RESEARCH ARTICLE

Htt is a repressor of Abl activity required for APP-induced axonal growth

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Abstract

Huntington's disease is a progressive autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract at the N-terminus of a large cytoplasmic protein. The Drosophila huntingtin (htt) gene is widely expressed during all developmental stages from embryos to adults. However, Drosophila htt mutant individuals are viable with no obvious developmental defects. We asked if such defects could be detected in htt mutants in a background that had been genetically sensitized to reveal cryptic developmental functions. Amyloid precursor protein (APP) is linked to Alzheimer’s disease. Appl is the Drosophila APP ortholog and Appl signaling modulates axon outgrowth in the mushroom bodies (MBs), the learning and memory center in the fly, in part by recruiting Abl tyrosine kinase. Here, we find that htt mutations suppress axon outgrowth defects of αβ neurons in Appl mutant MB by derepressing the activity of Abl. We show that Abl is required in MB αβ neurons for their axon outgrowth. Importantly, both Abl overexpression and lack of expression produce similar phenotypes in the MBs, indicating the necessity of tightly regulating Abl activity. We find that Htt behaves genetically as a repressor of Abl activity, and consistent with this, in vivo FRET-based measurements reveal a significant increase in Abl kinase activity in the MBs when Htt levels are reduced. Thus, Appl and Htt have essential but opposing roles in MB development, promoting and suppressing Abl kinase activity, respectively, to maintain the appropriate intermediate level necessary for axon growth.

Author summary

Understanding the normal physiological roles of proteins involved in neurodegenerative diseases can provide significant insight into disease mechanisms. Drosophila offers a powerful system in which to ask these fundamental questions. Both Htt, related to Huntington’s disease, and Appl, related to Alzheimer’s disease, have well-conserved single orthologs in the fly genome. Appl has been shown to be a conserved modulator of a Wnt-
PCP signaling pathway required for axon outgrowth in the mushroom body (MB) in the Drosophila brain. However, roles for Htt in fly brain development have not been reported. Unexpectedly, we found that htt mutations suppress the axon outgrowth defects of Appl mutants in the MB, indicating a link between these two neurodegenerative proteins and a cryptic role of Htt during development. Abl tyrosine kinase is a downstream effector of the Appl receptor, and we show here that Abl is also required for MB axon outgrowth. Importantly, Abl activity must be tightly regulated as evidenced by our observations that both under and overexpression of Abl result in similar axonal defects. We demonstrate that Htt is an inhibitor of Abl activity and provide evidence that the phenotypic rescue of αβ axons in Appl mutants by reducing htt is mediated by the restoration of proper levels of Abl signaling. These data, therefore, suggest that Appl and Htt act antagonistically to maintain an optimal balance of activation and inhibition of Abl, and thereby promote the growth of MB αβ axons.

Introduction

Neurodegenerative disease (ND) encompasses a large and heterogeneous group of maladies, including many that are associated with accumulation of specific misfolded proteins [1]. Despite their variety, however, these diseases share a number of cellular pathologies [2,3], raising the question of whether different ND-associated genes might function in shared genetic pathways. Several of these disease genes, moreover, have been implicated in neurodevelopmental processes [4–8], suggesting that studies of development may be an effective strategy to reveal initially cryptic connections among genes implicated in ND.

Huntington’s disease (HD) is a progressive, autosomal dominant, neurodegenerative disorder. It is a monogenic disease caused by the expansion of a polyglutamine (polyQ) tract at the N-terminus of a large cytoplasmic protein (3144 a.a.), huntingtin (Htt) [9]. Several studies indicate that an alteration of wild-type Htt function might also contribute to disease progression [4]. Consistent with this, numerous biochemical and in vitro studies have suggested that Htt functions in mammalian neuronal development, synaptic function and axonal trafficking [9]. While HD has been characterized as a neurodegenerative disease, a recent study indicates it is also required for normal human brain development [8].

Drosophila has been useful previously as a model to examine the effects of polyQ-expanded human huntingtin transgenes on neuronal form and function [10,11]. The fly huntingtin protein (Htt, 3583 a.a.), although lacking a polyQ tract, is similar to the human Htt protein, with four regions of high sequence homology clustered along the protein in the N-, central- and C-terminal regions. Fly Htt is expressed ubiquitously at low level in embryos, larval and adult tissues, with no specific pattern of expression. Fly Htt is found predominantly in the cytoplasm, even when overexpressed [12]. Despite htt being highly conserved across all Drosophila species, indicating an essential role for biological fitness, null htt mutants display no gross developmental defects [12,13], although brains from Drosophila Htt mutants have reduced axon complexity [12]. This suggests that it could be necessary to alter the expression of another gene (or genes) during development to be able to detect a htt loss of function phenotype. Therefore, we asked whether mutant htt modifies a brain axon growth defect present when another neurodegeneration-related protein is lacking, i.e., in a sensitized genetic background.

