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 ciliogenesis 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. 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. 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. 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. IFT is an evolutionarily conserved protein trafficking system that mediates anterograde and retrograde movement along ciliary microtubule axonemes and is essential for cilia formation and maintenance. We and others have demonstrated that components of the BBSome participate in IFT in mammals and lower eukaryotes, 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 cilia biogenesis. Therefore, the penetrance of BBSome phenotypes in different organ systems is variable. The olfactory epithelium (OE) is one location where ciliation is dramatically decreased, 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 pleiotropic 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, 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...
restore olfactory cilium function and enable sensory detection in a hypomorphic mouse model of a severe prenatal lethal ciliopathy.\textsuperscript{22,26} 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.\textsuperscript{22,25} 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 defects in BBS patients.

RESULTS

Reduced OSN Cilium Length and Number in BBS Mutant Mice

Olfactory deficits have been described in several BBS mouse models.\textsuperscript{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\textsuperscript{osnKO}). This strain was generated by combining a floxed Bbs1 allele\textsuperscript{27} with an OMP-Cre allele\textsuperscript{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\textsuperscript{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\textsuperscript{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,\textsuperscript{4,20} Bbs1\textsuperscript{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\textsuperscript{osnKO} mutants (Figures S1D–S1F).\textsuperscript{1,16} To confirm that decreased acetylated α-tubulin, adenylyl cyclase III (ACIII), and CNGA2 signals corresponded to OSN cillum loss, we performed scanning electron microscopy on the olfactory turbinates of Bbs1\textsuperscript{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 cillum 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, cillum structure, and protein trafficking dynamics limiting the contribution of artifacts.\textsuperscript{14} Combined with AV5-mediated expression of the myristoylated-palmitoylated form of mCherry (MryPalm-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.\textsuperscript{4,18} 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 adenylyl cyclase III (ACIII-GFP) and MryPalm-mCherry showed ACIII-GFP presence in residual OSN cilia of Bbs1\textsuperscript{osnKO} animals (Figure S2A), suggesting that BBS1 was not essential for ACIII entry into OSN cilia. This is consistent with previous reports\textsuperscript{4,18} and diminished endogenous ACIII and Cnga2 immunostaining in Bbs1\textsuperscript{osnKO} mutant coronal sections (Figure S1).

Using AV5-mediated expression of MryPalm-mCherry, we next examined the degree of OSN ciliation across the turbinates of the OE. Analysis of cilia from control animals showed uniform OSN cillum lengths and numbers across the turbinate surface of the OE (Figure S3), which are consistent with past reports.\textsuperscript{29–32} We next quantified the extent of OSN cillum loss in Bbs1\textsuperscript{osnKO} animals. Compared with controls, Bbs1\textsuperscript{osnKO} animals had significantly reduced cillum length, resulting in a leftward shift in the cumulative distribution of total cilia (Figure 1J; Figure S3). In Bbs1\textsuperscript{osnKO} mutants, OSN cillum length was reduced by 77% (6.11 ± 0.15 μm) from control (26.61 ± 0.63 μm), whereas the cillum number per OSN was reduced by half in Bbs1\textsuperscript{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\textsuperscript{osnKO} mutants retain the capacity to build OSN cilia but are unable to attain or maintain normal OSN cillum length or number.

Impaired BBSome Trafficking in Bbs1\textsuperscript{osnKO} Mutant Mice

Our findings of shorter and fewer OSN cilia in Bbs1\textsuperscript{osnKO} mutants prompted us to analyze cillum protein trafficking in the animals. Previous work has uncovered specific interactions between BBS proteins\textsuperscript{13} and their assembly into the BBSome.\textsuperscript{14} 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 cillum protein trafficking,\textsuperscript{14} we examined IFT within the residual cilia. Interestingly, we found that IFT was retained in Bbs1\textsuperscript{osnKO} mutant OSN cilia (Figures 2A, 2B, and 2E). Components of the heterotrimeric kinesin II (Kap3a) and cytoplasmic dynein motor (Dyn2li1) complexes, which associate with the IFT particles, showed cillum trafficking and bidirectional transport in Bbs1\textsuperscript{osnKO} mutants (Figures 2A and 2B; Figure S2B). Next we assessed the cillum trafficking of BBSome proteins. We demonstrated that BBS1, BBS2, BBS4, and BBS5 undergo IFT in OSN cilia.\textsuperscript{14} In Bbs1\textsuperscript{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\textsuperscript{osnKO} mutants and did not find co-localization.
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات