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Gene Therapeutic Reversal of Peripheral Olfactory Impairment in Bardet-Biedl Syndrome

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Olfactory dysfunction is a pervasive but underappreciated health concern that affects personal safety and quality of life. Patients with olfactory dysfunctions have limited therapeutic options, particularly those involving congenital diseases. Bardet-Biedl syndrome (BBS) is one such disorder, where olfactory loss and other symptoms manifest from defective cilium morphology and/or function in various cell types/tissues. Olfactory sensory neurons (OSNs) of BBS mutant mice lack the capacity to build/maintain cilia, rendering the cells incapable of odor detection. Here we examined OSN cilium defects in *Bbs1* mutant mice and assessed the utility of gene therapy to restore ciliation and function in young and adult mice. *Bbs1* mutant mice possessed short residual OSN cilia in which BBSome protein trafficking and odorant detection were defective. Gene therapy with an adenovirus-delivered wild-type *Bbs1* gene restored OSN ciliation, corrected BBSome cilium trafficking defects, and returned acute odor responses. Finally, using clinically approved AAV serotypes, we demonstrate, for the first time, the capacity of AAVs to restore ciliation and odor detection in OSNs of Bbs1 mutants. Together, our data demonstrate that OSN ciliogenesis can be promoted in differentiated cells of young and adult *Bbs1* mutants and highlight the potential of gene therapy as a viable restorative treatment for congenital olfactory disorders.

INTRODUCTION

Human ciliopathies are a growing class of hereditary disorders in which altered cilium formation and/or function underlie pathogenesis. Ciliopathies encompass syndromes that affect single organs as well as highly pleiotropic diseases that exhibit systemic penetrance. Phenotypes include bone anomalies, situs inversus, heart malformation, neurological defects, ataxia, infertility, renal dysplasia, and sensory deficits.[1] Bardet-Biedl syndrome (BBS) (Online Mendelian Inheritance in Man #209900) is an autosomal recessive and broadly pleiotropic ciliopathy that features postaxial polydactyly followed by the onset of obesity, retinal degeneration, and renal failure.[2,3] In addition, BBS patients have variably penetrant olfactory deficits that range from mild microsmia to full anosmia.[4,5]

BBS is a genetically heterogeneous disease with 21 identified loci to date (http://www.ncbi.nlm.nih.gov/pubmed/27008867). Eight BBS gene products interact together in a core complex known as the BBSome.[6] The BBSome is postulated to function as a membrane coat complex that drives ciliary membrane biogenesis and regulates the ciliary trafficking of polytopic membrane proteins through an interaction with intraflagellar transport (IFT) machinery.[7–12] IFT is an evolutionarily conserved protein trafficking system that mediates anterograde and retrograde movement along ciliary microtubule axonemes and is essential for cilium formation and maintenance.[13] We and others have demonstrated that components of the BBSome participate in IFT in mammals and lower eukaryotes;[12–17] however, the exact functional role of the BBSome in the mammalian IFT is unclear. Importantly, loss of BBSome function in murine BBS models typically alters ciliary signaling capabilities and polytopic membrane protein localization in different cell types with diverse effects on cilium biogenesis. Therefore, the penetrance of BBS phenotypes in different organ systems is variable. The olfactory epithelium (OE) is one location where ciliation is dramatically decreased,[4,18–20] accounting for anosmia observed in BBS patients. This body of evidence, across several tissues and organisms, suggests that the precise role of the BBSome in normal ciliary trafficking and/or function varies by cell type, which may underlie the pleotropic nature of BBS.

Although clinical treatments for BBS and other ciliopathy patients are limited, our expanding comprehension of ciliopathy genetics enables the pursuit of gene therapy as a curative measure. It is estimated that roughly 80% of all BBS cases can be attributed to one of the known disease loci,[21] indicating that personalized medicine is a viable option for most patients. Previously, we demonstrated that ectopic gene introduction via intranasal viral delivery is an effective measure to

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restore olfactory cilium function and enable sensory detection in a hypomorphic mouse model of a severe prenatal lethal ciliopathy.[22] Here we tested the potential of gene therapy to restore odor detection in a BBS1 murine disease model that represents one of the three most commonly mutated BBS genes.[2,23] We report that noninvasive intranasal delivery of the wild-type (WT) BBS1 gene via adenovirus serotype 5 (AV5) is sufficient to restore ciliation of olfactory sensory neurons (OSNs), correct ciliary trafficking defects, and improve odor detection in both young and adult BBS mutant animals. Importantly, we demonstrate that clinically relevant adeno-associated virus serotype 9 (AAV9) is also effective for transduction of the OE and restoration of ciliation and odor detection in mutant animals. Our data indicate that BBS gene therapy can initiate ciliogenesis in differentiated mutant cells in vivo and that it represents a viable approach for treating olfactory deficits in BBS patients.

