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**Functional analysis of BBS3 A89V that results in non-syndromic retinal degeneration**

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Objectives

Bardet-Biedl syndrome (BBS) is a genetic autosomal recessive disorder that has characteristics of retinal pigmentosa, obesity, polydactyly, renal abnormalities, hypogenitalism, and cognitive impairment. There have been 14 BBS genes (BBS1-14) that have been found to cause BBS. In previous studies, it was shown that knockdown of bbs3 causes delays in intracellular melanosome transport and visual impairment, which is present with knockdown of all BBS genes. BBS3L is the longer eye-specific transcript of BBS3 that is required for retinal organization and function. Knockdowns of bbs3 or bbs3L lead to vision impairment in zebrafish. In a consanguineous Saudi Arabian family, a missense mutation (A89V) in BBS3 was found that lead to non-syndromic retinitis pigmentosa. The family had the visual defect associated with BBS but none of the other symptoms that BBS patients have. By a comparison of vertebrate species, it was also found that the A89V mutation occurs in a highly conserved region of the protein.

The objective of the researchers was to determine what the function of the A89V mutation had on BBS3 and BBS3L. Also, how can non-syndromic retinitis pigmentosa be caused by a mutation in a gene that normally causes BBS.

The BBS3 A89V mutation was examined for the intracellular transport of melanosomes through rescue experiments and visual function using a vision startle assay utilizing zebrafish.

Experimental Approach & Results

To study the effects and functions of the A89V mutation in BBS3 or BBS3L, a variety of experiments were performed using the zebrafish as the animal model. Knockdown of bbs3, a gene which is associated with BBS, involved using an antisense oligonucleotide (Morpholino [MO]) caused delays in intracellular melanosome transport and visual impairment in zebrafish. Using the antisense MO, the researchers were able to test how disrupting the expression of the BBS3 gene would affect phenotypes displayed by the model animal. Also to determine the functional requirement of each transcript, RNA encoding human BBS3 or BBS3L were injected with bbs3 aug MO which targets both transcripts. The antisense MOs were injected into one to four cell stage embryos at a concentration of 12 ng. It was found that BBS3 RNA suppressed the melanosome transport delays but not the vision defect while BBS3L RNA suppressed the vision defect but not the melanosome transport delay.

To study the A89V mutation, experiments were performed to determine the stability of the expression of BBS3L A89V. Because only BBS3 A89V mutation was found in the Saudi Arabian family, the researchers wanted to see if BBS3L A89V could be present as well because BBS3L was the longer, vision specific transcript of BBS3. The stability expression tests were measured through western blot analysis. The embryos were injected with C-terminal *myc*-tagged human BBS3L or BBS3L A89V RNA at a quantity of 8pg. They were then collected at time intervals of 72 hours, 4 dpf and 5 dpf. The blot showed similar bands after 5 days post fertilization (dpf). The similar bands showed that even with the mutation present in BBS3L, the area in which the mutation is in is a highly conserved region present in both BBS3 and BBS3L. BBS3L A89V was able to be expressed after 5 dpf meaning that mutation did not impact BBS3L expression.

The BBS3 A89V mutation was then tested for its effect on the intracellular melanosome transport delay, which are associated with the phenotypes that are attributed to the knockdown of bbs3 and all BBS genes. Zebrafish alter their skin pigmentation through intracellular melanosome transport in response to light or hormonal stimuli. Using the response system of the zebrafish, 6 day old zebrafish were first dark adapted and treated with epinephrine to cause retrograde melanosome transport. Retrograde melanosome transport results in the melanosomes moving towards a perinuclear location. The test measured the rate in which the melanosomes returned to their normal positions. Rescue experiments were then performed on zebrafish that were injected with RNA encoding human BBS3 or BBS3 A89V with bbs3 aug MO. BBS3 was used instead of BBS3L because in previous research, BBS3 was shown to suppress the delayed intracellular melanosome transport. Wild type zebrafish showed rapid melanosome aggregation with an average of 1.45 min. The knockdown bbs3 showed a significant delay in transport at 2.42 min. BBS3 RNA restored the transport time of melanosome back to the wild type times at 1.94 min respectively. BBS3 A89V RNA also restored the transport time back to wild type levels at 1.63 min.

