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Microfluidic platform to evaluate migration of cells from patients with DYT1 dystonia

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Abstract

Background—Microfluidic platforms for quantitative evaluation of cell biologic processes allow low cost and time efficient research studies of biological and pathological events, such as monitoring cell migration by real-time imaging. In healthy and disease states, cell migration is crucial in development and wound healing, as well as to maintain the body's homeostasis.

New Method—The microfluidic chambers allow precise measurements to investigate whether fibroblasts carrying a mutation in the *TOR1A* gene, underlying the hereditary neurologic disease - DYT1 dystonia, have decreased migration properties when compared to control cells.

Results—We observed that fibroblasts from DYT1 patients showed abnormalities in basic features of cell migration, such as reduced velocity and persistence of movement.

Comparison with Existing Method—The microfluidic method enabled us to demonstrate reduced polarization of the nucleus and abnormal orientation of nuclei and Golgi inside the

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Conflict of interest

Authors have no conflict of interest.

moving DYT1 patient cells compared to control cells, as well as vectorial movement of single cells.

Conclusion—We report here different assays useful in determining various parameters of cell migration in DYT1 patient cells as a consequence of the *TOR1A* gene mutation, including a microfluidic platform, which provides a means to evaluate real-time vectorial movement with single cell resolution in a three-dimensional environment.

Keywords

Microfluidic device; Cell polarization; Migration; Dystonia; DYT1; torsinA

1. Introduction

Dystonia is the third most common neurological movement disorder in humans. Dystonia is a complex syndrome characterized by motor dysfunction in a number of neurological diseases (for review see Albanese and Lalli, 2012). Dystonia is clinically diagnosed with physical symptoms such as involuntary twisting movements and abnormal postures (Fahn, 1984; Fung et al., 2013; Albanese et al., 2013). Fourteen genes have been implicated in 25 forms of hereditary dystonia allowing for mutational testing in diagnosis and genetic counseling (Lohmann and Klein, 2013). DYT1 dystonia, also known as early onset generalized torsion dystonia, is an autosomal-dominant form of dystonia with low penetrance with most cases caused by a common mutation - a GAG deletion in the coding region of the *TOR1A* gene that encodes torsinA (Bressman et al., 2002). Mutant torsinA, torsinA^E appears to act in a dominant-negative manner to suppress wild-type activity, which supports functions of the endoplasmic reticulum (ER) and nuclear envelope (NE) (Hewett et al., 2007; Nery et al., 2008; Nery et al., 2011; Atai et al., 2012). TorsinA participates in a number of cellular functions, including migration of cells through a role in nuclear polarization (Nery et al., 2008), egress of viral and large ribonucleoprotein particles out of the NE (Maric et al., 2011; Jokhi et al., 2013), and protection from cellular stress (Nery et al., 2011; Bragg et al., 2011; Chen et al., 2010; Cao et al., 2010).

Cell migration is an evolutionarily conserved mechanism that underlies the development and functioning of uni- and multicellular organisms and takes place in normal and pathogenic processes, including various events of embryogenesis, wound healing, immune responses, cancer metastases and angiogenesis (Kurosaka and Kashina, 2008). Functionally torsinA^E is believed to reduce activity of wild-type torsinA thereby weakening the connection between the cytoskeleton and the outer nuclear membrane and the contiguous ER membrane (Nery et al., 2008; Atai et al., 2012). The relationship between deficient cell migration and the abnormalities in synaptic plasticity found in dystonia remains to be elucidated (Albanese and Lalli, 2012; Quartarone and Pisani, 2011). The current study focuses on quantitation of changes in cell migration in DYT1 patient fibroblasts as a model for delayed migration documented for neurons in DYT1 knock-out embryos (McCarthy et al., 2012). During brain development torsinA is highly expressed in dopaminergic neurons in the central nervous system located in the substantia nigra, as well as in neurons in the striatum, cerebral cortex, thalamus, hippocampus, cerebellum, midbrain, pons and spinal cord (Rostasy et al., 2003; Augood et al., 1998, 1999, 2000; Vasudevan et al., 2006).

