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The importance of elderly genomes

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The difficulty in classifying a rare genetic variant as "likely pathogenic," "likely benign," or VUS (variant of unknown significance) represents a significant challenge in genetic counseling (GC) when trying to establish a diagnosis or as a result of incidental findings. This classification may impact the communication of prognosis in late-onset conditions, such as neuromuscular disorders, and the consultants' reproductive decisions regarding future offspring. Here, we report two unrelated families, one Brazilian and one of East Asian ancestry, where a rare and previously unreported deletion in the dystrophin gene was identified. In these two families, the analysis of older male relatives (from 56 to 89 years old) who were fully asymptomatic provided relevant information to their families about the potential pathogenicity of this dystrophin variant. These cases support our previous suggestion highlighting the relevance of genome sequencing of older healthy individuals or family members, above the age of 50 and going into the 80's ad 90's, and the importance of sharing new relevant information for decision-making with families who previously underwent genetic counseling. In addition, these case reports contribute to the classification of VUS, enhancing our knowledge of the impact of specific mutations in functional studies.

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Next-generation sequencing (NGS) has allowed immense improvement in diagnosing genetic disorders, facilitating precision medicine. Furthermore, the current cost of whole exome sequencing (WES) and whole genome sequencing (WGS) makes them increasingly accessible diagnostic tools. However, we frequently have to deal with variants of unknown significance (VUS), which could cause a major illness or just be a rare genetic variant not yet deposited in the international genomic data banks.

Another ethical challenge geneticists face when sequencing a genome is accidental findings that could be utterly unrelated to the disease of the problem. For example, a mutation in the *BRCA1* gene, responsible for breast cancer, in an 8-year-old boy with an undiagnosed myopathy. Should the proband or the family be informed? The American College of Medical Genetics and Genomics (ACMG) published a list of genes and genetic variants that should be reported as incidental findings (or secondary findings) when they are discovered during genomic testing, even if they are unrelated to the suspected diagnosis (1, 2).

Our strategy has been to sequence the genome of healthy elderly individuals in Brazil, as those sequences could (1) contribute to databanks of our admixed Brazilian population, (2) help to classify the pathogenicity of rare unknown variants, and (3) provide essential insights on conditions that are prevalent later in life, such as hypertension, type 2 diabetes, Parkinson's, and cancer, among others. To pursue this strategy, in 2008, we launched the 80+ project, aiming to sequence the genomes of older Brazilians. A first draft, with 609 exomes, was published in 2017 (3), and a second study, including WGS of 1171 individuals, was published in 2022 (4), representing the most extensive genomic databank of older individuals in Latin America.

A comment in *Cell* published several years after our first study was initiated called attention to the importance of studying the genomes of admixed populations, as available databanks have been constructed mainly with individuals of European ancestry (5). Indeed, in our recent WGS study of more than 1000 individuals, we identified 2 million genetic variants not reported previously. More recently, the All of Us Research Program (6), a longitudinal cohort study aiming to enroll a diverse group of at least one million individuals across the United States, involved 77% of participants from communities that are historically under-represented in biomedical research and 46% individuals from under-represented racial and ethnic minorities. The All of Us Research Program identified more than 1 billion genetic variants, including more than 275 million previously unreported ones. This reinforces the value of studying the genomes of admixed populations. In addition to population genetic data banks, the genome study of older probands' relatives can be extremely valuable in real-life decision-making, as illustrated by two examples below.

Case 1

In 2012, a 44-year-old man was referred to our center because he had a mutation in the dystrophin gene, which was identified in a genetic center in the United States. He was perfectly healthy and robust, but he was investigated as a result of that accidental finding because his 10-year-old daughter had a diagnosis of coloboma and some hearing difficulties (7). The genome study of the young girl did not uncover any variant that could explain her condition. However, it revealed that she carried an unrelated mutation in the dystrophin gene, encompassing exons 38–44, inherited from her 44-year-old father. Most mutations in the dystrophin gene are responsible for Duchenne muscular dystrophy (DMD), a severe lethal condition that affects about 1 in 5000 male newborns (8). Those are disruptive mutations that result in the absence of muscle dystrophin. Affected boys usually lose ambulation by age 10–12 and are entirely dependent on all activities in their second decade. However, some mutations can result in a partially functional dystrophin and a milder but highly variable phenotype, as seen in Becker muscular dystrophy (BMD). Depending on the type and site of the mutation along the gene, BMD patients can be confined to a wheelchair around age 16 or remain ambulant in their sixties or seventies. For example, it is known that some mutations in the rod domain (central part of the gene) that maintain the RNA reading frame (in-frame deletions) can cause only cardiopathy later in life but no muscular weakness. Therefore, mutations in the dystrophin gene should be classified as dystrophinopathies and not Duchenne mutations, as they are responsible for a wide range of clinical variability.