BBS3L A89V mutation was then tested for its function in vision. Mouse and zebrafish models have previously demonstrated that BBS3L is necessary for proper retinal function and previous experiments have shown that BBS3L can suppress the vision defect when injected with bbs3 aug MO. A vision startle assay was performed on bb3 knockdown zebrafish. They were injected with BBS3L or BBS3L A89V RNA. The vision function was measured by the zebrafish’s natural escape response that is induced when the fish embryos are exposed to rapid changes in light intensity. For the test, 5 dpf embryos were light adapted for 1 hour and then exposed to five short trials of bright light at 30 second intervals. The embryos were monitored and recorded based on a distinct C-bend shape the embryos would create in response to the bright light. The control for the vision impaired embryo utilized a cone-rod homeobox (crx) gene knockdown that is essential for photoreceptor formation in zebrafish. The wild type embryos responded to an average of 3.77 times while the crx knockdown embryos responded to an average of 2.39 times. The bbs3 aug MO embyros showed a reduction in the number of responses at 1.91 times. BBS3L RNA with bbs3 aug MO was able to restore the visual response back to the wild type levels at 3.46 times. BBS3L A89V with bbs3 aug MO was unable to restore vision back to wild type levels with a response of only 1.60 times.

Conclusion

By studying the effects of the A89V mutation in BBS3 and BBS3L, it was seen that the mutation plays a large role in maintaining proper vision function. It was previously discovered in the Saudi Arabian family, that they had a mutation that was located in a gene that normally causes BBS. But with the A89V mutation, only the phenotype of retinitis pigmentosa occurred. Combination of the melanosome transport tests and the vision startle assay would explain how non-syndromic retinitis pigmentosa occurred in a gene that normally causes BBS. In the melanosome rescue experiment, BBS3 A89V was able to restore the rescue times back to the wild type times. This would be consistent on why individuals who do possess the A89V mutation do not possess any of the other phenotypes associated with BBS since BBS3 A89V is able to restore proper function of intracellular melanosome transport. The tests involving the vision startle assay showed how individuals could be present with non-syndromic retinitis pigmentosa. The test showed BBS3L A89V was unable to restore vision while comparative tests involving BBS3L did restore vision in the embryos. The A89V missense mutation was shown to be essential to proper vision function.

They also emphasized the importance of mutations that fall within different splice variants of single genes because even though the BBS3 A89V mutation does not fall within a splice site, it is located such that it impacts both BBS3 and BBS3L isoforms. Multiple splice isoforms with potentially diverse functions can be generated from a single gene which would contribute to phenotypic complexity in disease. This could infer how BBS3 A89V or BBS3L A89V can lead to just the phenotype of retinitis pigmentosa.

The importance of this study was to show how retinitis pigmentosa could occur in a non-syndromic form involving a mutation in an area that normally causes BBS.

Future Research

For future studies, I would suggest more research to be performed on the BBS3 A89V mutation. Even though in the previous studies, it was shown that BBS3 RNA was able to suppress the melanosome transport delays but not the vision defect, it is curious that the A89V mutation was discovered in BBS3 in the Saudi Arabian family and not BBS3L, the eye specific transcript required for vision function.

Specific research should be performed on the region that is different between BBS3 and BBS3L. Even though the region of difference was small, BBS3 and BBS3L both had opposite results in functionality when co-injected with the MO.

BBS3 was a known as part of the Ras family of small GTP binding proteins. How does the mutation affect the function of BBS3 regarding it as a small GTP protein.

Also, another suggestion for future research is to try and determine why the change from alanine to valine causes such a large change in visual function. Even though it is known that a mutation that changes an amino acid can causes a mess of problems, why does that change make such a large difference in retinal function.

Critique

The authors focused on two different transcripts of the mutation, BBS3 and BBS3L. But since the mutation was found in BBS3 and not BBS3L, their conclusion of how exactly non-syndromic retinitis pigmentosa could occur can be questioned. Since it appears they are combining two separate experiments, involving BBS3 A89V and BBS3L A89V, into a single conclusion. BBS3 can inhibit the melanosome transport defect but not the impaired vision while BBS3L can do the opposite.