In contrast to HD, Alzheimer’s Disease (AD) is highly genetically complex [14–16]. Like HD, AD is also viewed as a proteinopathy, since it is associated with accumulation of amyloid fibrils derived from the Amyloid Precursor Protein (APP). APPs have therefore been
investigated intensely, however their normal function in the brain remains unclear and controversial. *Drosophila* encodes a single APP homologue, called *Appl*, that is expressed in all neurons throughout development. It has been shown that *Appl* is a conserved neuronal modulator of a Wnt planar cell polarity (Wnt/PCP) pathway [5]. This signaling pathway is essential for proper axon outgrowth in the learning and memory center of the fly, a bilaterally symmetric pair of structures called the Mushroom Bodies (MB). In this context, it has been proposed that *Appl* is part of the membrane complex formed by the core PCP receptors, and further that it promotes phosphorylation of the Dishevelled (Dsh) cytoplasmic adaptor protein. Dsh is a core component required for all known Wnt pathways, including the Wnt/PCP pathway [17,18]. Specifically, *Appl* recruits a non-receptor protein tyrosine kinase, called Abl, to the PCP receptor complex and positively modulates its phosphorylation of Dsh [19]. Consistent with this view, it has been shown that a 50% reduction of *Abl* leads to enhancement of the *Appl* mutant phenotype. Conversely, overexpression of wild-type *Abl*, but not a kinase-dead version, in the MB neurons led to a strong rescue of the *Appl* mutant phenotype. Together with accompanying biochemical experiments, these data suggested that *Appl* promotes the phosphorylation of Dsh by Abl kinase, and further showed that this mechanism is conserved in mammals [5]. Thus, Abl is a key downstream effector of *Appl* required for it to stimulate MB axon outgrowth.

The Abl family of non-receptor tyrosine kinases includes human ABL1 and ABL2 as well as *Drosophila* Abl. Each Abl protein shares a conserved domain structure consisting of a SH3-SH2-TK (Src homology 3-Src homology 2-tyrosine kinase) domain cassette which confers autoregulated kinase activity. A carboxy-terminal F (F-actin-binding) domain ties Abl-dependent phosphoregulation to actin filament reorganization [20]. ABL1 has been implicated in a range of cellular processes including actin dynamics and cell migration. Abl was discovered as a cellular proto-oncogene that is constitutively active in human chronic myelogenous leukemia and acute lymphocytic leukemia [21]. Kinase activity of Abl *in vivo* is limited both by intramolecular interactions [22] and by cellular inhibitors, such as Pag/Msp23 [23]. After removal of inhibition, Abl acquires substantial catalytic activity that is further enhanced by primary and secondary (auto)phosphorylation [24]. The Abl kinases have also been shown to play a crucial role in the development of the nervous system. Overexpression of active Abl in adult mouse neurons results in neurodegeneration and neuroinflammation and activation of Abl has been shown to occur in human neurodegenerative disease [25]. In contrast to mammalian Abl, *Drosophila* Abl has not been shown to directly cause tissue hyperplasia or cell fate transformation *in vivo*, but rather is essential for cell adhesion and morphogenetic processes such as axonogenesis and growth cone motility [26–28]. Several studies have shown that the precise level of Abl activity is critical to its axonal function, with loss- and gain-of-function both leading to severe defects in neural patterning [29–31]. Recent experiments revealed the cell biological and biophysical basis of this relationship, showing that either increased or decreased levels of Abl activity induce disorder in growth cone actin and thereby greatly augment the frequency of stochastic errors in growth and guidance [32,33]. Of particular relevance here, Abl has been implicated in axonal arborization and growth in the *Drosophila* brain [34], including the MBs [5], though the precise role of Abl in normal MB development has not been documented.

The MBs, together with the central complex, form the core of the adult central brain of *Drosophila*. Due to extensive study, they offer an exceptionally powerful system for analyses of genetic and molecular mechanisms of development and function. The MBs are two bilaterally symmetric structures that are required for learning and memory [35,36]. Each MB is comprised of 2000 neurons that arise from 4 identified neuroblasts. Three types of neurons appear sequentially during development: the embryonic/early larval γ, the larval α′β′ and the
late larval/pupal αβ. Each αβ neuron projects an axon that branches to send an α branch dorsally, which contributes to the formation of the α lobe, and a β branch medially, which contributes to the formation of the β lobe [37]. Both lobes require the PCP mechanism for efficient axon extension [38]. The PCP genes, however, do not act cell-autonomously to promote MB axon growth. Rather, PCP produces a “community effect” that coordinates the growth decisions of large groups of MB axons, and that overrides the effect of mutations in single PCP components in any single axon or cluster of axons. [38]. Appl is required for this PCP-dependent ‘community effect’. In β-branches, Appl is evidently also required for some other mechanism that acts cell-autonomously, in addition to its contribution to the non-cell-autonomous PCP mechanism [5]. The molecular nature of this second autonomous Appl function remains unknown.

