Answers to Audience Questions - WSPOS Worldwide Webinar 13:
Inherited Retinal Diseases

WWW 13 Panellists


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1. **Is gene therapy a proven solution for retinitis pigmentosa, if it is not a systemic disease?**

AOK: At this time there is approved gene therapy for only the form of retinitis pigmentosa due to biallelic mutations in the gene *RPE65*. This is not a systemic disease. Gene therapy for this form of retinitis pigmentosa improves visual function if there are viable retinal cells but it is not a cure. Other forms of gene therapy for other forms of retinitis pigmentosa are under development.

ES: No

ET: Only in one type of RP caused by mutations in the gene RPE65

BL: Actually only one gene therapy is approved i.e. Luxturna (voretigene neparvovec) for RPE65-mutation associated IRD which may manifest as LCA/EOSRD or a.r. juvenile RP

2. **Is segregation Analysis essential for therapy?**

AOK: Before doing gene therapy, one must be absolutely sure that the patient's disease is due to mutations in that gene. In biallelic disease that means each of the two identified mutations needs to be on each of the two copies of the gene (rather than the two mutations both being on one copy of the gene). Often segregation analysis is needed to prove this. However, there are circumstances where segregation analysis is not needed to prove the presence of biallelic disease (e.g. homozygous mutations).

ES: It should be performed, should parents DNA be available.

ET: Unsure

BL: You have to be sure that in a.r. disease (like RPE65-associated IRD) disease-causing mutations are present on both alleles. In case of homozygous mutations, no segregation analysis is needed, as one mutation has to come from the father and the other same mutation from the mother. This is typically the case in consanguineous families. In case of 2 different mutations it is important to be sure that the mutations are not located on the same allele, but one on the maternal and the other on the paternal chromosome. The easiest way is to examine both parents. If only one parent is available and two different mutations are present, it is almost certain that the mutations are Biallelic as required in a.r. disease. You could also test siblings or offspring. If they are healthy and carry only one mutation, this also supports that the mutations in the proband are Biallelic. In Germany, it is compulsory to show Biallelic inheritance in order to get approval from the health insurance for treatment with Luxturna, and this corresponds to the German guidelines

for treatment with Luxturna. In addition, the mutations need to be considered as pathogenic or highly likely to be pathogenic.

3. **Hand held ERGs are becoming popular. Do we need to establish controls for each lab? Also do we need to ideally put this normative database in the results. like you showed control vs patient? Reteval has its own inbuilt database, right?**

   AOK: Hand held ERG can be useful for gross data but do not allow the same level of sophistication for diagnostic purposes as standard ERG setups. Most machines (both handheld and standard) come with control data and this can be adequate but it is preferred to have your own control database.

   AL: Attached is an extract from the ISCEV standards:
   Each laboratory should establish or confirm typical reference values for its own equipment, recording protocols and patient population giving attention to appropriate sample sizes. Because some ERG parameters (such as b-wave amplitude) are not normally distributed, interpretations based on standard deviation may be misleading. The median value (not the mean) should be used to define reference values, and the actual values on either side of the median that bracket 90 percent of the reference range of ERGs (in other words, the 5th and 95th % should be determined by direct tabulation of ERGs).
   McCulloch, D.L., Marmor, M.F., Brigell, M.G. et al. ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 130**,** 1–12 (2015). https://doi.org/10.1007/s10633-014-9473-7
   With equipment that comes with normative data the users should be aware of what the age range of the normative data and how it compares it to the patient data. For example, is the data age matched year by year or multiple years. This is important especially when under 1 year of age patients are compared to normative data.
   Normative waveforms in a report are not required especially of the values of the normative range are given

4. **Any practical protocol published for good pedigree and H & P?**

   AOK: This is a large topic. As starting point consider the on-line free resource “Understanding Genetics: A New York, Mid-Atlantic Guide for Patients and Health Professionals.” There is a chapter “Pedigree and family history” - https://www.ncbi.nlm.nih.gov/books/NBK115557/

   BL: Available in genetic textbooks or electronic pedigree programs

5. **May electrophysiology be useful in follow up of cases with genetic treatment? Defining the success in genetic treatment should also be discussed.**

   AOK: Electrophysiology is currently the only way to measure retinal function and is thus needed. One example is follow-up after genetic treatment. However, specialized testing other than full-field ERG is typically used.

