

Hello, my name is Gema García. I'm a post-doctoral researcher on José María Millán's team. Our laboratory is located on the Health Research Institute of La Fe in Valencia. And we are also a member of the CIBER of Rare Diseases. Today, I'm going to talk to you about the genetic testing for Usher syndrome. Usher syndrome is a disorder of genetic origin and the pattern of inheritance is autosomal recessive. For each gene, we have two copies: one from the mother, and one from the father.

For the disease to be manifested, the two copies of a gene have to be altered. And these alterations are called mutations. So, the genetic testing for Usher syndrome must be able to detect both mutations responsible for the disease. Traditionally, the molecular diagnosis for Usher syndrome has been performed by analyzing it gene by gene. Starting with the gene with a higher prevalence in each clinical type.

However, the high number of Usher genes, the large size of the genes, and the existence of atypical cases, where the clinical features don't fit into our exact clinical type make the molecular diagnosis difficult. Another strategy is the genotyping microarray. It's a rapid strategy, but it's only able to detect mutations previously described.

Several years ago, the complexity of diagnosing Usher syndrome has been solved by the Next Generation Sequencing technology. This technology allow us to screen all the known Usher genes in several patients at the same time. We have developed a panel of genes, including the six genes responsible for type one, the three genes relating with Usher syndrome type two, the two genes responsible for Usher syndrome type three, and the two additional genes associated with the disease.

Moreover, we have included also four regions in USH2A gene, where intronic mutations have been described. Briefly, the strategy consists in several steps. The first step is to obtain the material, the DNA. And we extract the DNA from blood or saliva from the patients. The next step is to cut all the genome in high number of pieces. And then, using different systems, we are able to select only the fragments corresponding to the Usher genes, in white in the picture, and to remove the other ones in grey.

Then, we have two sequence to analyze all the smaller fragments corresponding to the Usher genes. The last step is to align, to map, all the small fragments to a reference genome. As example, imagine that the sentence, "When I saw you I fell in love, and you smiled because you knew" is the genome of reference the normal sequence of a gene. And the small fragments, the small pieces of the sentence, are the sequence we have obtained. With all the fragments, we are able to construct the original sentence, and comparing the fragments with the genome of reference with the sentence, we are able to detect all of the differences.

For example, the A has changed to I, and this difference is called a variant. One of the problems we have to address is the interpretation of the variants. Why? Because when we analyze all the Usher genes, we detect more than 100 variants per patient. And we have to discriminate the benign variants from those having a pathogenic effect. For the assignment in polymorphic, or pathogenic state, we rely on public databases. We actually use bioinformatic tools to predict the effect of the variant. Usually, we analyze also the relative. And sometimes, additional functional studies are needed. Large deletions duplications are not easy to detect using Next Generation Sequencing.

For this reason, to detect this type of mutations, we use the CGH array. Comparative genome hybridization array. We have the DNA from the patient, and we have the DNA from a healthy control. The patient DNA is marked in red, and the control DNA in green. And then, we have to compare the color intensities.

If, for a region, our patient has an intensity lower than for the control, the patient has fewer copies than the control, so the patient has a deletion in this region. We've used Next Generation Sequencing since 2015, and we have analyzed more than 100 patients. In 89% of the patients, we have detected at least one mutation. In 71% of the cases, we have detected both mutations responsible for the disease. In 18% of the patients, we have detected only one mutation, and in 11%, mutations were not detected.

In this picture, we can see the distribution of the mutations in the genes. We can say that 43% of the mutations are located in Usher 2A gene. The two other genes with a higher prevalence are MYO7A and GPL98. Sequencing all the Usher genes allow us to detect the mutations in patients with clinical features, typically Usher syndrome type two. Having mutations in genes typically involved in Usher syndrome type one, as MYO7A or Cadherin 23.

As I said on the other slide, in 18% of patients, we detect only one mutation, and in 11%, mutations were not detected. So, the question is, why don't we detect all mutations in all the patients? So maybe the mutations are located in other genes. In other genes, associated to the Usher syndrome, but not yet described. Or maybe these patients have other clinical features, and they have other syndromes, not Usher syndrome but with overlapping characteristics.

Another possibility is that the mutations are located in non-coding regions. Why? Because the genes are composed by coding regions called exons. And also by non-coding regions, in red in the picture. Usually, for a molecular diagnosis, we analyze only the coding regions, the exons, in yellow, but not the non-coding regions. Why? Because usually, the mutations are localized in the exons, and because the non-coding regions are very, very large. So maybe some of our patients have mutations in these non-coding regions. We hope that these patients with only one mutation or with mutations will be solved analyzing the whole exome of the whole genome.

It has been a pleasure to me to give this talk. To end, I would like to thank to all the patients, and their relatives,

for always collaborating in our projects, and also to all the associations for their help. Thank you very much.