Here we investigate the genetic and functional interactions of Htt, Appl, Abl and the core PCP gene dsh in Drosophila. We find that htt mutations suppress the MB axonal outgrowth defects observed in Appl mutants. Since Abl is known to act downstream of Appl it seemed a potential target for htt mutant-induced suppression of the Appl phenotype. We therefore next characterized the role of Abl in normal MB development. Using analysis of Abl loss-of-function (LOF) alleles in single-cell MARCM clones we show that Abl is required for axonal growth in the developing αβ neurons of the MBs and that it is expressed in these neurons. Importantly, the overexpression of Abl in these neurons also leads to axonal growth defects, suggesting the possible existence of cellular proteins that negatively control neuronal Abl activity. Finally, we demonstrate that Htt acts as a cellular inhibitor of Abl activity, both genetically, as it functions antagonistically to Abl in MB axon growth, and biochemically, as FRET measurement of Abl kinase activity in vivo reveals that is derepressed by reducing Htt expression. These results indicate that Appl and htt, whose human homologs are central players in neurodegeneration, regulate Abl kinase in opposite directions to maintain its activity in the narrow range necessary for normal axon outgrowth in MB αβ neurons.

Results
Reduction of htt rescues the MB β axon outgrowth phenotypes of Appl mutants
Appl null (Appl<sup>Δ</sup>) flies are viable, fertile and display no gross structural defects in the brain [39]. However, Appl<sup>Δ</sup> MBs display modestly-penetrant axonal defects in αβ neurons [5]. For simplicity, in our quantification of this phenotype we will focus our attention on the role of Appl in β-branches, where Appl is required cell-autonomously [5]. From 3110 Appl<sup>Δ</sup> MBs, 451 (14.5%) showed an absence of β lobe phenotype (see details in Material and methods), in accordance with what was previously reported [5]. Two control MBs (0.26%) displayed β lobe absence out of 759 MBs (see details in Material and methods). In line with our interest in potential developmental associations of neurodegenerative genes, we wondered whether htt would modify the effect of Appl in MB αβ neurons. By itself, as expected from published data [12] the loss of one or even two copies of htt did not result in any significant MB developmental defects (S1A Fig). However, we found that mutation of htt potently suppresses the MB Appl<sup>Δ</sup> mutant phenotype in αβ neurons (Fig 1). Specifically, the Appl<sup>Δ</sup> MB phenotype was rescued by reducing htt expression using three different genetic manipulations: 1) htt heterozygosity using two mutant alleles of htt (htt-ko or htt<sup>int</sup>), 2) heterozygosity for a 55 kb chromosomal deficiency uncovering the htt locus and most of the adjacent CG9990, and 3) htt RNAi knock down in the αβ MB neurons (Fig 1A–1E; RNAi expression was driven in this experiment by the αβ neuron-specific GAL4 line c739). Conversely, when htt was overexpressed in the MB αβ neurons using UAS-htt-fl-
CTAP, a UAS-C-terminally TAP-tagged full length htt, it did not rescue htt/int (+16% absence of β lobe in Appl; UAS-htt-fl-CTAP/c739-GAL4; htt/int/+ vs 2% in Appl; +/+; htt/int/+ and 16% in Appl) (Fig 1E). As expected from the above genetic results, htt-fl-CTAP protein, like wild type Htt, was present throughout the axon when expressed in αβ neurons (Fig 1F).

Since Dsh is a core intracellular component of the Appl-dependent Wnt/PCP pathway in the MBs, we tested the potential interactions between htt and dsh. We found that removing one copy of htt also suppressed the dsh MB phenotype in the β-lobe (21% absence of β lobe in dsh; +/+ vs 4% in dsh; htt-ko /+—Fig 2A, 2B and 2E upper bars). Moreover, hemizygosity for both Appl and dsh resulted in a strong MB axon outgrowth phenotype (Appl w dsh; 60% absence of β lobe, vs 21% in dsh and 16% in Appl). We found that heterozygosity for htt also strongly suppressed the Appl-dsh phenotype (12% of absence of β lobe —Fig 2C–2E lower bars). Taken together, these data strongly suggest that Htt is a negative regulator of the Appl-dependent Wnt-PCP signaling pathway acting during MB β-axon outgrowth.
Abl loss-of-function and gain-of-function mutants induce similar MB αβ neuron phenotypes

Since Abl is a key component of the Appl-dependent Wnt-PCP signaling pathway required for axon growth in MBs [5] and Abl was previously shown to phosphorylate Dsh [19], we sought to clarify the potential relationship between Abl, Appl, and Dsh in MB αβ neurons. Indeed, we found that the axonal phenotypes of Appl and dsh MB αβ neurons can be rescued by modest enhancement of Abl expression, through transgenic expression of an Abl-GFP fusion under control of Abl upstream genomic sequences (S1B and S1C Fig). We therefore characterized in more detail the axonal morphology phenotype in Abl loss- and gain-of-function MB αβ neurons.