RESULTS

Reduced OSN Cilium Length and Number in BBS Mutant Mice

Olfactory deficits have been described in several BBS mouse models.[4,18,20,24–26] To examine gene therapy as a potential treatment option in BBS-associated anosmia and limit the effect of exogenous tissues, we used an OSN-specific knockout of *Bbs1* ($Bbs1^{osnKO}$). This strain was generated by combining a floxed *Bbs1* allele[27] with an *OMP-Cre* allele[28] that expresses Cre recombinase specifically in mature OSNs. Homozygous floxed *Bbs1* mice carrying a single *OMP-Cre* allele were used as mutants throughout this study; control animals carried at least one WT *Bbs1* allele or lacked the *OMP-Cre* allele. We first assessed the status of OSN cilia in the OE of $Bbs1^{osnKO}$ animals. The OE is a pseudo-stratified epithelium in which the OSN dendrites extend apically toward the nasal cavity lumen, forming knobs decorated with cilia. These cilia form a meshwork on the OE surface that can be visualized by immunostaining of acetylated α-tubulin, a marker of ciliary microtubules. Compared with control mice, $Bbs1^{osnKO}$ mutants showed global acetylated α-tubulin signal reduction on the OE apical surface (highlighted via OMP immunoreactivity), suggesting a loss of OSN cilia (Figures 1A, 1B, 1D, and 1E). Similar to reports on other BBS models,[4,20] $Bbs1^{osnKO}$ mutants did not show acetylated α-tubulin reduction on the apical surface of the respiratory epithelium (where OMP is not expressed) (Figures 1D and 1E). The reduction in acetylated α-tubulin immunostaining is concomitant with diminished endogenous ACIII (Figures S1A–S1C) and cyclic nucleotide gated channel alpha 2 (Cnga2) immunostaining in $Bbs1^{osnKO}$ mutants (Figures S1D–S1F).[4,18] To confirm that decreased acetylated α-tubulin, adenylate cyclase III (ACIII), and CNGA2 signals corresponded to OSN cilium loss, we performed scanning electron microscopy on the olfactory turbinates of $Bbs1^{osnKO}$ animals and found diminished ciliation (Figures 1C and 1F). Notably, OSNs possessed residual cilia among the exposed microvilli of the underlying supporting cells (Figure 1F). To examine the composition and morphology of the residual OSN cilia of BBS mutants, we next employed adenovirus (AV)-mediated ectopic expression of fluorescent protein-tagged cilium markers and live en face confocal imaging of the OE surface (Figures 1G and 1H). Compared with examination of coronal cryosections of fixed tissues, live en face confocal imaging allows for detailed examination of intact cilia, cilium structure, and protein trafficking dynamics limiting the contribution of artifacts,[14] Combined with AV5-mediated expression of the myristoylated-palmitoylated form of mCherry (MyrPalm-mCherry), an inert probe that marks the cell and ciliary membrane inner leaflet, we are able to visualize and confirm the full length of OSN cilia.[14] We next assessed the localization of polytopic membrane proteins that are part of the olfactory signaling pathway and enriched in the cilia. Ectopic co-expression of GFP-fused adenylate cyclase III (ACIII-GFP) and MyrPalm-mCherry showed ACIII-GFP presence in residual OSN cilia of $Bbs1^{osnKO}$ animals (Figure S2A), suggesting that BBS1 was not essential for ACIII entry into OSN cilia. This is consistent with previous reports[4,18] and diminished endogenous ACIII and Cnga2 immunostaining in $Bbs1^{osnKO}$ mutant coronal sections (Figure S1). Using AV5-mediated expression of MyrPalm-mCherry, we next examined the degree of OSN ciliation across the turbinates of the OE. Analysis of cilia from control animals showed uniform OSN cilium lengths and numbers across the turbinate surface of the OE (Figure S3), which are consistent with past reports.[29–32] We next quantified the extent of OSN cilium loss in $Bbs1^{osnKO}$ animals. Compared with controls, $Bbs1^{osnKO}$ animals had significantly reduced cilium length, resulting in a leftward shift in the cumulative distribution of total cilia (Figure 1J; Figure S3). In $Bbs1^{osnKO}$ mutants, OSN cilium length was reduced by 77% (6.11 ± 0.15 μm) from control (26.61 ± 0.63 μm), whereas the cilium number per OSN was reduced by half in $Bbs1^{osnKO}$ mutants (12.22 ± 0.49 cilia) from the control (23.07 ± 0.95 cilia) (Figures 1K and 1L; Figure S3). Overall, our results indicate that $Bbs1^{osnKO}$ mutants retain the capacity to build OSN cilia but are unable to attain or maintain normal OSN cilium length or number.

Impaired BBSome Trafficking in $Bbs1^{osnKO}$ Mutant Mice

Our findings of shorter and fewer OSN cilia in $Bbs1^{osnKO}$ mutants prompted us to analyze cilium protein trafficking in the animals. Previous work has uncovered specific interactions between BBS proteins[33] and their assembly into the BBSome;[34] however, BBS protein function in mammalian protein trafficking in the cilia and BBSome ciliary targeting are unclear. We therefore examined the effect of BBS1 disruption on the ciliary localization and IFT of other BBSome proteins. Using total internal reflection fluorescence (TIRF) microscopy, which allows visualization of cilium protein trafficking,[14] we examined IFT within the residual cilia. Interestingly, we found that IFT was retained in $Bbs1^{osnKO}$ mutant OSN cilia (Figures 2A, 2B, and 2E). Components of the heterotrimeric kinesin II (Kap3a) and cytoplasmic dynein motor (Dync2li1) complexes, which associate with the IFT particles, showed cilium trafficking and bidirectional transport in $Bbs1^{osnKO}$ mutants (Figures 2A and 2B; Figure S2B). Next we assessed the cilium trafficking of BBSome proteins. We demonstrated that BBS1, BBS2, BBS4, and BBS5 undergo IFT in OSN cilia.[14] In $BBS1^{osnKO}$ mutants, ectopically expressed BBS2-GFP, BBS4-GFP, and BBS5-GFP fail to localize within the cilia despite heavily accumulating in OSN dendritic knobs (Figures 2C and 2D). To confirm this result, we co-expressed BBS4-mCherry and Kap3a-GFP in $Bbs1^{osnKO}$ mutants and did not find co-localization

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