PedsCases Podcast Scripts

This podcast can be accessed at www.pedscases.com, Apple Podcasting, Spotify, or your favourite podcasting app.

Genetic Diseases 3: Genetic testing technologies

Developed by Rozlyn C.T. Boutin and Dr. Caitlin Chang for PedsCases.com.
February 7, 2023

Introduction:

Hi, my name is Rozlyn Boutin and I am a senior medical student at the University of British Columbia. This podcast was developed together with Dr. Caitlin Chang, a medical geneticist at the British Columbia Children's Hospital in Vancouver, BC, Canada. This is the final podcast in a 3-podcast series on Genetic Diseases. Today we will be discussing different types of genetic testing technologies using the case of X-linked hypophosphatemia from the second podcast in this series as an example.

By the end of this podcast, learners should be able to:

1. Describe four types of genetic testing technologies.
2. Describe the advantages and disadvantages of direct-to-consumer genetic testing.
3. Define personalized medicine.

Genetics play an important role in all areas of medicine, and an understanding of the genetic basis of disease is essential for any clinician. Thanks to recent scientific advances in technology and computational tools, many different types of genetic tests are now available in Canda. Having a basic understanding of the indications and utility of each test can help to counsel patients and inform treatment options for patients with genetic conditions. To illustrate, let's review our case.

Case

You are a 4th-year medical student working in an outpatient Medical Genetics clinic. You are seeing David, a 5-year-old male referred by his pediatrician for lower extremity bowing noted after he started to walk. David is suspected to have rickets, a skeletal disorder most commonly caused by vitamin D deficiency. He was seen by endocrinology and contrary to what would be expected for vitamin-D-deficiency-associated rickets, the workup demonstrated normal calcium and vitamin D levels, but low blood phosphate levels and high alkaline phosphatase. Additional urine studies were done by the endocrinologist, who suspects hypophosphatemic rickets (urine phosphate wasting), but these are not back yet.

You have already taken David's medical and family history, and suspect that David may have either an autosomal dominant or X-linked form of hypophosphatemic rickets[1]. Based on the absence of male-to-male transmission, you consider that X-linked inheritance is still a possibility in this family. After presenting your findings and differential diagnosis to your preceptor, she is impressed that you have used the pertinent positives and negatives on history to present a prioritized differential! She asks what you would like to do next for the genetic workup.

Genetic Testing

Seeing your puzzled expression, she offers some guidance by reviewing the various genetic testing modalities available in Canada. These include a karyotype, chromosomal microarray, gene panel, and whole exome or whole genome sequencing[2-4]. Each testing modality has certain advantages and disadvantages depending on the clinical context, and it is important to note that as advances in technology continue to be made at a rapid pace, practice guidelines on recommendations for testing are continually being updated.

Recall from our first podcast that the genome can be visualized as a bookshelf of books where each chromosome is represented by an individual book on the bookshelf. Within each book are letters that can be put together to make understandable words and sentences, just like how each chromosome contains many individual genes that each encode the blueprint for making a specific protein.

A karyotype analysis was one of the first types of genetic analyses developed. A karyotype is an image of an individual's chromosomes that have been stained during a specific phase of the cell cycle[2,5]. Think of a karyotype as an image of all the books on the bookshelf. This test can be used to look for abnormal numbers or structures of chromosomes[2,5]. In current testing, karyotypes are primarily used to detect abnormal numbers of chromosomes, known as aneuploidy[2,5]. An example would be trisomy 21 or Down syndrome. It can also detect chromosome rearrangements such as translocations[2,5]. For example, this is offered in the setting of unexplained recurrent miscarriages.

Smaller changes in chromosome material can be detected by a genetic test called chromosomal microarray. This test can look for missing or extra chromosomal information in greater detail or resolution than karyotypes[6-8]. Using our book analogy from the first podcast in this series, if a karyotype looks for entire bookshelves or chapters of books that are missing or extra, a chromosome microarray can detect as few as a couple pages missing or extra, known as "microdeletions or microduplications" [6-8]. This test does not look at structural rearrangements (or what order the books are in). It also does not read through specific spelling differences in genes[6-8].

Gene panels are another type of test that can be extremely useful if a presentation is known to be associated with disease-causing variants in different genes[3]. Using our library analogy, instead of looking at the entire genome, or reading an entire bookshelf full of books, which can be very expensive and time consuming, clinicians can tailor their assessment to only certain pages of different books to detect specific spelling changes in genes of interest. Gene panels allow clinicians to examine hundreds of genes at a time[3]. They can be ordered

as pre-determined or “off the shelf” sets designed by companies and laboratories, or can be customized according to a clinician’s clinical suspicions.

