Genetics

Introduction

Dr. Catalina Betancur, MD, PhD presented on the "Genetic Basis of PMS". Her presentation focused on the known genetic mechanisms underlying PMS, emphasizing the multiple pathways to PMS based upon specific chromosomal alterations or genetic mutations. She also highlighted for families the importance of completing genetic testing with additional first- and second-degree family members.

PMS is currently diagnosed molecularly, rather than by a specific phenotypic presentation. The chromosomal abnormality causing PMS is the deletion of chromosome 22q13.33, hence the secondary name of "22q13 deletion syndrome". This syndrome predominantly involves a deletion of the gene SHANK3; however, the size of the chromosomal deletion varies substantially (e.g., 5kb – 9Mb). The larger the size of the deletion, the more genes lost; however, this does not necessarily mean more loss of function. In addition, PMS can result from sequence mutations in the SHANK3 gene, involving a change in a single or multiple nucleotides. In both deletions and mutations, the SHANK3 protein is not produced in sufficient quantity or it is defective. SHANK3 is a scaffolding protein that is important for the communication between neurons at the synapses. Because neurological functioning relies on the adequate communication between neurons (e.g., learning, memory, motor), disturbances in the SHANK3 protein likely have widespread effects.

Primary characteristics of PMS include intellectual disability (ID), absent or delayed speech, features of autism spectrum disorder (ASD), and hypotonia (i.e., low muscle tone). The prevalence is equivalent among males and females, and it accounts for approximately 1% of all cases of ASD. Because this is one of the highest prevalence of genetic mutations in ASD, it is recommended that any individual with ASD receive a chromosomal microarray.

Several types of chromosomal abnormalities within 22q13 may lead to a PMS diagnosis, including a deletion (e.g., simple, terminal, interstitial), an unbalanced rearrangement (e.g., translocation), or ring chromosome. Of the PMS cases, approximately 80% are *de novo* and 20% are inherited. Because PMS is defined molecularly, genetic testing must be completed in order to diagnose PMS. Briefly, a microarray is able to identify whether a deletion is present or not; however, follow up genetic testing with FISH or karyotype is necessary to determine the presence of an unbalanced rearrangement or ring chromosome.

All inherited cases of PMS are chromosomal rearrangements (though, not all chromosomal rearrangements are inherited), which arise when a parent who has a balanced rearrangement in which no genetic material is lost, transmits an unbalanced rearrangement to the child. Thus, it is important that families seek FISH testing of first- and second-degree family members to determine possible carrier status. If an affected individual is determined to have a deletion of 22q13 and a duplication in another chromosome, there is a strong possibility that the individual has an unbalanced translocation. This individual also needs to be followed up with FISH, and the parents need to be tested to determine if one of them has a balanced translocation. If there is a duplication in a region near the deletion, it usually means there is an inversion. An inversion can be the result of a *de novo* event or it can result from a parental balanced inversion. The parents need to be tested with FISH to determine the origin of the inversion and to understand the risk of recurrence. Individuals with unbalanced translocations and deletions or duplications of other chromosomes may be at risk of other health problems not associated with PMS.

