Ciliary Beating Recovery in Deficient Human Airway Epithelial Cells after Lentivirus Ex Vivo Gene Therapy

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Abstract

Primary Ciliary Dyskinesia is a heterogeneous genetic disease that is characterized by cilia dysfunction of the epithelial cells lining the respiratory tracts, resulting in recurrent respiratory tract infections. Despite lifelong physiological therapy and antibiotics, the lungs of affected patients are progressively destroyed, leading to respiratory insufficiency. Recessive mutations in Dynein Axonemal Intermediate chain type 1 (DNAI1) gene have been described in 10% of cases of Primary Ciliary Dyskinesia. Our goal was to restore normal ciliary beating in DNAI1–deficient human airway epithelial cells. A lentiviral vector based on Simian Immunodeficiency Virus pseudotyped with Vesicular Stomatitis Virus Glycoprotein was used to transduce cultured human airway epithelial cells with a cDNA of DNAI1 driven by the Elongation Factor 1 promoter. Transcription and translation of the transduced gene were tested by RT–PCR and western blot, respectively. Human airway epithelial cells that were DNAI1–deficient due to compound heterozygous mutations, and consequently had immotile cilia and no outer dynein arm, were transduced by the lentivirus. Cilia beating was recorded and electron microscopy of the cilia was performed. Transcription and translation of the transduced DNAI1 gene were detected in human cells treated with the lentivirus. In addition, immotile cilia recovered a normal beat and outer dynein arms reappeared. We demonstrated that it is possible to obtain a normalization of ciliary beat frequency of deficient human airway epithelial cells by using a lentivirus to transduce cells with the therapeutic gene. This preliminary step constitutes a conceptual proof that is indispensable in the perspective of Primary Ciliary Dyskinesia’s in vivo gene therapy. This is the first time that recovery of cilia beating is demonstrated in this disease.

Introduction

Primary Ciliary Dyskinesia (PCD, OMIM #242650) is an inherited disease mainly characterized by dysfunction of airways’ motile cilia. The prevalence is approximately 1 in 12,000–20,000 [1–3]. About 50% of patients affected by PCD have a situs inversus which results from monocilia dysfunction at the embryonic node [4]. This association is referred to as Kartagener’s syndrome (OMIM #244400) [5]. PCD causes chronic sinus and bronchial respiratory infections that begin early in life, leading to nasal polyps and bronchiectasis. Males are frequently sterile due to dysfunctional spermatozoa flagella [6]. Other symptoms can also be associated with PCD like hydrocephalus, anosmia, retinitis pigmentosa and congenital heart diseases [7–10].

The disorder is genetically heterogeneous and in most cases, inheritance is autosomal recessive but X-linked inheritance patterns were also described [11]. Several loci and some genes have been identified, as DNAH1 (Ensembl ENSG00000122735), DNAH5 (ENSG00000039139), DNAH11 (ENSG00000105877), RPGR (ENSG00000156313), TXND5 (ENSG00000086288), ODF1 (ENSG000000046651), DNAI2 (ENSG00000171595) and KTU (alias C14orf104; OTTHUMG00000152331) genes [10,12–19]. The first gene described to be responsible for PCD and Kartagener syndrome was DNAH1 gene [18,20]. Eighteen mutations in DNAH1 gene were reported, and Zariwala et al. evidenced a founder effect for the most frequent mutation (c.48+2_48+3insT) [21]. Moreover, the authors estimated that mutations in DNAH1 gene represent about 10% of PCD cases.

DNAH1 encodes an axonemal dynein intermediate chain, a component of the outer dynein arm (ODA). Dyneins are molecular motors which produce energy for microtubules doublets sliding in the axoneme. To date, no etiological treatment of PCD is available and on the long range, PCD leads to respiratory insufficiency and lung transplant.