The phenotype of MB αβ neurons with alterations in Abl expression has not previously been described thoroughly. We have used three Abl alleles (Abl1, Abl2 and Abl4) to characterize the requirement of Abl function in MB morphology (S2A Fig). Each of three double heterozygous combinations (1/2, 2/4 and 1/4) are largely adult lethal but are viable at 48 hours after puparium formation (hAPF) enabling their effects to be investigated at that stage when the αβ neurons are present [37,40]. Interestingly, all the three allelic combinations gave similar MB phenotypes with a mixture of wild-type (WT) lobes (~20%) and MB lacking α-lobes, β-lobes, or both (~80%) (Fig 3A–3D). These Abl mutant phenotypes, including the adult lethality, were significantly rescued by introducing the Abl-GFP genomic transgene (from 20% WT MBs to 80%, P < 10^-5; Fig 3D). Abl is known to be toxic when expressed in an unregulated fashion [21,25], and to cause axon growth and guidance defects when overexpressed in some neurons [29–31]. We therefore overexpressed Abl specifically in the MBs using the GAL4/UAS system [41]. Overexpression of WT Abl in MB neurons with the OK107-GAL4 driver produced αβ lobe loss phenotypes similar to those observed with Abl LOF alleles (Figs 3E and S2B–S2E). Note that the use of a the weaker c739-GAL4 driver resulted in no lobe loss phenotype (n = 100). The penetrance of the gain-of-function (GOF) mutant phenotype was even higher than that in the Abl LOF alleles; WT MBs were never detected. In contrast, expression of a kinase dead version of Abl failed to produce a MB mutant phenotype, although expression...
Fig 3. Either loss or overexpression of Abl affect MB αβ neuron morphology. (A–C) Anti-Fas2 staining on wild-type (WT) brain (A) and on Abf2/Abf1 brain (B–C). In a wild-type (WT) brain, the α lobe (indicated by yellow arrowhead) projects vertically and the β lobe, indicated by pink arrowhead, projects toward the midline and stops before reaching it. The loss of the β and α lobes (B) and of both the α and β lobes (C) is emphasized by white dashed lines. * shows the ellipsoid body. (D) Quantitation of the αβ neuron mutant phenotype in the Abf2/Abf1 mutant and rescued brains with the Abl-GFP genomic transgene. n = number of MB observed and *** P < 0.001 (Fisher exact test). Transgenic expression of
Abl-GFP rescued the morphological defect and lethality of the double heterozygous Abl+/Abl+ combination, but failed to rescue Abl homoyzogote lethality, indicating that this lethality is due to associated modifiers on the chromosome independent of Abl. (E) Quantification of the αβ neuron mutant phenotype when a wild-type or a kinase dead form of Abl expression is driven in the MBs by OK107-GAL4 (n = number of MB observed). (F-F’) Two-cell WT αβ neuron MARCM clone in a WT brain (F) associated with anti-Fas2 staining in red (F’). (G-G’) Two-cell WT-looking αβ neuron clone (G) associated with anti-Fas2 staining in red (G’) in an Abl+/Abl+ brain. (H-H’) A single-cell αβ neuron clone with an α branch growth defect (H) associated with anti-Fas2 staining in red (H’) in an Abl+/Abl+ brain displaying an absence of α lobe. Note the α branch which stops just after the branching point in H (yellow arrowhead). (I-I’) A single-cell αβ neuron clone with α and β branch growth defects (I) associated with anti-Fas2 staining in red (I’) in an Abl+/Abl+ brain displaying an absence of α and β lobes. Note the small α and β branches in I. (I-F’) Expression of Abl within the MB using an Abl-GFP genomic transgene (I). α and β lobes are revealed by anti-Fas2 staining in red (I’). Merge of GFP and anti-Fas2 staining (I”). All panels correspond to 48 hAPF brains except for E and the rescue experiment in D which are from adult.

Images are composite stacks to allow the visualization of axon trajectories along their entire length. Full genotypes: (A) wild type: y w67c23; Abl+/Abl+. (F) two-cell WT αβ neuron MARCM clone in a WT brain displaying an absence of α lobe. Note the small α and β branches in F. (F-F’) Expression of Abl within the MB using an Abl-GFP genomic transgene (F). α and β lobes are revealed by anti-Fas2 staining in red (F’). Merge of GFP and anti-Fas2 staining (F”). All panels correspond to 48 hAPF brains except for E and the rescue experiment in D which are from adult brains. White arrowheads show the peduncle or common part of the αβ axon, white arrows show the α lobes and pink arrowheads show the β lobe. The scale bar in panels A-C and F-J indicates 30 μm. Images are composite stacks to allow the visualization of axon trajectories along their entire length. Full genotypes: (A) wild type: y w67c23; (B and C) y w67c23:: Abl/FRT2A / Abl+FRT2A. (D) top to bottom: y w67c23; Abl/FRT2A / Abl+FRT2A. y w67c23; Abl-GFP / +; Abl/FRT2A / Abl+FRT2A. (E) top to bottom: y w67c23 / y; UAS-Abl / UAS-mCD8-GFP; OK107-GAL4/+; y w67c23 / y; UAS-Abl427 / UAS-mCD8-GFP; TM6B,Tb+; +; OK107-GAL4/+; (F) w F0607b GAL80 hs-FLP122 FRT19A / w; sn FRT19A; c739-GAL4 UAS-mCD8-GFP / UAS-mCD8-GFP. (G-H-I) w; tubP-GAL80 hs-FLP122 FRT19A / w; sn FRT19A; c739-GAL4 UAS-mCD8-GFP / UAS-mCD8-GFP. To set a small window for these “visualization clones” since only the membrane marker is clonal, and not the Abl mutation. For this experiment, clones are initiated in L3 larvae, and examined at 48 hAPF (to avoid the substantial late-pupal/adult lethality of homozygous Abl mutations). Two-cell/single cell visualization clones in Abl mutant animals revealed highly-penetrant growth defects (87% n = 15) (Table 1B and Fig 3F–3I”). We conclude that Abl, like Appl, is required for MB αβ axon outgrowth. To visualize the localization of Abl protein we employed a transgenic fly bearing a genomic Abl-GFP construct, which rescues both Abl mutant lethality and the mutant MB phenotypes (see above) and therefore is a bona fide endogenous marker for Abl. Abl-GFP is expressed broadly and homogenously in the brain from L3 to adult with elevated levels in the MB αβ axons of 48 hAPF brains (Fig 3J–3I”). Taken together, these data show that Abl is expressed in the MBs and that Abl function is required for MB αβ axon outgrowth. Additional
neuroblast and more than two-cell visualization clones in Abl mutant animals confirmed the Abl requirement for MB αβ axon outgrowth (S3 Fig). In order to know if other MB neurons, in addition to the αβ’s, are sensitive to the lack of Abl we have also looked at the α’β and the γ MB neurons. We could not assess the α’β neurons because neither the anti-Trio antibody nor the c305a-GAL4 line labelled the MB α’β neurons adequately before 48 hAPF. However, anti-Fas2 revealed a clear defect in Abl2>Abl4γ neurons (S3 Fig). Therefore, at least the γ and the αβ MB neurons are sensitive to the lack of Abl function. Since both αβ and α’β are sensitive to Abl overexpression (see above), all MB neurons appear to require normal levels of Abl function.