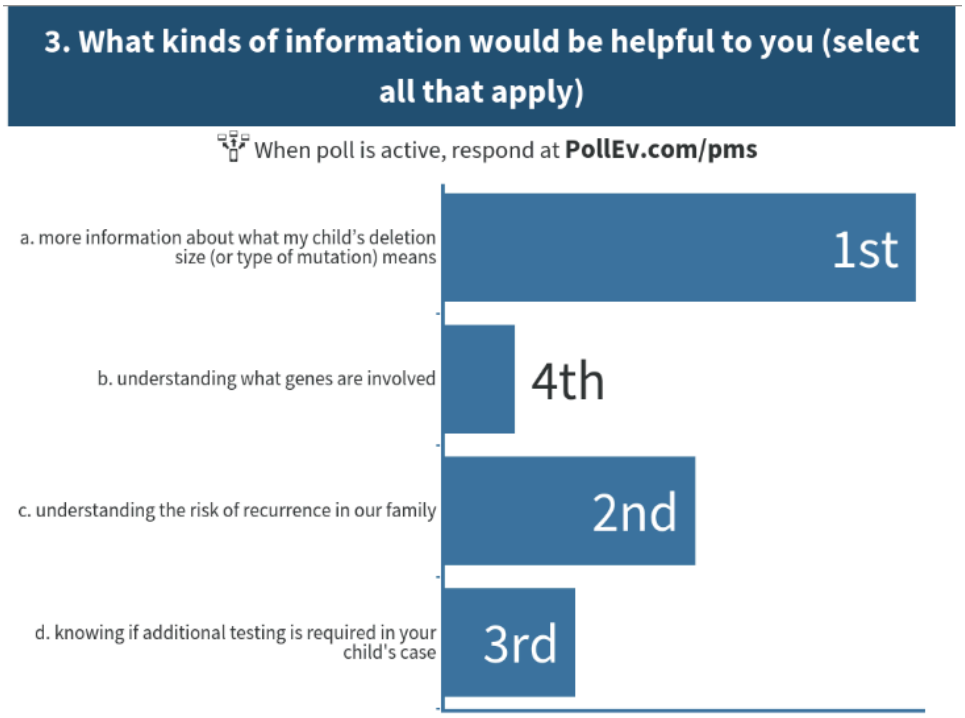
In addition, some individuals with PMS may have Ring 22, in which chromosome 22 forms a ring. A ring chromosome is detected by a genetic test called a karyotype study. Individuals with Ring 22 have additional risk of health problems. Because ring chromosomes are unstable, sometimes the entire chromosome is lost. As a consequence, individuals with Ring 22 have an increased risk of neurofibromatosis, a disorder that is associated with tumors.

Mutations (so called “spelling errors”) of SHANK3 also are a cause of PMS. However, a microarray cannot detect a mutation of SHANK3. Thus, if the microarray did not reveal a cause of the individual’s developmental delays (e.g., autism spectrum disorder, intellectual disability), the individual is recommended to undergo Whole Exome Sequencing (WES). WES detects very small “spelling errors”, called variants. While not every variant in SHANK3 is pathogenic or leads to PMS, nonsense, frameshift, and splice mutations are more likely to be pathogenic than missense mutations. Parental testing is important in order to determine if the variant is *de novo* or inherited. The geneticist will need to look up the particular variant to know if it has ever before been identified in an individual with PMS or in controls in order to determine if the variant is truly the cause of PMS. Sometimes it is not clear if a variant is disease-causing, in which case it is classified as a variant of unknown significance. Furthermore, it is possible that other variants could be identified during WES, however, unless SHANK3 is affected, PMS will not be diagnosed.

Identified Problems

1. **The clinical implications for the type and size of genetic mutation my child has is unclear or unknown.**

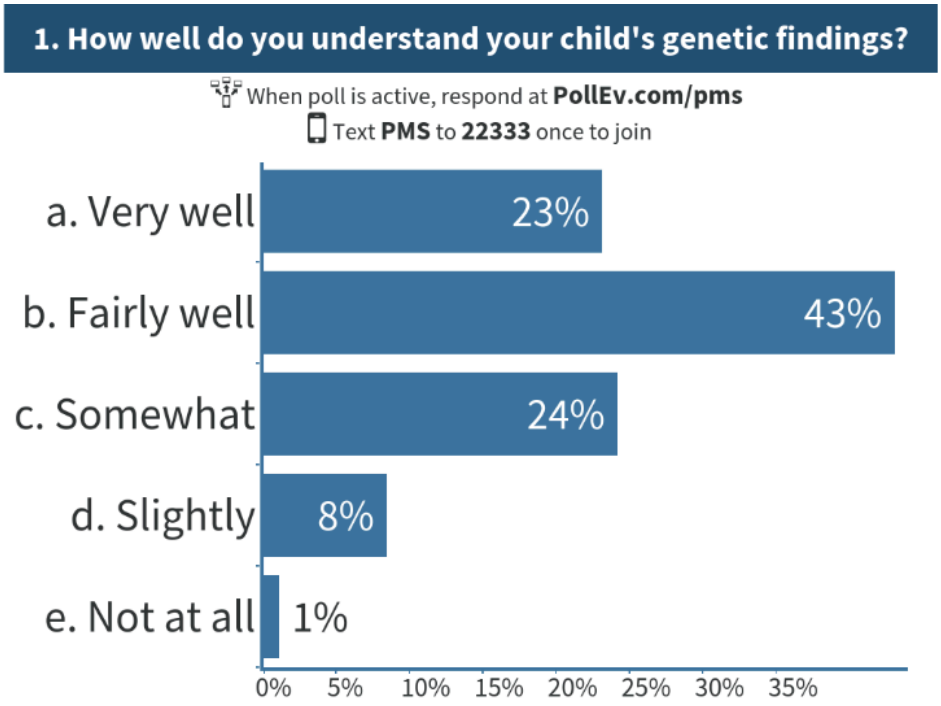
Based upon Poll Everywhere (see Question 3 graph below) and Group/Panel discussion topics, the primary problem identified by parents was that they along with clinicians had poor understanding of the clinical implications of their child's genetic results. During the Group discussion, parents asked questions such as "How much does deletion size correlate with [my child's] health issues?" and "What does the mutation mean for my child?" Parents indicated specific concerns regarding comorbid health issues, severity of disability, and long-term prognosis. Parents also indicated confusion regarding anecdotal evidence that small deletions occasionally had larger impacts than large deletions. Several parents also shared their frustration with researchers who were conducting correlational analyses with deletion size data instead of identifying other possible impactful genetic mutations. At least 72 parents indicated this to be relatively significant concern (4.4 out of 5).



2. The genetic results my family received were confusing and poorly explained by the doctors, and the doctors did not seem to know about PMS.

Although this topic ranked fourth in Question 3 and the majority of parents indicated that they understood their genetic findings "fairly well" during the group and panel discussions, this topic was the second most prevalent. An estimated total of 71 parents indicated this as a concern, and rated it as being a fairly high concern, with an average rating of 4.1. Parents commented that they did not know "the meaning of a lot of terms used when listening to [doctors] or talks on genetics". Additionally, parents also endorsed concerns regarding the level of education medical providers had about PMS.

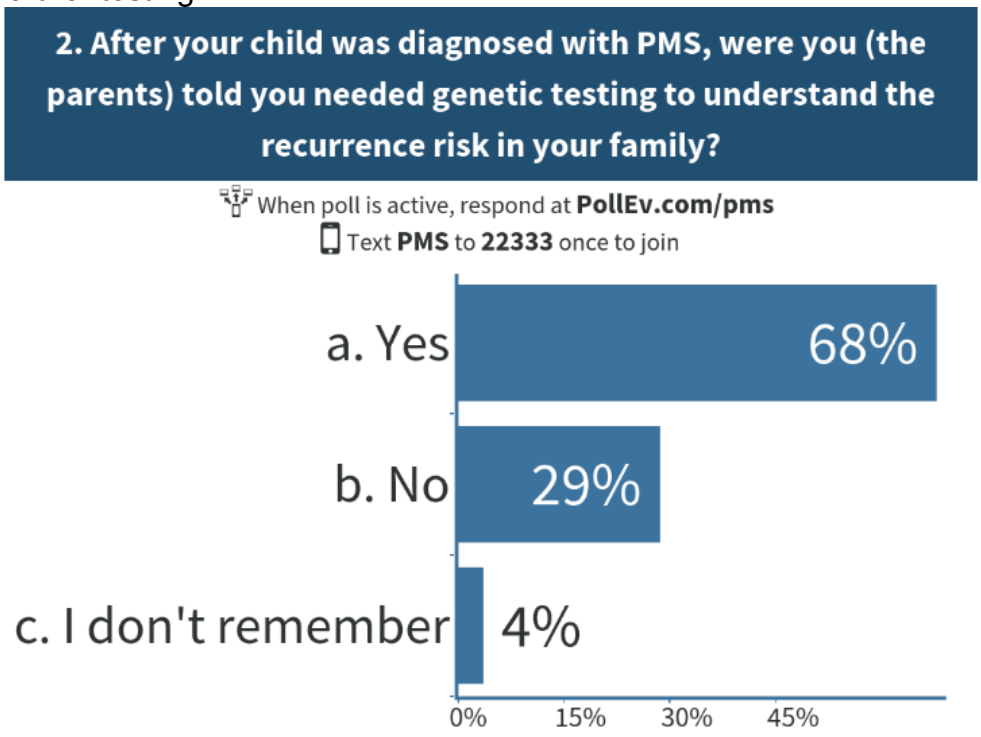
For example, one parent commented on the "lack of knowledge of genetic counselors when parents receive the diagnosis" as well as the "language disconnect between families and physicians". One parent noted that their experience with the geneticist was "horrible" due to way in which the diagnosis of PMS was delivered. Thus, both parents and medical providers have a less than optimal understanding of PMS.



3. **Genetic testing is expensive and not typically covered by insurance, and the necessity for additional genetic testing for my child and additional family members is unclear.**

A total of 58 parents with an average parent rating of 3.8 indicated that determining whether additional genetic testing was needed for their children, themselves, or extended family members was a concern. Despite 68% parents in the Poll Everywhere poll indicating they were told to receive follow-up testing (see Question 2 graph below), during the group and panel discussion, parents still identified lack of understanding whether they also needed genetic testing, especially for future family planning. For example, one group indicated receiving conflicting information regarding parental genetic testing, with some parents being told to complete genetic testing regardless of plans to have additional children, whereas other parents told they do not need testing at all. Additionally, parents shared specific concerns regarding whether their children needed further testing to determine exact type of genetic mutation or whether previous testing had to be repeated due to updates in technology. Furthermore, multiple parents raised concern regarding the cost of genetic testing, especially for repeat testing and family member testing, noting that these were rarely covered by insurance. Parents

commented that due to the cost of genetic testing it "discourage[d]" them from getting further testing.



4. **The definition of PMS based upon its molecular underpinning is too inclusive/exclusive.**

The definition of PMS was included in the initial presentation on the genetics of PMS, but it often came up during the Group and Panel discussions from parents, researchers, and clinicians, with individuals judging it as being too inclusive or too exclusive. At least 26 parents identified this as a concern during the Genetics topic and they indicated as a relatively high concern (4.4 out of 5). The majority of questions focused on the necessity of SHANK3 mutation to be considered PMS. For example, families commented on being excluded from research studies and financial support based upon the specific diagnosis and/or genetic mutation their child received.

Proposed Solutions

1. **Research dedicated to better understanding how specific genetic mutations of PMS relate to phenotypic presentations.**

This may be accomplished through existing research protocols, especially those detailing natural histories; however, this also may be a primary aim of future research projects. Given the complexity of PMS, it may be important to identify what genetic

mutations outside SHANK3 may have a casual role in PMS. Furthermore, this may be an important question within the Resilience Project to identify which genes are protective in the presence of 22q13.33 mutations.

2. **Development of detailed guide for family members and medical providers outlining types of chromosome 22q13.33 mutations associated with PMS and their potential clinical implications.**

Because both family members and medical providers are impacted by a relative lack of understanding of PMS, information in the form of practice parameters and lay-friendly guidelines or handbooks are needed for both audiences. These materials must be accessible to each of these groups. In other words, a family handbook should have an appropriate reading level, whereas practice parameters may have more technical terms. Furthermore, family handbooks should help empower families to advocate for necessary resources for the individual with PMS. In addition, physician guides also should help medical providers speak with families in an appropriate and empathic manner. The existing practice parameters as derived from Kolevzon et al., 2014 may be used as a general framework, but would ideally contain additional information about the genetic underpinnings of PMS and be adapted for both families and physicians. For example, within the family version, definitions of key words such as gene and chromosome should be included. Also, as outlined by Dr. Betancur and discussed below (Proposed Solution 3), such practice parameters and handbooks should outline process of genetic testing.

3. **Development of consensus guidelines regarding genetic testing for an individual suspected to have PMS and their family members.**

Dr. Betancur's presentation provided a basic outline of genetic testing for PMS. This outline should be published in a more formal way through consensus guidelines, which would serve as a road map with specific check points to help families determine necessary next steps regarding genetic testing. For example, it can help families determine whether additional testing is needed for their affected child and/or whether testing is needed for parents and/or siblings. These guidelines also would provide pertinent information regarding when it is necessary and/or appropriate to share genetic findings with extended family members. The development of these guidelines would be grounded in research and ideally would serve as justification for insurance companies regarding coverage of genetic testing that is deemed "necessary" as opposed to superfluous.

4. **Consensus regarding the definition of PMS.**

Currently, the PMSF has a very inclusive definition of PMS and welcomes all families whose children have any deletion of 22q13 or mutations of SHANK3, regardless of specific genotypes. However, due to an often more exclusive definition within the

medical and research realms, it is important to develop a more clear definition that may be used across settings. Further research is needed to understand the contribution of deletion size, genetic background, and SHANK3 variants to the broad spectrum of phenotypes in PMS.