We hypothesized that gene therapy could restore ciliary function in DNAH1-mutated airway epithelial cells to prevent patients from infectious complications. To introduce genetic material into cells we focused on lentiviral gene transfer because lentivirus has the property to integrate its genetic material into host cell genome even in non-replicating cell [22]. Moreover, lentivirus...
Author Summary

This manuscript reports on a successful gene therapy attempt on human airway epithelial cells of a patient suffering from Primary Ciliary Dyskinesia. In this autosomal recessive disease, cilia of the epithelial cells that border the upper and lower respiratory tracks are not functioning. As a result, patients suffer from recurrent airway infections leading progressively to respiratory insufficiency. There is no treatment as of today that could restore normal ciliary beating. In this report, we showed that it is feasible to transfer a therapeutic gene to human airway epithelial cells with a lentivirus. This transferred gene is transcribed and expressed. Moreover, defective cells that had immotile cilia due to compound heterozygous mutations in the DNAI1 gene recovered ciliary beating after treatment with a lentivirus containing a normal DNAI1 gene. This is the first report on gene therapy in Primary Ciliary Dyskinesia. Since lentivirus is able to insert therapeutic genes into the cell genome, this result may have impact on in vivo gene therapy in this disease and in diseases related to human epithelial airway cells such as cystic fibrosis.

is weakly immunogenic unlike recombinant adenovirus which efficiency was reported to decrease after several administrations in a clinical study of patients suffering from cystic fibrosis [23]. Lentiviral-derived vectors used in gene therapy were principally based on SIV (simian immunodeficiency virus) or HIV (human immunodeficiency virus). For this latter one, gene transfer efficiency was reported to decrease after several administrations in a clinical study of patients suffering from cystic fibrosis [23].

We decided to modify a SIV-based vector, previously described by Negre et al. to efficiently transduce mature human dendritic cells [25,26], and to transduce human airway epithelial cells (HAECs) cultured as described by Jorissen et al. [27]. First, we showed here that normal HAECs were efficiently transduced by SIV-based vector containing eGFP. Then, we validated lentiviral vectors’ constructions containing DNAI1 cDNA sequence and showed that transduced DNAI1 is transcribed and expressed. Finally, we demonstrated that transduction of DNAI1-mutated HAECs with wild-type DNAI1 can restore ciliary beating and that ODA are binding again to microtubules.

Results

Transduction of HAECs with pGFP

To estimate whether HAECs could be transduced by a SIV-based lentivirus pseudotyped with VSV-G, normal HAECs were infected with pGFP a vector containing eGFP as a reporter gene in a variety of conditions [25,26]. Three parameters were investigated: (1) the multiplicity of infection (MOI), (2) the moment of infection and (3) whether using a polycation, Polybrene, or not.

Two different moments of infection during Jorissen’s culture were tested: at J+1, cells were ciliated and in suspension or at J+3, cells were de-differentiated and adherent. Two days post-infection, reporter gene expression was analyzed by FACS (Figure 1). In any of the selected conditions, HAECs were transduced but the proportion of transduced cells seemed to be dependant on MOI irrespective of the other parameters, and apparently higher at MOI 75. Then, transduction of cells infected at J+1 seemed more efficient compared to cells infected at J+3. At MOI 75, approximately 38% of cells infected at J+1 were transduced versus 20% for cells infected at J+3. Moreover, these results seemed to be improved by the use of Polybrene. Finally, at MOI 75, transduction efficiency was quantified at about 38% for cells infected at J+1 without Polybrene compared to approximately 50% for cells infected at J+1 with the use of Polybrene. These experiments which were not repeated, demonstrated that HAECs could be transduced in a variety of conditions and that J+1 with Polybrene at a MOI of 75 were presumably the best experimental conditions for HAECs infection. Therefore, we selected these conditions for further experiments.

DNAI1 cDNA Cloning in Lentiviral Vectors

Full-length DNAI1 cDNA was cloned into the lentiviral vector in place of eGFP gene. Two constructions were obtained using BamHI and XhoI restriction sites (pK-DNAI1) or Neo I and XhoI (pK+pDNAI1), and resulted respectively in an intact DNAI1 cDNA associated with a modified Kozak sequence which could prevent normal translation, or an intact Kozak sequence associated with a modified DNAI1 cDNA which could result in a dysfunctional protein (Figure 2A). Then, to differentiate endogene from exogene DNAI1, a hemagglutinin tag (HA) was added at the 3’ side of DNAI1 cDNA sequence in each plasmid which resulted in two additional vectors: pK-HA or pK+pHA (Figure 2B).

Detection of DNAI1 Gene Expression in Transduced Normal HAECs

Normal HAECs were transduced at J+1 with Polybrene at a MOI of 75 since these conditions gave satisfactory results as evaluated with eGFP. First, mRNA extracts from transduced HAECs were controlled by PCR with alpha-tubulin specific primers for absence of genomic DNA (gDNA) contamination (not shown). Second, we confirmed that non-ciliated HAECs (NC) do not express DNAI1 because DNAI1 specific RT primer (P5) led to an absence of amplification by contrast to re-ciliated HAECs (RC) template (Figure 3, lanes 1 and 2). Third, DNAI1 mRNA was not amplified using a HA-tag RT specific primer (HA) from non-infected re-ciliated HAECs (Figure 3, lane 3). To test DNAI1 transcription from lentiviral vectors, HA-tagged DNAI1 gene transcription was revealed by RT-PCR using HA-DNAI1 specific primers. Re-ciliated HAECs infected by particles containing HA-tagged DNAI1 with either an exact Kozak sequence (pK+HA) or a modified Kozak sequence (pK-

Figure 1. Transduction efficiency analysis by FACS two days after HAECs infection with pGFP vector. MOI, multiplicity of infection (3 values of MOI were tested 7, 35, and 75); J+1, one day after biopsy, ciliated cells in suspension; J+3, three days after biopsy, de-differentiated and adherent cells; +, with use of Polybrene; −, without use of Polybrene. These results were obtained from a single experiment in each condition. MOI is the ratio of infectious agents (e. g. lentivirus) to infection targets (e. g. cells).

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DNAI1 protein was detected by Western blot with specific anti-DNAI1 antibodies after 293Bosc cells' transfection with the Kozak modified (pK-DNAI1) or Kozak conserved (pK+DNAI1) DNAI1 sequence (Figure 4, lane 1 and 2). DNAI1 protein was also detected after 293Bosc cells' transfection with the vectors containing HA appended to the 3' end of DNAI1 sequence (Figure 4, lane 3; modified Kozak and lane 5; conserved Kozak sequence). By contrast, no DNAI1 protein could be detected by Western blot in protein lysate of non-transfected 293Bosc cells with anti-DNAI1 antibodies (Figure 4, lane 4).

Moreover, immunofluorescence assays showed that in pK-HA transfected 293Bosc cells, cytoplasmic HA-tagged DNAI1 proteins could be specifically detected by HA antibodies (Figure S1).

Transduction of DNAI1-Mutated HAECS

In the second set of experiments, transduction was performed on DNAI1-mutated HAECS at J+t and cells were cultured to generate ciliated vesicles. DNAI1-mutated HAECS were transduced with pK-DNAI1 or pK-HA to determine if immotile cilia might recover a beat. The same protocol of transduction was used as for normal HAECS. We confirmed DNAI1-mutated HAECS transduction efficiency with pGFP vector, as de-differentiated cells at J+3 and re-ciliated cells at J+17 (17 days post-collagen digestion) expressed GFP protein (Figure S2).

After re-differentiation, GFP transduced HAECS were covered with cilia (Figure 5A-a) but these cilia were immotile. The variation of optic signal along a line crossing the cilia during 400 msec does not show any movement (Figure 5A-b). This immotility is also visible on video recording (Video S1). By contrast, ciliary beating was recorded on DNAI1-mutated HAECS transduced with either pK-DNAI1 or pK-HA vector (Figure 5A-c). This beat is demonstrated by recording the variation of optic signal along a line crossing cilia. Waves are clearly visible (Figure 5A-d) that evidenced the periodic beat of cilia. This active beat is also visible on video recording (Video S2). At J+30, a ciliary beat frequency (CBF) was measured for pK-DNAI1 and pK-HA transduced cells. CBF of HAECS with beating cilia were 9.95±1.23 Hz and 11.31±0.85 Hz, for pK-DNAI1 and pK-HA treated cells, respectively. These values fall into the range of control HAECS (from 7 to 11 Hz). Cilia length in DNAI1-mutated HAECS was estimated at 6 μm in all cases and did not depend on DNAI1 treatment and cilia beating.

In DNAI1-mutated HAECS' axoneme, ultrastructure analysis by TEM showed that ODA were absent or shorter than in normal HAECS (Figure 5B-a). TEM on axonemes of DNAI1-treated cells were analyzed and some cilia had normal amounts of ODA, while in other cilia the ODA were partially absent (Figure 5B-c and 5B-d).
The average number of ODA per axoneme was 3.29 ± 1.53 in pGFP infected DNAI1–mutated HAECs. ODA increased significantly to 5.67 ± 1.83 (p = 0.0001) and 5.73 ± 2.10 (p = 0.002) in DNAI1–mutated HAECs treated with pK–DNAI1 and pK–HA, respectively. By contrast, there was no significant difference between DNAI1–mutated HAECs treated by pK–DNAI1 or pK–HA. The distribution of the number of ODA per axoneme presented a single peak in pGFP infected DNAI1–mutated HAECs but two peaks in pK–DNAI1 and pK–HA treated cells (Figure 6). IDA analysis showed no difference between control and DNAI1–treated cells (data not shown).

**Discussion**

In the present study, we demonstrated that a SIV-based vector pseudotyped with VSV-G protein, previously described to efficiently transduce human dendritic cells [25,26], could also efficiently infect normal cultured HAECs [27]. Previous studies showed that Murine Leukemia virus (MuLV) [28] and Feline Immunodeficiency Virus pseudotyped with VSV-G envelope [29] could transduce HAECs in culture. Though, this transduction was only possible from the basolateral surface of polarized HAECs. We did not know whether a Simian Immunodeficiency Virus would transduce although we could foresee that transduction would be more efficient from the basolateral surface of HAECs since it was pseudotyped with VSV-G. We did not know also whether de-differentiated cells would be transduced. It appeared that whatever the conditions used in this study, a certain proportion of cultured HAECs were infected. We then selected the conditions that seemed to provide the higher percentage of infected cells in culture. In any case, these conditions are strikingly different from *in vivo* situation where HAECs are a component of a complex epithelium recovered by a thin layer of mucus. Moreover, Polybrene which seemed to improve viral infection in cultured cells cannot be used *in vivo* due to side-effects. In a next step, we demonstrated transcription and translation of the transduced DNAI1 cDNA. Finally, we showed that DNAI1–mutated HAECs treated by DNAI1 gene transfer recovered ciliary beating whereas GFP-treated cells’ cilia remained immotile, thus supporting our concept that PCD gene therapy was possible using DNAI1 gene. This conceptual proof is essential in the perspective of a human gene therapy.

Since it was impossible to obtain a vector containing a conserved Kozak sequence associated with a normal DNAI1 cDNA sequence, we decided to construct two vectors: one with a modified Kozak sequence upstream a conserved DNAI1 cDNA sequence (pK–DNAI1), and another one with a conserved Kozak sequence.

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**Figure 5. Images of transduced DNAI1–mutated HAECs and electron microscopic sections of cilia.** (A) At J′+31, vesicles had cells with cilia transduced with either pGFP (A-a) or pK–DNAI1 vectors (A-c). However, cilia of cells transduced with pGFP were immotile whereas cells transduced with pK–DNAI1 vectors were beating. Figures (A-a) and (A-c) represent the variations of the video signal during the time of recording (400 msec) along a virtual line delimited by the “>” and “<” signs on figures (A-a) and (A-c), respectively. The periodic beat of cilia is clearly visible on figure (A-a) compared to the immotility of cilia on figure (A-c). Black bar on figure (A-a): 10 μm. Black bar on figure (A-c): 100 msec. DNAI1–mutated HAECs transduced with pK–HA vector containing HA-tagged DNAI1 cDNA sequence gave identical results (data not shown). See Video S1 and Video S2. (B) Axoneme ultrastructural analysis by TEM (a-c): (B-a) Normal HAEC. (B-b) DNAI1–mutated HAEC treated with pGFP. (B-c) DNAI1–mutated HAEC treated with pK–DNAI1 vector. (B-d) DNAI1–mutated HAEC treated with pK–HA vector. Arrowheads indicate ODA. Axoneme diameter is about 0.2 μm. doi:10.1371/journal.pgen.1000422.g005

**Figure 6. Distribution of the number of ODA per axoneme in DNAI1–mutated infected HAECs.** X axis, number of ODA per axoneme; Y axis, percentage of axonemes with a given number of ODA. Empty bars, DNAI1–mutated HAECs infected with pGFP; filled bars, cells infected with pK–DNAI1; striped bars, cells infected with pK–HA. doi:10.1371/journal.pgen.1000422.g006
Airway ciliated cells are differentiated cells which do not
transdifferentiate into other cell types and restored the airway
after an injury event, murine ciliated epithelial cells could
type II cells generation [34]. Nevertheless, Park reported that
short. In the mouse model, ciliated cells renewal was reported to
proliferate and their cycle life span is supposed to be relatively
resulting in higher titres of infected cells than with VSV-G [33].

We demonstrated that HA envelope protein of H7N1 and H5N1 avian
receptors, as
be specifically localized on ciliated airway and type II alveolar
cells. Finally, Szecsi demonstrated that his cilia were 6 µm-long and totally immotile. Analysis of his DNA demonstrated that he harbored heterozygous
mutations: c.487+2_487+3insT and c.1543G>A [20]. This patient signed an informed consent allowing us to
experiment on nasal biopsies which consisted in removing the
most anterior part of his middle nasal turbinate on both side. This
study complies with the rules of the local ethical committee.

Material and Methods
Patient Data
The diagnosis of a male patient affected by PCD was based on
clinical signs (recurrent upper and lower respiratory tract
infections since early in life, nasal polyps and complete situs
inversus – Kartagener syndrome). In addition, he was sterile.

Human Airway Epithelial Cell Culture
Human airway epithelial cells (HAECs) from normal subjects
were obtained from nasal turbinates which were removed and
discarded in the process giving access to the ethmoidal sinus.
Patients were operated for tumours located in the ethmoidal
region and had no respiratory disease.

Cells from control subjects and the patient were grown using the
immured cell culture previously described by Jorissen et al. [27].
Briefly, ciliated cells were isolated and cultured the day following the
biopsy [J+1] in collagen-coated flasks to de-differentiate in non-
ciliated cells. When they reached 80–90% confluence, collagen
was digested (J’, 7–10 days post-seeding) and cells were suspended in
flasks with rotation to re-differentiate in the form of ciliated vesicles.

Cells were infected at J+1 or J+3. Non-ciliated cells were
harvested at J+7 to 10 (J’) and re-ciliated cells were fully re-
differentiated at J’+28.
Cloning of DNAI1 cDNA
To generate DNAI1 cDNA (AF091619), we extracted total RNA from ciliated HAEC and synthesized the cDNA. A pair of primers was designed to amplify the full-length cDNA. Six supplementary sets of specific primers were used to sequence DNAI1 cDNA. The full-length DNAI1 cDNA PCR product was cloned.

Addition of enzyme restriction sites in 5' and 3' DNAI1 cDNA sequence was performed using two sets of primers. The BamHIDNAI1_for and NCOIDNAI1_for primers (forward) added BamHI and XhoI restriction sites upstream DNAI1 cDNA sequence, respectively. The XHOIDNAI1_rev primer (reverse) added a XhoI restriction site downstream DNAI1 cDNA sequence.

Addition of hemagglutinin (HA) tag downstream DNAI1 cDNA sequence was performed by PCR using lentiviral vectors containing DNAI1 cDNA (pK+DNAI1) as template and upDNAI1_for (forward)/lowHA_rev (reverse) primers. For more information see Text S1.

Lentiviral Vectors
The lentiviral vector system used in this study was derived from SIV vectors described by Negre et al. [25,26]. Five different lentiviral vector constructs were used all under the transcriptional control of the human Elongation Factor-1 promoter (EF1): (1) pRS4A-EFS-GFP-W (pGFP), (2) pK-DNAI1, (3) pK+DNAI1, (4) pK-HA, (5) pK+HA. The pGFP vector contains the gfp cDNA sequence. The pK-DNAI1 and pK+DNAI1 vector constructs are essentially the same as the pGFP vector but with the DNAI1 cDNA sequence replacing the gfp gene sequence, whereas pK-HA vector has a modified Kozak sequence with an intact eGFP sequence. The pK+DNAI1 and pK+DNAI1 vector constructs are essentially the same as the pGFP vector but with the DNAI1 cDNA sequence replacing the gfp gene sequence, whereas pK+DNAI1 has an intact Kozak sequence with a modification at position 4 of the DNAI1 cDNA sequence, resulting in DNAI1 second amino acid modification: isoleucine>valine. The pK-HA and pK+HA vector constructs correspond to pK-DNAI1 and pK+DNAI1 vectors, respectively, with a 3' DNAI1 cDNA hemagglutinin (HA) tag addition, using Blp I/Xho I restriction sites (Figure 2B).

Lentiviral Transduction
Three sets of experiments were carried out.
In the first set, normal HAECs were transduced the day of seeding (J+1) on collagen-coated flasks or at J+3, and the cells were incubated for 24 hours with the lentivirus at MOI (Multiplicity Of Infection) of 7, 35 and 75 in the presence or absence of 6 μg/mL Polybrene (1,5-dimethyl-1,5-diazanuclecamethylene polymethylene bromide, hexadimethrine bromide), MOI is the ratio of infectious agents (e.g. lentivirus) to infection targets (e.g. cells). Then, medium was completely changed in order to remove debris and inactive lentiviruses. Two days post-infection, reporter gene expression was analyzed by FACS.
In the second set of experiments, normal HAECs were transduced or not the day of seeding (J+1) at MOI 75 with Polybrene with pK-HA or pK+HA. RNA was extracted before or after re-ciliation.
In the third set of experiments, transduction was performed on DNAI1-mutated HAECs at J+1, at MOI 75, with the use of Polybrene and cells were cultured to generate ciliated vesicles.

RT–PCR Analysis
Tagged DNAI1 gene expression was revealed by reverse transcription PCR (RT–PCR) on infected HAEC. Non-ciliated cells were collected the day of collagen digestion (J') and ciliated cells were collected when they were fully covered by cilia (J'+28).

Poly(A)+ mRNA was isolated by the Dynabeads Oligo(dT)25 purification kit, according to the manufacturer's protocol (Dynal Biotech, Norway).

Western Blotting Analysis
Transient transfection of 293Bosc cells was performed with each four different plasmids: pK-DNAI1, pK+DNAI1, pK-HA or pK+HA. Western blot analysis was carried out according to standard techniques (Text S1).

Video Analysis and Ciliary Beat Frequency (CBF)
In order to assess the functional activity of the ciliated DNAI1-mutated cells after lentiviral transduction, video recordings were performed with a ×40 objective lens in the light path at different steps of the culture in flasks, by using an Olympus IX50 inverted phase-contrast microscope. The control GFP fluorescence was observed with a magnifying digital camera SCION CFW 1308M (Scion Corporation, Frederick, MD) and the recovery of ciliary beating was recorded with high speed digital video camera pco. 1200 hs (PCO, Germany). The digital image-sampling rate was software-controllable using CamWare and for all experiments the sampling rate was set at 500 frames per second (fps).
For measurements of ciliary beat frequency (CBF), the video images of active ciliated cells were captured with a ×100 oil-immersed objective lens, using Leica DMRXA microscope and pco. 1200 hs camera. A time-motion representation was obtained and analyzed using ImageJ software (National Institutes of Health, USA). Briefly, a line cutting cilia close to their tip was drawn. The “redice” function of ImageJ was then used to obtain the video signal along this line (y axis) during 400 msec (x axis). Finally, the data were expressed as mean±SD from at least three regions of interest. Values for normal HAECs were used as controls.

Transmission Electron Microscopy (TEM) and Statistical Analysis
Transmission electron microscopic analyses were processed on ciliated vesicles obtained after HAECs culture, as described by Jorissen et al. [27]. Amounts of ODA were expressed as mean±SD from n axonemes (n = 17 for pGFP, n = 21 for pK-DNAI1, n = 11 for pK-HA). Values of HAEC infected with pGFP, pK-DNAI1 or pK-HA were statistically analysed by Student t test and the significance was calculated for a two-tailed test.
See Text S1 for more details.

Supporting Information
Text S1 Supporting Material and Methods.
Found at: doi:10.1371/journal.pgen.1000422.s001 (0.10 MB PDF)

Figure S1 HA-tagged DNAI1 expression in 293Bosc cell line transduced with pK-HA vector. (A) Nuclei were labelled with DAPI (blue) and image was superimposed with transmission image. (B) HA tag was immunostained using anti-HA antibody associated with biotinylated goat anti-mouse and FITC-conjugated ExtrAvidin (green). A specific cytoplasmic localization was observed. Scale bars, 10 μm.
Found at: doi:10.1371/journal.pgen.1000422.s002 (2.21 MB TIF)

Figure S2 DNAI1-mutated HAEC transduced with pGFP vector. eGFP fluorescence superimposed with transmission image. (A) At J+3, HAEC are de-differentiated and adherent (bar, 50 μm). (B) At J'+17, HAEC are re-differentiated and in suspension as ciliated vesicles. Arrowheads indicate cilia (bar, 20 μm).
Found at: doi:10.1371/journal.pgen.1000422.s003 (2.54 MB TIF)

Ciliary Beating Recovery

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**Video S1** DNAII-deficient Human Airway Epithelial Cells transduced with GFP. DNAII-mutated Human Airway Epithelial Cells were transduced with a GFP containing lentiviral vector. Cilia are clearly immotile as in non-transduced cells of this patient. The video was recorded at 500 frames per second. This video includes 201 frames.

Found at: doi:10.1371/journal.pgen.1000422.s004 (7.23 MB AVI)

**Video S2** DNAII-mutated Human Airway Epithelial Cells after transduction with DNAII lentiviral vectors. DNAII-mutated Human Airway Epithelial Cells were transduced with a pK-DNAII and pK-HA lentiviral vectors. In both cases, beating cilia were clearly visible as on this video from pK-DNAII treated cells. The video was recorded at 500 frames per second. This video includes 200 frames.

Found at: doi:10.1371/journal.pgen.1000422.s005 (7.19 MB AVI)

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**Author Contributions**


**References**