**Table 1. Abl mutant MARCM clones.**

(A) Ablmut / Ablmut MARCM clones.

| Genotype | WT | Absence of α lobe | Absence of β lobe | Absence of α/β lobes | n   |
|----------|----|-------------------|-------------------|---------------------|-----|
| Control  | 22 | 100%              | -                 | -                   | 22  |
| Abl2 / Abl2 | 43 | 90%              | 5                 | 10%                 | 48  |
| Abl1 / Abl1 | 37 | 82%              | 5                 | 11%                 | 45  |
| Abl1 / Abl1 | 71 | 92%              | 3                 | 4%                  | 77  |

(B) Visualization Abl2/Abl4 MARCM clones.

| Genotype | WT | α branch growth defect | β branch growth defect | α/β branch growth defects | n   |
|----------|----|------------------------|------------------------|---------------------------|-----|
| Control  | 30 | 00%                    | -                      | -                         | 30  |
| Abl2 / Abl4 | 2 | 13%                    | 10                    | 67%                      | 15  |

> Two-cells clones

| Genotype | WT | Absence of α lobe | Absence of β lobe | Absence of α/β lobes | n   |
|----------|----|-------------------|-------------------|---------------------|-----|
| Control  | 9  | 100%              | -                 | -                   | 9   |
| Abl2 / Abl4 | 6 | 23%              | 14                | 54%                 | 26  |

Neuroblast clones

| Genotype | WT | Absence of α lobe | Absence of β lobe | Absence of α/β lobes | n   |
|----------|----|-------------------|-------------------|---------------------|-----|
| Control  | 9  | 100%              | -                 | -                   | 9   |
| Abl2 / Abl4 | 2 | 17%              | 7                 | 58%                 | 12  |

WT: wild-type clones. n: number of clones analyzed.

Full genotypes: A) Control: y w67c23 hs-FLP122 / +; c739-GAL4 UAS-mCD8-GFP / +; tubP-GAL80, FRT2A / FRT2A. Mutant: y w67c23 hs-FLP122 / +; c739-GAL4 UAS-mCD8-GFP / +; tubP-GAL80, FRT2A / Abl2 FRT2A. y w67c23 hs-FLP122 / +; c739-GAL4 UAS-mCD8-GFP / +; tubP-GAL80, FRT2A / Abl4 FRT2A. y w67c23 hs-FLP122 / +; c739-GAL4 UAS-mCD8-GFP / +; tubP-GAL80, FRT2A / Abl1 FRT2A. B) Control: w tubP-GAL80 hs-FLP122 FRT19A / w sn FRT19A; c739-GAL4 UAS-mCD8-GFP / UAS-mCD8-GFP. Abl2/Abl4: w tubP-GAL80 hs-FLP122 FRT19A / w sn FRT19A; c739-GAL4 UAS-mCD8-GFP / UAS-mCD8-GFP, Abl2 / Abl4.