The problem in this case is that the dystrophin mutation found in our proband had never been reported before. Could it be responsible for a late-onset disorder, or was it just a likely benign rare variant? Although he was healthy and strong at age 44, he wanted to know whether he might develop muscular weakness later in life. If he had carried a novel

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mutation, it would not have been possible to anticipate his clinical status later in life. The only alternative was to investigate his older relatives, hoping their genomic data might be informative. In other words, we needed to investigate whether those elderly relatives carried the same dystrophin mutation. That was the case: we studied several family members and found out that the proband's mother and one maternal uncle, who was 56 years old at that time, also carried the same mutation, and they were asymptomatic. It was good news. We published this case report with a take-home message: if you want to sequence your genome, keep your older relatives' DNA. They can bring important information (7).

Case 2

More recently, I received an email from a young woman of East Asian ancestry who wrote to me because she discovered that she carries a DMD mutation encompassing exons 38–44, the same rare deletion of the Brazilian family in case 1. Her mutation had been inherited from her 60-year-old mother. Because of the lack of information in genome data banks, the mutation was classified as likely to be pathogenic, and she underwent an abortion at 27 weeks of pregnancy. She wrote in her email that this "led her to be devastated, but also to conduct tons of research." Searching references, she discovered that her mutation was the same in our previously reported family, and she wanted more information about our case. Her questions were: (1) could you please provide more context on what "asymptomatic" means for that family? (2) Do they not show any signs of DMD/BMD, including no signs of CK increase/cardiomyopathy? (3) If this research shows exon deletion 38–44 is asymptomatic for this Brazilian family, can I safely assume it will also be asymptomatic for my family? (4) What is the current status of your patients 12 years after your report? (5) How much should I (she) be worried about this mutation in my (her) future offspring?

Our report reinforcing the importance of testing older relatives prompted her to study her grandparents. Her maternal grandmother was already deceased, and she had three brothers who refused to be tested. However, her maternal grandfather underwent genetic testing, which revealed that he carries the 38–44 mutation. It could not be better news since he is currently 89 years old, fully ambulant, and has no cardiomyopathy.

Asymptomatic genetic variants in patients of different ethnic backgrounds: "VUS or likely benign?

Following these last genetic results, I contacted the Brazilian family to share the excellent news about the healthy 89-year-old man carrying the 38–44 deletion, and they informed me that they also continue to be healthy and strong. Our proband and his maternal uncle carrying the dystrophin deletion are currently 56 and 68, respectively. The new observation that this same variant is not associated with any muscular weakness in two families with different ethnic backgrounds supports the hypothesis that it is a "likely benign" variant. However, some geneticists would still classify it as a VUS. Most importantly, it reinforces the relevance of genomic screening of older populations and probands' family members.

The pathogenicity of VUSs can also be studied using in *silico* strategies that include computational structural biology or in *vivo* experiments in which a new variant is created via CRISPR and inserted in a living organism. However, we believe that such models will lack the input from other putative protective variants; moreover, the outcomes of gene-gene interactions may be missed. Therefore, we advocate for the study of elderly genomes as a key tool to determine the clinical significance of VUSs.

In a recent review of the literature and database, Fortunate et al. reported 22 cases of patients who carried *in-frame* deletions in the dystrophin gene and were fully asymptomatic (9). They were older than 43, while the three individuals reported were older than 55. According to Fortunate et al., some deletions should be carefully considered when identified as incidental findings, and genetic counseling must always be offered to help interpret these rare dystrophin genotypes. Indeed, in the current case, sharing new data with our family was very helpful in their decision about future offspring, which also reinforces the importance of re-visiting previously counseled families with new, relevant information.

Fowler and Reham recently questioned whether VUS would still be present by 2030 (10). They suggest that investing in eliminating VUSs is worthwhile because their predominance remains one of the biggest challenges to precision genomic medicine. Sharing case reports such as those synthesized here can not only bring relief to families with such genetic variants but can also contribute to classifying the pathogenicity of VUS and rare variants.

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