The most comprehensive genetic tests are whole genome and whole exome sequencing. These are most useful when a patient is suspected to have a severe undiagnosed genetic syndrome, has intellectual disability with multisystem involvement, if there is a wide genetic differential, or there is a suspicion for two or more well-defined conditions requiring multiple targeted gene panels. Whole exome sequencing involves sequencing the protein-coding genes of the genome (~20 000-25 000 genes) and is considerably cheaper and more rapid than whole genome sequencing[2,3]. The exome only represents 2% of the genome, but is where a large proportion of disease causing variants are located. Whole genome sequencing looks at the protein-coding regions, as well as the non-coding regions of the genome, for which our interpretation is currently more limited[2,3].

Whole genome and whole exome sequencing can be done on a single patient or proband only, or as a trio with the proband as well as both parents. Sometimes whole exome sequencing on an individual returns a result known as a variant of unknown signficiance, or VUS. This result indicates that the individual has a variant in a gene that is either not reported in the literature or has insufficient evidence in the literature to be able to draw conclusions about the functional impact of the variant. In these cases, parental testing can be informative, as if a parent is unaffected but carries the same gene variant, the variant is unlikely to be pathogenic or contributing to the patient’s phenotype. Trio testing therefore can increase the yield of genetic testing and assist with interpretation of the results. Whole genome and whole exome sequencing in Canada both remain very expensive, as this testing is not currently done within the country and requires extensive bioinformatics and data processing expertise to interpret the results[2,3]. There is also the possibility of finding “incidental” test results that are unrelated to the reason for doing the test (such as a disease predisposition for an unrelated condition), and uncertain results, which can be anxiety-provoking.

Case

Now, let’s return to the case. You review David’s condition in the context of each available testing modality. While karyotypes are useful for detecting aneuploidy, genetic forms of Rickets are typically caused by spelling differences in specific genes, and you decide that this is not an appropriate test to offer David. Chromosomal microarrays are a first-line test for children with congenital anomalies or global developmental delay in Canada given the association of these conditions with variation in gene copy numbers[9], but Rickets is not typically associated with these conditions or caused by microdeletions or insertions, so you decide this is also not the right test for David. Gene panels are extremely useful for detecting spelling changes to specific genes known to be associated with certain phenotypes, and commonly used genetic testing companies have excellent gene panels available for Rickets. You and your preceptor agree this would be a good test option. You decide to offer a “Hypophosphatemic Rickets Panel” for David[10]. You review this with the family and offer to test either David or his mother, as you suspect that David inherited a disease-causing variant from his mother. When discussing the option of genetic testing, David’s mother mentions that she and her husband recently sent their saliva to 23andme.

Developed by Rozlyn C.T. Boutin and Dr. Caitlin Chang for PedsCases.com.
February 7, 2023.

She remembers there being a section in the results on health predispositions, but no abnormalities were detected on her report. She wonders why additional genetic testing is needed.

23andme is an example of direct-to-consumer genetic testing, rather than clinical testing. Direct-to-consumer testing is available to the public for purchase through private for-profit companies and is done without the involvement of health care professionals[11]. With 23andme, consumers send in a saliva sample to the company and receive a report back that provides information on a person's risk for developing various health conditions, as well as non-health-related information such as ancestry and personal traits such as curly hair. Direct-to-consumer testing can be informative for patients who know little about their family history, but this type of testing also has some limitations that patients are often unaware of before they purchase a testing kit[11]. For instance, when assessing for health-related conditions, direct-to-consumer tests often report a person's risk of developing certain complex medical conditions that develop as a result of both genetic and environmental factors, such as diabetes or heart disease[11]. The test has no way of knowing about a person's environment, so can sometimes be falsely reassuring or cause unnecessary worry.

This is different from testing for disorders caused by specific differences in a particular gene, such as suspected for David. With regards to direct-to-consumer testing used to assess for single-gene disorders and carrier status, it is important to remember that companies offering direct-to-consumer testing are commercial companies looking to make a profit, so they limit their analyses to common disorders and common disease-causing gene variants in the population at large[11]. This means that a large number of clinically relevant genes, including those associated with David's condition, are not assessed by these tests. Even among the genes that are tested, companies often limit their analyses to only a few common disease-causing variants[11]. As an analogy, you explain to David's mother that the DNA that makes up genes can be thought of as a zipper, with each tooth on the zipper representing a different letter. Direct-to-consumer testing looking for single gene disorders may assess only a few teeth along the zipper for a given gene, thereby missing any possible disease-causing variation in the DNA at all the other teeth. In contrast, genetic testing ordered by a medical professional has the capacity to more closely assess each tooth on the zipper, and is only ordered once appropriate counselling has been provided.

David's mother thanks you for explaining and expresses an interest in having the gene panel testing done for David.