https://doi.org/10.1371/journal.pgen.1009287.t001

**Htt modifies the Abl mutant phenotype in MB axon outgrowth**

As discussed above, the Appβ2 MB phenotypes in β axons can be rescued by UAS-driven overexpression of Abl [5], and both the Appβ3 and dsh1 β axonal phenotypes can be rescued by modest overexpression of Abl using a genomic transgene, (S1B and S1C Fig). Taken together
with the finding that \textit{htt} suppresses both \textit{Appl} and \textit{dsh} MB phenotypes, we hypothesized that \textit{htt} might be a suppressor of \textit{Abl} action. To test this hypothesis, we conducted three sets of experiments to examine the effects of reducing \textit{htt} dosage on \textit{Abl} phenotypes. First, we found that the increased severity of the MB mutant phenotype in \textit{Appl\textsuperscript{D}; Abl\textsuperscript{2}/+} individuals was completely abolished when one copy of \textit{htt} was also removed (31% absence of \(\beta\) lobe in \textit{Appl\textsuperscript{D}; Abl\textsuperscript{2}/+} compared to 14% in \textit{Appl\textsuperscript{D}; Abl\textsuperscript{2} htt\textsuperscript{m1}/+}—Fig 4A upper and lower bars). Second, a modest but significant increase in the proportion of WT MBs in \textit{Abl\textsuperscript{2}/Abl\textsuperscript{1}} individuals was observed when one dose of \textit{htt} was removed (from 20% to 32%—Figs 4C and S4). Third, enhancement of the \textit{Abl} GOF mutant phenotype, measured by the simultaneous absence of both \(\alpha\) and \(\beta\) lobes, was seen when one copy of \textit{htt} was removed (from 21% to 75%—Fig 4D upper and middle bars). Conversely, rescue of this phenotype was observed when \textit{htt} was overexpressed using \textit{UAS-htt-fl-CTAP} driven by \textit{c739-GAL4} (from 21% to 3%—Fig 5D upper and lower bars). This interpretation of a phenotypic rescue is further supported in this comparison by the increase in the number of wild-type MBs (0% in \textit{UAS-Abl}, vs 16% in \textit{UAS-Abl; UAS-htt-fl-CTAP}). The ability of \textit{htt/+} to suppress the \textit{Abl} LOF phenotype or to enhance the \textit{Abl} GOF phenotype appears to correlate with the amount of kinase-competent residual protein in the various mutant backgrounds (see Discussion). Taken together, these data strongly indicate that Htt is a repressor of Abl function during MB axon outgrowth.

\textbf{Reduction of \textit{htt} increases Abl kinase activity in the developing MBs}

One potential explanation for the suppressor effect of \textit{htt} on the \textit{Abl} mutant MB phenotype is that Htt inhibits Abl kinase activity. To test this idea, we employed a fluorescence resonance energy transfer (FRET) biosensor probe that allows Abl kinase activity to be assayed \textit{in vivo} in \textit{Drosophila} [42]. Using this tool, we first showed that Abl kinase activity is detectable in the MBs by comparing the FRET activity of wild type \textit{UAS-Abl-FRET} versus the FRET activity of a mutant \textit{UAS-Y\textsuperscript{-}F Abl-FRET} that lacks its phosphorylatable tyrosine (Figs 5D–5F and S5). We then tested the FRET efficiency in the developing MBs of control (+/+ versus \textit{htt\textsuperscript{m1}/+} larvae and detected a significant increase of the FRET efficiency when one copy of \textit{htt} was removed (Fig 5E and 5G). Note that the exact value of the FRET ratio depends on various microscope and laser settings during imaging. Consequently, this number can only be compared directly between samples imaged together in a single imaging session and cannot be compared from separate experiments. In these experiments, images for validation of the FRET(WT) probe by comparison to FRET(Y\textsuperscript{-}F) were collected in a single session (D,F) as were images comparing FRET activity in a wild type vs \textit{htt/} genetic background (E,G). Quantitative PCR (qRT-PCR) analysis of control versus \textit{htt\textsuperscript{m1}/+} larval brains revealed no difference in \textit{Abl} mRNA expression (Fig 5H). Furthermore, there were no apparent differences in the overall levels of Abl protein in the \textit{htt/} heterozygous MBs relative to WT controls (Fig 6A–6C). The expression level of Abl protein is fairly homogenous in the larval brain rendering the quantitation of Abl in the MBs difficult. In order to reliably quantitate Abl protein levels, we measured the level of Abl protein in 48 hAPF MBs. As noted above, Abl is enriched during this stage. Finally, reduction or increase in Htt expression did not alter the total levels of neuronal Abl protein in \textit{Drosophila} heads (Fig 6D–6I). Taken together, these data strongly suggest that Htt is a repressor of Abl kinase activity in the developing MB axons.