   ET: Good question. Yes, it can be. Specific criteria are established for any given study and treatment modality.

   BL: Electrophysiology is useful in the presence of measurable ERGs at baseline. In patients with RPE65-associated IRD, it has not been shown that ERG becomes measurable after gene therapy (there was only one exceptional case in the London RPE65 study with some ERG post treatment). The improvement in mobility using the MLMT and the improvement of FST are accepted read-out parameters in RPE65 gene therapy. Arrest in retinal degeneration seen with OCT and other imaging techniques is another read-out parameter besides the classic ones like VA and VF.

   AL: The use of electrophysiology is of benefit when reporting outcome measures for gene therapy as quantitative measures can be compared before and after treatment. Electrophysiologists need however to devise new protocols as many patients receiving gene therapy have extinguished ERG responses and although behavioral measures of visual function can demonstrate an improvement following treatment, improvement in retinal function it is below the resolution of current electrophysiological techniques.

6. **Do you test to diagnose or do you test to understand your patients vision?**

   AOK: Genetic testing is done after clinical exam. Phenotype guides genetic testing. Genetic testing based solely on vision would not be useful. Genetic testing enables genetic counseling, identifies the potential for extraocular disease, and determines eligibility (or non-eligibility) for gene-based treatments or trials.

   ES: Genetic testing allows molecular confirmation of the CLINICAL diagnosis, establish genotype phenotype correlations, and may answer some lifetime expectations that the patient/family have.

   ET: Both. Testing allows a precise diagnosis so predictions are better understood in light of the specific gene and mutation

   BL: Both

7. **How long does therapeutic effect of Luxturna last? Is retreatment inevitable?**

   AOK: In the clinical trials, maximum effect was measured approximately 1 month after treatment and has been sustained for 4 years. Follow-up is on-going.

   ES: No one knows the right answer. Factors like the age of treatment, the severity of clinical phenotype at treatment total number of live cells and areas of live cells) are just a few determinants.

   ET: So far, retreatment does not appear to be necessary

   BL: So far, long term therapeutic effect has been shown in the dog (10 y, the dog then died), in humans it has been shown that the treatment effect lasts for at least 5 years.

8. **Can you please post the reference for preservation of morphology?**

   AOK: I believe this question asks how to determine if there are viable retinal cells. This is a decision left to the discretion of the treating physician based on multimodal imaging and specialized testing.

   BL: This has been presented by the McLaren group from Oxford, UK, in their RPGR gene therapy trial. The paper has been published recently.

9. **How common is it, that the gene has been inherited with the change from the parents with 2 slightly different structure and the gene is therefore not active and the disease is stable, not progressive.**

   AOK: There are some inherited retinal diseases that are relatively stationary rather than progressive. One example is congenital stationary night blindness. However, even in these conditions, over a lifetime, there can be progression.

   ES: Different classes of mutations have different impact on the amount of produced protein. The gene simply codes for a sequence of amino acids. A null mutation is determined by a premature stop, producing a smaller protein that may be devoid of function. A missense mutation where one amino acid is replaced by another amino acid, may influence function by changing the secondary structure (the shape) of the protein, thus changing its function.

   BL: I am not sure about this question. When the gene is not active in a.r. disease, this would correspond to a null mutation associated with disease.

10. **What do we do in cases where the rods in the retina are active but the cones aren't, but the cones continue to damage the Vitreous cavity?**

AOK: I think the question is how to intervene in cone degenerations. This is an active area of research but there is no official treatment at this time.

BL: I am not sure what the question is. I do not know a condition where pure cone disease damages the vitreous cavity.

11. **How comparable are results from one lab to another for ERG? 2- is ERG no longer needed with available genetic testing?**

AOK: ISCEV standards ensure standardization. Normal values tend to be similar across labs but there can be variation and thus it is recommended for labs to generate their own normative data. Electrophysiology is the only way to assess retinal function and thus is needed when this assessment is desired, even with available genetic testing. Genetic testing results do not tell us retinal function.