Microfluidic platforms are emerging to study cell migration with great spatial and temporal resolution, for precise measurements of velocity, directionality, and persistence. These tools have allowed monitoring of the vectorial movement of individual neutrophils around obstacles (Ambravaneswaran et al., 2010), cancer cells in conditions of three-dimensional confinement in linear channels (Irimia and Toner, 2009), and microglia in the presence of amyloid beta within channels (Cho et al., 2013). The unprecedented precision of speed, directionality, and persistence measurements enabled by these tools provided the support for unexpected findings regarding the alterations of neutrophil migration after burn injuries (Butler et al., 2010), the role of self-generated gradients during epithelial cell migration through mazes (Scherber et al., 2012), and the contribution of asymmetric location of mitochondria in front of the nucleus to the fast and persistent migration of cancer cells (Desai et al., 2013).

The limitations in developing neuronal models have led scientists to examine the role of proteins involved in human neurologic diseases in non-neuronal model systems (Falkenburger and Schulz, 2006). The published literature indicates this approach is not only viable, but has proven very successful, providing very useful and informative results (Ferraiuolo et al., 2013; Burbulla and Krüger, 2012; Connolly, 1998). Recently, there has been increased interest in the use of patient-derived fibroblasts, as induced pluripotent stem cells can be derived from them and studied directly or after differentiation into neurons and glia for studying neurological diseases (Koch et al., 2011; Qiang et al., 2011; Yu et al., 2007; Gibbs and Singleton, 2006). In this study we used primary skin fibroblasts from DYT1 patients and healthy controls to monitor variations in cell movement relevant to neuronal migration. We found delayed rates of migration, reduced polarization of the nucleus in migrating cells, and abnormal orientation of nuclei and Golgi in migrating DYT1, as compared to control cells using different culture assays. For the first time we report a single cell vectorial movement assay in microfluidic chambers that allows real-time evaluation of migration dynamics.

2. Materials and methods

2.1. Cell culture

The following cell lines were used, human control fibroblast lines: HF6, HF19, GM02131, GM02912B, GM0024, HF77, HF71, and DYT1 fibroblast lines: HF49, GM02551, GM02306, GM03208 and GM03211. HF lines were obtained by skin biopsy generated in our laboratory under IRB approved guidelines (Breakefield et al., 1981); GM lines were purchased from Coriell (Camden NJ). Fibroblasts were grown in Dulbecco's Modified Eagle Medium (Gibco, Rockville, MD; Invitrogen Life Technologies; Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco, Invitrogen Life Technologies). To simplify the text, we have used the last two/four numbers of each line of fibroblasts from Coriell in the text and figures.

2.2. Oris cell migration assay

In some experiments cell migration was analyzed using the Oris Cell Migration Assay (Platypus Technologies, Madison, WI) with some modifications. The cell-seeding stopper

was inserted into wells of a 96-well plate in wells bottoms with 10 μ /ml concentration (Invitrogen). The fibroblasts were diluted to 2.5×10^5 cells/ml in 100 μ l of the cell suspension were seeded per well with a stopper inserted to allow seeding only in the outer annular regions of the wells. After appropriate cell attachment was achieved (24 hours), the culture insert/stopper was removed and the wells were filled with cell-free medium. Removal of the stopper resulted in opening of a round, unseeded region into which the cells migrated. Cells were examined microscopically to monitor progression of migration into the cell-free zone 72 hours after stoppers were removed. Cells were fixed in 4% formaldehyde in 1X phosphate buffered saline (PBS) and stained by immunocytochemistry for an ER protein, protein disulfide isomerase (PDI) for fluorescence quantification of cells in this zone using a confocal microscope. The images were captured and the data analyzed in pre-migration ($t = 0$ hour) and post-migration ($t = 72$ hours) wells. The average fluorescence signal from the detection zones (unseeded region or cell-free zone created by insertion of the Oris™ stopper) was calculated as the mean $\pm$ S.D. for each condition.