\textbf{Discussion}

Tumorigenesis and neurodegeneration may be two sides of the same coin [43]. Indeed, defining the overlap of molecular pathways implicated in cancer and neurodegeneration may open
the door to novel therapeutic approaches for both groups of disorders [43]. Correlative studies have highlighted a decreased cancer incidence in the population with the neurodegenerative disorder Huntington’s disease and both wild-type and mutant huntingtin (Htt) have been implicated in tumor progression [44]. Interestingly, it has been proposed that, in the normal...
Fig 5. Reducing Htt expression increases Abl-FRET biosensor phosphorylation state during MB development. (A) Schematic representation of an imaged MB in the brain. (B) The Abl-FRET biosensor is based on mammalian CRK protein scaffold with two additional fluorescent proteins (CFP and YFP). ABL kinase activity induces phosphorylation of UAS-Abl-FRET biosensor leading to its spatial rearrangement and increased FRET efficiency [59]. (C) Representative maximal projection of the YFP and FRET signals recorded in the MB lobes of adult flies using confocal microscopy. These are presented as examples of the kind of image data that goes into the FRET ratio calculation. Scale bar: 10μm. (D) FRET and YFP signals are recorded on
expressed in MB neurons using the (E) Reducing reduced for tyrosine located in ABL target site (PYAQP) was replaced by a phenylalanine (PFAQP) impairing phosphorylation [42]. FRET efficiency is significantly reduced for UAS-Y>F Abl-FRET relative to UAS-Abl-FRET biosensor for all confocal planes considered except for the nine most anterior and six most posterior planes. Two-tailed Mann-Whitney tests on non-Normally distributed data. Results are mean ± SEM with n ≥ 7 MB for each confocal plane. (E) Reducing htt expression increases FRET efficiency of UAS-Abl-FRET biosensor in third instar larval MB lobes. The UAS-Abl-FRET biosensor is expressed in MB neurons using the c739-GAL4 driver in control (+/+) and htt\(^{-}\)/+ flies. FRET efficiency is significantly increased in htt\(^{-}\)/+ flies for all confocal planes along the anterior-posterior axis except for the three located 3 to 4 μm from the initial confocal plane. Two-tailed Mann–Whitney tests on non-Normally distributed data. Results are mean ± SEM with n ≥ 13 MB for each confocal plane. (F) FRET efficiency is globally reduced in UAS-Y>F Abl-FRET mutant versus UAS-Abl-FRET. FRET efficiency is averaged for all confocal planes and all along the anterior-posterior axis. Two-tailed Mann–Whitney test on non-Normally distributed data. Results are mean ± SEM with n ≥ 462; ** p < 0.001. (G) FRET efficiency is globally increased when htt expression is reduced. FRET efficiency is averaged for all confocal planes and all along the anterior-posterior axis. Two-tailed Mann–Whitney test on non-Normally distributed data. Results are mean ± SEM with n ≥ 1055; *** p < 0.001. (H) Abl mRNA expression is not changed in L3 brains following htt partial inactivation. Abl expression was assessed using RT-qPCR in L3 brains of htt\(^{-}\)/+ versus WT (+/+). Male flies. Results show three independent biological replicates. Full genotypes: Genotypes: y \(w^{67C23}\)/ Y; c739-GAL4/+; UAS-Abl-FRET/+; y \(w^{67C23}\)/ Y; c739-GAL4/+; UAS-Y>F Abl-FRET/+; y \(w^{67C23}\)/ Y; c739-GAL4/+; htt\(^{-}\)/+ UAS-Abl-FRET/+.

https://doi.org/10.1371/journal.pgen.1009287.g005

physiological situation, the neurodegeneration-related Amyloid precursor protein (APP) recruits the oncogenic Abelson (Abl) kinase in order to promote axonal outgrowth [5]. It is, therefore, tempting to propose that different neurodegenerative diseases (ND) may share components and mechanisms and that Abl may also have a role in ND.

In this study, we have shown that Abl is required for axonal growth in the MBs, a brain structure that is involved in memory. Furthermore, we show that both Abl overexpression and lack of expression in the MBs result in similar phenotypes, indicating the need to tightly regulate Abl activity during MB axon outgrowth. This raises the question of how Abl activity is normally negatively regulated during MB axon outgrowth. We confirmed the previous observation that overexpression of Abl rescues the App\(^{L}\) MB phenotypes [5]. Furthermore, we found that Abl overexpression also rescues the dsh\(^{L}\) MB phenotype. These two results support the model that Appl activates Abl, which in turn phosphorylates Dsh. At the genetic level, we expected an increase of Abl activity in an individual bearing a loss-of-function mutation of a putative Abl repressor. We therefore hypothesized that reducing the levels of an Abl repressor would result in suppression of the Appl and dsh mutant MB phenotype. We found that Htt is such an inhibitor of Abl activity in the MBs. The loss of one dose of htt increased the activity of the wild-type Abl still present in Abl\(^{L}\)/+ individuals and therefore prevented the enhancement of the MB mutant phenotype. While a number of studies have concluded that Htt deficiency results in significant alterations to kinase signaling pathways [45], to our knowledge this study is the first to implicate the crucial tyrosine kinase, Abl. Together, these data demonstrate the power that neurodevelopmental studies have to reveal close functional relationships between genes implicated in different forms of neurodegenerative disease.

It was a surprise that null htt mutants show no obvious developmental defects in Drosophila although strong defects could have been expected from the lack of such a conserved protein [12]. One possible hypothesis is that, due to its fundamental importance, some functional redundancy has been selected to buffer against variation in the production of the Htt protein. We reasoned that altering the levels of another protein, particularly another protein known to be implicated in neurodegeneration, could reveal cryptic phenotypes of htt mutation during brain development. Following this reasoning, we combined a null Appl mutant with heterozygous null htt mutations in double mutant individuals. Unexpectedly, we found that mutant htt suppressed the Appl MB axonal outgrowth defect.