ES: WRT 'How comparable are results from one lab to another for ERG?': Can't compare apples with pears. Even labs that supposedly follow ISCEV norms may deliver slightly different results. Compare results from the same lab, it's easier.
WRT '2- is ERG no longer needed with available genetic testing?': Still important in clinical follow-up of patients

ET: This was addressed in the presentation the other day. Relatively comparable. ERG still necessary in some cases, especially when genetic testing is not available

BL: I am not an electrophysiologist, so I prefer not to answer this question about comparison of results from different labs.

AL: A study in 2015 recorded Standard ISCEV flash ERGs in 2 subjects across 15 units that revealed an inter unit variability of around 25% for corneal electrodes and slightly higher for skin electrodes, see reference below. Studies have demonstrated that the inter trial variability in 1 unit is above this value.
Hamilton R, Al Abdlseaed A, Healey J, et al. Multi-centre variability of ISCEV standard ERGs in two normal adults. Doc Ophthalmol. 2015;130(2):83-101. doi:10.1007/s10633-014-9471
Although genetic testing is becoming a standard in retinal pathology, electrophysiology testing is able to provide a functional phenotype.

12. **Does Luxturna improve the nystagmus?**

AOK: I have seen a 2-year-old boy whose nystagmus decreased after he was treated.

ES: There are reports of such improvement.

ET: Uncertain

BL: This has been shown in the dog and in patients. In some at least. Nystagmus maybe minimal or even absent in RPE65-associated IRD.

13. **Cost of gene therapy if it is not covered? Who should be doing this therapy? Only specialized centers?**

AOK: Gene therapy should only be done at specialized centers. Cost is an issue in healthcare in general but particularly for gene therapy. Government and advocacy groups need to address this important point.

ES: WRT 'Cost of gene therapy if it is not covered?': Varies from country to country
WRT 'Who should be doing this therapy? Only specialized centers?': Therapy is only performed in certified Specialized Centers.

ET: Yes, only specialized centers in my opinion.

BL: Cost coverage: This will be country-specific. Besides health insurances there may be charity etc.
Who should do gene therapy: only specialists in IRD who will insure extensive pre- and postoperative phenotyping. The data are still sparse and we should do everything to increase our knowledge on what gene therapy can do.

14. **With regards to the cost of gene therapy, what are the possibilities of bringing down costs for wider applications?**

AOK: It is the role of government and advocacy groups to address this important point.

DM: Under the current costs associated with making the gene therapy for research purposes, this is still expensive (in the order of $50,000 per treatment). Ed Stone had proposed treating patients as part of ongoing research rather than developing more approved and marketed treatments that the majority of patients cannot afford. If technologies improve this may drop. Given that some of the COVID-19 vaccine trials are using gene therapy technology, this wide-scale use may lead to a significant drop in price.

ES: Yes

BL: Governmental institutions would be best placed to help to bring the price down.

15. **Is there a maximum (or even minimum for that matter) age when it is too late for gene therapy?**

AOK: It is more a question of viable retinal cells. In general, older patients tend to have less viable retinal cells. However, there is variable expressivity and there is no absolute contraindication regarding age. In the USA, LUXTURNA was FDA-approved for older than 1-year-old, although most surgeons would treat at a slightly older age.

ET: Depends on extent of retinal damage. You need some surviving photoreceptors for recovery of better function.

BL: Maximum age: It is not so much a matter of age but of disease stage that vary with different mutations. Minimum age: surgical issues and a debate of ongoing maturation of neuroretinal cells that could suffer more with earlier age at retinal detachment needed for the subretinal application of Luxturna. The youngest patient treated so far to my knowledge was about 2 y old.

16. **Could the panel give a checklist for IRD (purely from a clinical point of view)?**

AOK: There are many such checklists in textbook chapters regarding retinal dystrophy. One of the panelists is submitting his checklist as a separate document.

ET: Please use the checklist attached here.

BL: Extensive family history for ocular and extraocular congenital disorders. Extensive individual medical history. Typical symptoms like night blindness, light adversity, color vision problems, nystagmus, visual field restrictions, biomicroscopy anterior and posterior segment, OCT, FAF.