One month later, you join your preceptor to review David's test results with the family via telehealth. His gene panel reports a pathogenic variant in the *PHEX* gene. Prior to the appointment, you spend some time reading around the diagnosis of X-linked dominant hypophosphatemic rickets. This condition develops when a patient is unable to appropriately resorb phosphate at the level of the kidney due to a mutation in *PHEX*, resulting in hypophosphatemia and abnormalities of the bones and teeth[1,10]. This can result in bone demineralization with symptoms similar to those of vitamin-D-deficient rickets.

Based on the discussion, treatment for David is initiated with oral phosphate and calcitriol (activated vitamin D) supplements, with ongoing monitoring through endocrinology. He is

referred to a pediatric dentist for regular checkups and the importance of good dental care is emphasized. David will likely require therapy until his growth plates have sealed and will need annual x-ray imaging of his legs to ensure his bones are responding to therapy, in additional to regular renal ultrasound assessments for nephrocalcinosis[1].

David's mother asks what will happen if he does not respond to standard therapies. Based on your reading, you recall that targeted therapies are available in this condition for cases which do not respond to usual treatment. Targeted therapy means that by identifying the specific gene or molecular abnormality resulting in a clinical phenotype, treatment options can be tailored to meet an individual patient's needs. In this case, a specific monoclonal antibody targets part of the signaling pathway, allowing for phosphate re-absorption by the kidney[1]. This is an example of personalized medicine, one of the exciting and rapidly advancing aspects of Medical Genetics.

David's mother is reassured and thanks you for your help. Before she ends the call, your preceptor discusses that other members of David's family may carry the pathogenic variant identified in David, and that they may benefit from treatment as well. You and your preceptor review X-linked inheritance with David's mom and explain that she, her mother, her brother, and David's brother likely also have this condition. The mother, uncle, and grandmother are referred to adult endocrinology for discussion and ongoing care. David's younger brother is referred to endocrinology and started on oral phosphate and calcitriol, with good outcomes.

That brings us to the end of the case for today! Let's review some take-home points from this final episode in our 3-part series on Genetic Diseases.

1. Different genetic tests are available for investigating possible genetic causes of disease, including karyotype, chromosomal microarray, gene panels, and whole exome or whole genome sequencing. The best option for testing will depend on the specific clinical scenario.
2. Direct-to-consumer testing such as 23andme reports increased or decreased risks for developing certain common diseases or traits, but are unable to account for lifestyle or other environmental factors that can contribute to the manifestation of disease. Direct-to-consumer testing may falsely reassure patients if they carry a genetic finding that is not assessed as part of the test.
3. Personalized medicine involves tailoring treatment options to meet an individual patient's needs based on their genetic composition or other factors.

Thanks so much for listening and don't forget to listen to the first two episodes in this series on Genetic Diseases to learn more about other forms of genetic inheritance!

References:

1. Ruppe M. X-Linked Hypophosphatemia. In: Adam M, Ardringer H, Pagon R, Al E, editors. GeneReviews®. 2012 Feb 9. Seattle, WA: University of Washington; p. 1993–2021.

Developed by Rozlyn C.T. Boutin and Dr. Caitlin Chang for PedsCases.com.
February 7, 2023.

2. Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson Genetics in Medicine. 8th ed. Philadelphia, PA: Elesvier; 2016.
3. Wallace SE, Bean LJH. Educational Materials — Genetic Testing : Current Approaches This discussi. In: Adam M, Ardringer H, Pagon R, Al E, editors. GeneReviews®. 2017 Mar 1. Seattle, WA: University of Washington; p. 1993–2001.
4. Organization GECK. Technologies [Internet]. [cited 2021 Dec 13]. Available from: https://geneticseducation.ca/educational-resources/technologies/
5. O'Connor C. Karyotyping for Chromosomal Abnormalities. Nat Educ. 2008;1(1):27.
6. Theisen A. Microarray-based Comparative Genomic Hybridization (CGH). Nat Educ. 2008;1(1):45.
7. Morrison S, Sroka H, Allanson J, Carroll J. Prenatal Chromosomal Microarray. 2015. (GECKO on the run).
8. Morrison S, Carroll J, JE A. Microarray. 2019. (GECKO on the run).
9. Bélanger SA, Caron J. Evaluation of the child with global developmental delay and intellectual disability. Paediatr Child Heal. 2018;23(6):403–10.
10. Genetics B. Hypophosphatemic Rickets Panel [Internet]. 2021 [cited 2021 Dec 13]. Available from: https://blueprintgenetics.com/tests/panels/nephrology/hypophosphatemic-rickets-panel/
11. Morrison S, Allanson J, Carroll J. Direct to Consumer Genetic Testing. 2017. (GECKO on the run).

Developed by Rozlyn C.T. Boutin and Dr. Caitlin Chang for PedsCases.com.
February 7, 2023.