2.3. Monolayer wounding and determination of nucleus and centrosome position

Human fibroblasts were cultured, as described above, and plated in semiconfluent monolayer. Cells were starved in serum-free medium for 2 days, because serum may contain up to 10 μ M lysophosphatidic acid (LPA), a cell polarization stimulator. On the third day, three linear wounds were scratched in each well of cells with a p200 pipet tip. Then the wells were washed twice with serum-free Dulbecco's Modified Eagle's Medium (DMEM). After a wound area was created and washed, the culture medium was replaced with fresh serum-free medium containing 10 μ M LPA (Sigma-Aldrich, St. Louis, MO), as described (Nery et al., 2008). Three hours later, the cells were fixed in 4% paraformaldehyde (PFA) in PBS for 15 minutes at room temperature followed by methanol at -20°C for 5 minutes. Cells were stained for the centrosome marker, pericentrin (PRB-432C, from Covance, Princeton, NJ, 1:1000), nuclei (DAPI, Sigma-Aldrich 0.1%) microtubules (α -tubulin, Sigma-Aldrich, 1:1000), as described (Nery et al., 2008). Centrosome orientation relative to the position of the nucleus and leading edge of the plasma membrane was determined, as described (Gomes et al., 2005; Palazzo et al., 2001). Briefly, images were pseudocolored and analyzed with Image-J software scoring >80 cells per group. Centrosome orientation was defined by localization in the sector ($\sim 1/3$ of cell) between the nucleus and leading edge (Gomes et al., 2005). Random orientation would be expected to be $\sim 33\%$.

2.4. Microfluidic device manufacturing and setup

Microfluidic devices were fabricated using standard micro-fabrication technologies. Briefly, two layers of SU-8 photoresist (Microchem, Newton, MA) one 10 μ m thin and one 50 μ m thick were photo-patterned on a silicon wafer using standard microfabrication technologies. The thin and thick layers were aligned with 1 micron precision by using matching photolithographic features in the two layers. The design of the devices consists of four arrays of 80 parallel microchannels 10 μ m width 10 μ m height and 600 μ m length which are distributed around a circular well of 2 mm diameter and 50 μ m height. The silicon wafer with microscale SU-8 features was then used to cast polydimethyl siloxane (PDMS, Dow Corning Midland, MI) in a 2 mm thick layer. After baking for 8 hours at 65°C , the PDMS layer was peeled off the wafer, punched with a 1.5 mm puncher (Harris Uni-Core, Ted Pella,

Reading, CA) to define the cell loading chamber, and whole devices punched out using a 5 mm puncher (Ted Pella). The “donut” shaped devices were then exposed to oxygen plasma and bonded on glass-bottom multiwell plates (MatTek Co., Ashland, MA). To promote adhesion of fibroblasts inside the channels, the device was primed with 10 μ /ml fibronectin for about 10 minutes. After incubation, the device was washed with PBS (Fisher Scientific, Pittsburgh, PA) and primed with DMEM media.

2.5. Microscopy and image analysis

Ten microliters of fibroblasts (3000 to 10000 cells) were injected inside of the cell loading chamber of each microfluidic device. The migration of the cells from the chamber into the channels was recorded using a Nikon TiE microscope operating in epifluorescence mode and equipped with a culture incubation chamber. All fluorescence images were acquired with 12-bit resolution using a cooled-CCD camera (QImaging, Surrey, BC, Canada). Individual frames were recorded at 10 minute intervals and analyzed using ImageJ (<http://rsb.info.nih.gov/ij/>) and the manual tracking plugin. To improve the contrast of the images and better identify the moving cells, the initial frame was subtracted from all subsequent frames. Tracking was terminated as soon as the cell stopped migrating or reversed its direction. The collected data up until that point was imported into Microsoft Excel where the velocity of the cell was calculated using the distance achieved during the time of forward migration. The velocity and the time to cessation or reversal of movement were analyzed using the total amount of minutes until initial stop. The duration of the movement was calculated based on the time interval between the recorded individual frames. A total of 428 cells were tracked (control cells, 203; DYT1 cells, 225).

2.6. Live cells labeling

Viable fibroblasts were stained with Hoechst 33258 and BODIPY FL C5-ceramide dyes (both from Molecular Probe Inc., Eugene, OR), or PKH67 (Sigma-Aldrich), according to the manufacturer's instructions. Green-fluorescent BODIPY FL C5-ceramide localizes to the Golgi apparatus, blue-fluorescent Hoechst 33258 dye stains the nuclei, and the green fluorescent dye PKH67 stains cell membranes.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 3.0). Results are expressed as mean $\pm$ S.E.M. Multiple comparison analysis of variance between the groups was performed by a one-way ANNOV test followed by Tukey's Multiple Comparison test (MC) or Bonferroni's Multiple Comparison test to determine if velocity between control and DYT1 fibroblasts were significant during migration. The same was done for migration distance (data not shown), Golgi localization and centrosome orientation. A Spearman correlation was done between the velocity (μ m/min) and the time of initial stop (min) between the control and DYT1 fibroblasts.

3. Results

3.1. Migration stopper assay

In initial experiments the migration of DYT1 and control fibroblasts was evaluated using the Oris™ stopper wells. These wells have the advantage that they create a more precisely delineated cell-free zone to evaluate movement of cells over time as compared to the “hand cut” wound zones used in some assays (Araki-Sasaki et al., 1995). However, it is not a measure of vectorial cell movement as cells can migrate in different directions around the margin. The extent of migration into the cell-free zone over 72 hours was assessed by fluorescent staining of cells for an ER marker and quantitation of the fluorescence intensity (Fig. 1A). The random movement of cells around the detection zone/exclusion edge is notable at a higher magnification (Fig. 1B). In the post-migration wells the average fluorescence signal for control fibroblasts was 5,333 ($\pm$ 351 S.D.) and for patient fibroblasts was 3,700 ($\pm$ 473 S.D.) (Fig. 1C; $p < 0.002$). This represents an approximately 36% decrease in the extent of cell migration in patient fibroblasts expressing endogenous torsinA, as compared to control cells, consistent with previous reports using the hand cut wound assay for cells lacking torsinA (Nery et al., 2008).

3.2. Centrosome orientation assay

At the onset of cell migration the nucleus moves to a position posterior to the centrosome with respect to the leading edge of movement (Pouthas et al., 2008). Centrosome reorientation in cells is rate limiting in cell migration. It has been demonstrated that in fibroblasts polarizing for migration into wounds in culture, an actin-dependent nuclear movement is triggered by serum or the phospholipid serum factor, LPA, and this reorients the centrosome toward the leading edge (Gomes et al., 2005). Using a wound assay, as described (Starr, 2009), confluent monolayers were incubated in serum-free medium for 48 hours, then a hand-cut wound was made in the monolayer, and cells were stimulated with 10 μ M LPA (Gomes and Gundersen, 2006). After 3 hours incubation with LPA, we assessed the relative position of the nucleus, centrosome and wound margin by staining for the nucleus, centrosome and microtubules (Fig. 2A). Quantitation was carried out on three control lines and four DYT1 lines with significantly different ranges of 66-76% (average 68%) for correctly oriented nuclei for control cells and 25-34% (average 30%) or DYT1 cells (Fig. 2B; $p < 0.01$ between groups).

3.3. Microfluidic chamber assays

In order to obtain more precise measurements of the vectorial movement of cells, microfluidic chambers were prefabricated to contain a well for cell loading and channels radiating out from it, having 10 x 10 μ m cross section and 600 μ m length to allow single file migration of cells and imaging in real-time (Fig. 3A). Cells were labeled with the PKH67 membrane dye to monitor their extent and position within the chambers over time (Fig. 3B). Time-lapse image analysis was used to determine the forward velocity of four control cell lines and three DYT1 cell lines after they had entered the channels. Movement of individual cells was monitored visually and terminated whenever cells stopped or reversed their direction of movement. DYT1 cells showed an average 58% slower rate of forward movement within channels, as compared to control cells (Fig. 3C). This, and a greater

tendency of the DYT1 cells to stop and/or reverse the direction of movement, correlated with the velocity (Fig. 3D) and reduced the total distance traveled by DYT1, as compared to control cells over a fixed period of time.

Microfluidic chambers were also used to evaluate the relative position of cell nuclei and the Golgi apparatus within cells moving inside the channels. Golgi and nuclei were stained with intravital dyes and their relative position was assessed in living migrating control and DYT1 cells (Fig. 4A). Normally the Golgi moves in front of the nucleus under these conditions, but in some cases falls behind the nucleus or appears to surround it. In 75% of control cells (line 2131, n=40) the Golgi was positioned in front of the nucleus with respect to the direction of movement, while in DYT1 cells this dropped significantly to 44% (line 2551, n=57 cells) and 53% (line 2306, n=40 cells) (Fig. 4B; $p<0.001$).

4. Discussion

This study takes advantage of the high precision of measuring the speed and persistence of migrating cells through micron sized channels and the visual, real-time live cell monitoring at cellular and subcellular resolution. The mechanical confinement of fibroblasts inside channels of cross-section size comparable to that of the moving cells enables the cells to migrate for long time, at constant speed, unperturbed by interactions with other cells. These migration patterns are distinct from the traditional “random-biased walk” that is usually observous in the traditional methods used for measuring cell migration on flat surfaces, like the conventional stopper and wound healing methods, or the large majority of microfluidic devices for cell migration when cell migration also takes place on flat surfaces. Unlike the migration on flat surfaces, when the characteristic speed and directionality of migrating cells changes often, are perturbed by cell-cell interactions, and can only be estimated with limited precision by statistical methods, when inside channels, cells move at uniform speed and change direction far less frequently, enabling direct and precise measurements of migration parameters at single cell resolution. Taking advantage of the increased precision of the measurements, we observed and quantified the distinct motility behavior of DYT1 and wild-type fibroblasts. In addition, microfluidic devices permit single cell analysis as they are compatible with modern microscopic methods. The vectorial cell movement through channels helped significantly the study of movement of organelles inside the moving cells by providing a predictable reference frame for the position of the nucleus and leading edge at any time.

In addition, our findings confirm previous studies (Nery et al., 2008) indicating a reduced rate of migration of fibroblasts from DYT1 dystonia patients as compared to controls, due at least in part to a decreased nuclear polarization with respect to the centrosome and the leading edge of migration. Using microfluidic chambers we were able to show that the velocity and distance covered is also reduced in DYT1, as compared to control cells. In addition, DYT1 cells have a greater tendency to pause in migration and reverse direction. This also may explain an additional abnormal feature in which the relative positions of the Golgi and nucleus is more variable in DYT1, as compared to control cells in the process of migrating.

The longitudinal asymmetry of cellular structures relative to the nucleus is a common characteristic of various polarized cells. This asymmetry appears to be important during cell migration. For example, in epithelial cells the microtubule organization complex is often localized between the nucleus and leading edge (Yvon et al., 2002) and the ER and Golgi apparatus also occupy similar anterior positions (Miller et al., 2009; Pouthas et al., 2008). In moving lymphocytes, mitochondria accumulate behind the nucleus, toward the uropod, with consequences on the increased persistence of migration in a specific direction (Campello et al., 2006), and share asymmetrical localization with the microtubule organizing center (del Pozo et al., 1998). The effect of asymmetric organelle distribution inside moving cells can be precisely quantified when cell migration is confined in small channels, e.g. the anterior-localized mitochondria could account for an order of magnitude difference in invasive abilities between cells from otherwise homogenous cell populations (Desai et al., 2013). For moving fibroblasts, confinement along channels accentuates the correlations between intracellular organization and migration parameters, circumventing the limitations of studies on flat surfaces when cells change directions often and can extend multiple pseudopods at the same time (Uetrecht and Bear, 2009).

These studies are consistent with the findings that torsinA, located in the lumen of the contiguous NE and ER, has a role in morphology and orientation of the nucleus and that mutant torsinA interferes with this orientation (Naismith et al., 2004; Goodchild and Dauer, 2004; Nery et al., 2008). This function is due, at least in part, to associations between torsinA and the nesprin proteins which span the outer nuclear membrane and interact with different cytoskeletal elements in the cytoplasm, as well as SUN proteins that span the inner nuclear membrane and interact with nesprins in the lumen of the NE (Nery et al., 2008; Atai et al., 2012; Jungwirth et al., 2011; Vander Heyden et al., 2009). Since torsinA is expressed in most cells of the body the effects of mutant torsinA can be assessed in fibroblasts which can be obtained from patients, and normal torsinA can be assessed in fibroblasts from healthy, unaffected controls. Our current findings support both an initial delay in onset of migration in the culture wound paradigm as well as reduced forward velocity in primary DYT1 as compared to control cells, both of which may be explained by compromised relationship between the nuclear membrane and the cytoskeleton. Importantly, this is consistent with delayed migration of neurons during embryonic brain development in DYT1 torsinA knock-out mice both in culture and *in vivo* (McCarthy et al., 2012). Other studies have shown an altered morphology of neurons in DYT1 heterozygous knock-in mouse brains (Song et al., 2013; Zhang et al., 2011) and of the NE in neurons in DYT1 homozygous knock-out or knock-in brains (Goodchild et al., 2005). In parallel, *dtorsin*-null *Drosophila* has abnormal morphology of dopaminergic neurons and patterning of their axons in adult brains (Wakabayashi-Ito et al., 2011). Thus, a contribution to the pathophysiology of DYT1 dystonia may lie in subtle alterations in brain microcircuitry resulting from abnormalities in the association of the NE and ER with the cytoskeleton leading to reduced migration and extension of neuronal process in subsets of neurons during development.

5. Conclusion

These studies show that the microfluidic chambers designed to evaluate single cell migration in real-time provide a highly quantitative means for analysis of vectorial movement of cells, including positioning of organelles within migrating cells. We also confirm that cells expressing endogenous levels of both mutant and wild-type torsinA (from DYT1 patients) have a reduced rate of migration and misorientation of the nucleus during migration, as compared to control cells that express only wild-type torsinA.

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Abbreviations

DMEM	Dulbe co's Modi Eagle's Medium
ER	endoplasmic reticulum
LPA	lysophosphatidic acid
MCT	Multiple Comparison test
NE	nuclear envelope
PBS	phosphate buffered saline
PDI	protein disulfide isomerase
PDMS	polydimethyl siloxane
PFA	paraformaldehyde

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Highlights

- Microfluidic chambers provide a highly quantitative means for analysis of vectorial movement of cells, including positioning of organelles within migrating cells.
- Reduced rate of migration was observed in fibroblasts from DYT1 dystonia patients as compared to controls.
- Abnormal feature in the relative positions of the Golgi and nucleus is more variable in DYT1, as compared to control cells in the process of migrating.

Fig. 1. Cell migration of control and patient fibroblasts evaluated using fluorescent stain. Cell migration of control (HF77) and DYT1 fibroblasts (2551) was evaluated in the OrisTM stopper assay. A) Fibroblasts were plated at a final concentration of 2.5×10^4 per well in 96-well plates. Twenty-four hours after plating the stoppers were removed - creating a cell-free zone (dotted lines). Cells were fixed and stained by immunocytochemistry for PDI for fluorescence quantification of cells in this zone using a confocal microscope. The images were captured and the data analyzed from pre-migration ($t = 0$) and post-migration ($t = 72$ hours). B) High magnification of the "cell-free zone" for control and DYT1 fibroblasts. C) The average fluorescence signal from the detection zones (wound) was calculated as the mean $\pm$ S.D. for each condition ($n=8$ wells/cell type per experiment, from two experiments). In the post-migration wells the average fluorescence signal for control fibroblasts was greater 5333 (± 351 S.D.), as compared to patient fibroblasts 3700 (± 473 S.D.) ($p<0.002$; $n=3$ experiments), indicating reduced migration in patient fibroblasts.

Fig. 2. Nuclear movement is disturbed by torsinA E

Fibroblasts were treated with or without LPA for 3 hours after wound introduction on monolayer cultures on coverslips, then fixed with 4% PFA and methanol for immunohistochemistry. A) Diagram (top left) indicates oriented vs. non-oriented nucleus (N) with respect to the centrosome (yellow, C) and the wound edge. Staining (bottom left) shows alignment of cells along the wound edge for human control (HF 19) and DYT1 (2551) fibroblasts with centrosome (anti-pericentrin, yellow), nucleus (DAPI, blue) and microtubules (α -tubulin; green). The wound edge is toward the top in both images. Scale bars 10 μ m. B) The criteria used for quantification of centrosome orientation were as follows: cells touching the wound edge with the centrosome localized in the sector between the wound and leading edge in front of the nucleus were scored as oriented and centrosomes in other sectors were scored as non-oriented. The % of cells with oriented centrosomes relative to the wound edge and nucleus was significantly different ($p < 0.01$; $n = 3$ experiments) in control and DYT1 fibroblasts.

Fig. 3. Decreased velocity of DYT1 patient fibroblasts in microfluidic chambers

A) Control and DYT1 cells were plated in microfluidic devices positioned inside multiwell plates. Arrays of migration channels allowed the monitoring of single cell migration. B) The fibroblasts were stained with vital green fluorescent membrane dye PKH67 in microchambers. Fibroblast migration was evaluated with high resolution by time-lapse confocal microscopy at 37°C in cell culture chambers. C) The forward velocity of cells was monitored by time-lapse video microscopy. Single-cell tracking of the control human fibroblasts: HF19, n=72 cells; 2131, n=50; HF77, n=30; HF71, n=51 and DYT1 fibroblasts: 2551, n=100; 2306, n=100; HF49, n=25 were obtained over a 24 hour period. The average forward velocity of control cells was 0.24 μm/min, 95% confidence interval (CI)=0.18 to 0.34 μm/min, and DYT1 cells DYT1 0.14 μm/min, 95% CI=0.12 to 0.20 μm/min with a significantly decreased rate of forward movement of DYT1 compared to control cells ($p<0.001$; n=6 experiments). D) Correlation between the velocity (μm/min) and time to initial stop/reversal of movement (min). The human fibroblasts used were: controls - HF19, 2131, HF77, HF71 and DYT1 fibroblasts - 2551, 2306, HF49. There was a significant positive correlation between the migration velocity of control cell lines (blue), mean=0.25, S.E.M. $\pm$ 0.014 versus and DYT1 cells (red), mean=0.14, S.E.M. $\pm$ 0.008, and the time to initial stop/reversal of movement (controls, mean=1,072, S.E.M. $\pm$ 57.4, DYT1s cells, mean=548.7, S.E.M. $\pm$ 43.4), correlation $r=0.78$, $p<0.04$.

Fig. 4. Position of Golgi during migration of human control and DYT1 fibroblasts in microfluidic chambers

A) The position of the Golgi complex in human fibroblasts (2131) during the time-lapse microscopy is shown with the directions of movement indicated by arrows. The Golgi complex is stained green (BODIPY-Ceramide, Molecular Probes) and the nucleus, blue (Hoechst 33342, Pierce, Rockford, IL). B) Positions of the Golgi complex (in %) in relation to the nucleus in migrating control (2131) and DYT1 (2551 & 2306) fibroblast lines, indicate whether the Golgi was ahead of or behind the nucleus, or in an undefined position relative to the nucleus. There was a significant difference between the % of cells with the Golgi ahead in control and DYT1 groups (***) $p < 0.001$; $n = 2$ experiments).