delivery of care as a paradigm in medicine that could couple all the advances in medical science to the patients and families in need. This would advance our understanding of health and the processes that lead to the manifestation of symptoms that we refer to as diseases, enhance healthcare and save many billions of healthcare dollars.

Today, the required technologies exist and the experience of the UDN provides a model for delivery of vastly improved health outcomes and lower healthcare costs. A well-funded population-wide whole-genome sequencing program incorporated into newborn evaluation protocols is urgently needed and is the lynchpin of any solution that can make a real difference. Only when the genomes of all newborns are sequenced will we know the frequency of pathogenic variants. This will identify patients at risk, reduce the time and cost to achieve a diagnosis, and provide therapeutic opportunities before patients have progressed to the extent that the benefit of therapeutic interventions may be limited.

While population-wide genomic sequencing is necessary, the experiences of patient 001 and many other patients demonstrate that genetic sequencing of newborns, though laudable, is not sufficient. It needs to be coupled to the establishment of integrated genomic personalized medicine centers with the clear mandate to respond to the needs of patients with rare diseases and to integrate all the elements required to rapidly define the causative mutations, the functions of the affected gene, the nature of the mutation and the molecular pathological manifestation. Then that knowledge must be provided to an integrated medical team led by a physician and including nursing and social services professionals charged with providing holistic care to the patient, support for the family and knowledgeable recommendations about precision medicine treatment options.

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Author contributions

S.T.C. was the primary author and reviewed all of the data mentioned. K.D. compiled a natural history of patient 001. O.S.K.-M. reviewed the manuscript and is the treating physician for patient 001.

Competing interests

S.C. is the founder, chairman and CEO of n-Lorem Foundation, a non-profit foundation devoted to meeting the needs of nano-rare patients. K.D. is associate director of natural history and diagnostics in epilepsy at Praxis Precision Medicines. O.K.-M. declares no competing interests.