Abl is a key component of the Appl signaling pathway required for axonal arborization and growth in the fly brain and the functional relationship between these two proteins is likely conserved in mammals [5,34]. While the role of APP-mediated signaling has been shown most clearly in Drosophila, a number of lines of evidence suggest that mammalian APP also fulfills a
Fig 6. *htt* does not seem to affect the quantity of ABL in the MBs or the total levels of neuronal Abl in *Drosophila*. (A–B”) Expression of the Abl-GFP genomic transgene in a WT (A) and in a *htt*<sup>int</sup> heterozygous mutant background (B) at 48 hAPF. Anti-Fas2 staining marked αβ neurons (A’–B’). Merge of GFP and anti-Fas2 staining (A”–B”). (C) Quantitation of the GFP expression within MBs is not significantly different (N.S.) between WT and *htt*<sup>int</sup> heterozygous mutant background using a Mann-Whitney U test. The scale bar indicates 30 μm. Details of image quantification procedure and full genotypes are: (C) After having outlined the MB with the Fas2 staining, GFP and Fas2 intensities from MB shape were quantified for each slice of the stack. The GFP intensity of each slice was averaged and then normalized by the mean Fas2 intensity. Number of MB analyzed: control = 16, *htt* mutant = 14. Quantitation of the GFP expression within MBs is not significantly different between WT and *htt*<sup>int</sup> heterozygous mutant background using a Mann-Whitney U test. Quantitation were done with Imaj software. Images are composite stacks to allow the visualization of axon trajectories along their entire length. Genotypes: (A) *y w<sup>67c23</sup>; Abl-GFP / +. (B) *y w<sup>67c23</sup>; Abl-GFP / +; *htt*<sup>int</sup>/ +. (D) Western blots of whole cell lysates from adult heads from the following genotypes: +/+ , *htt*<sup>int</sup>/+ and *htt*<sup>int</sup>/*htt*<sup>int</sup>. Levels of Htt, Abl, and β-Tubulin (β-Tub) were assessed by probing blots using the indicated antibodies. 1, 2, and 3 indicate biological replicates for each line. (E and F) Quantitation of Htt (E) or Abl (F) protein levels in the indicated genotypes was assessed from western blots in (D) relative to β-Tubulin and plotted as relative band intensity. (G) Htt was over-expressed using *elav<sup>elav</sup>-GAL4 > UAS-htt-fl-CTAP*. Lysates from adult heads were subjected to western blotting to assess Htt, Abl, and β-Tubulin levels. 1, 2,
and 3 indicate biological replicates for each line. (H and I) Quantitation of Htt (H) or Abl (I) protein levels in the indicated genotypes was assessed from western blots in (G) relative to β-Tubulin and plotted as relative band intensity. In E and F, significance was calculated by one-way ANOVA. In H and I, significance was calculated by unpaired t-test. In E, F, H and I, errors indicate standard deviation. (n.s. not statistically different, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

https://doi.org/10.1371/journal.pgen.1009287.g006

signaling role [46]. Importantly, and in line with its proto-oncogenic role, Abl tyrosine kinase activity is tightly regulated by intramolecular inhibition [22]. Although Abl is clearly required as a downstream effector of Appl in the MB axon growth, its precise role and regulation in the MBs has not been described previously.

The three Abl alleles used in this study have all been shown to result in truncated proteins [47]. While Abl1 retains the SH3, SH2 and TK domains of Abl, Abl2 is mutated within the TK domain and only retains the SH3 and SH2 domains, while Abl4 is mutated in the SH2 domain, and only retains an intact SH3 domain. It therefore seems likely that very little or no residual Abl function remains in Abl1/Abl4 and Abl2/Abl4 individuals and may explain why no rescue was observed when one dose of htt was removed in these genetic backgrounds. In contrast, the Abl1/Abl2 allelic combination is likely less severe than the other two genotypes and Abl1/Abl2 animals do accumulate truncated Abl proteins. Indeed, while significant amounts of truncated Abl1 and Abl2 mutant proteins are detectable, only faint protein bands are observed in Abl4 pupae [48]. Therefore, some functionally significant kinase activity could remain in Abl1/Abl4 and Abl2/Abl4 individuals and the loss of one dose of htt might increase the activity of the remaining kinase activity. Finally, removing one dose of htt in animals overexpressing Abl would result in even more Abl activity, which in turn would exacerbate the mutant phenotype. Contrarily, overexpressing Htt, as in the UAS-Abl, UAS-htt doubly overexpressing individuals, would inhibit Abl function when compared to the UAS-Abl overexpression alone which thus might explain the observed rescue.

There are three different levels at which Htt might act to modulate Abl activity. First, Htt could either directly or indirectly affect Abl mRNA levels. Although fly Htt has been described as a cytoplasmic protein [12], htt has been shown to be a suppressor of position-effect variegation, suggesting a possible role in chromatin organization [13]. This hypothesis is unlikely for the MB phenotype described here since qRT-PCR analysis of third instar brains did not reveal a significant differences in Abl mRNA levels between httmut/+ and control individuals. Second, Htt could play a role regulating Abl protein level in the MBs. We also consider this unlikely since the quantity of the endogenous Abl is unchanged in httmut/+ relative to control individuals. Third, Htt could influence the kinase activity of Abl itself. Taking advantage of a FRET biosensor enabling Abl kinase activity to be assayed directly in the MBs, we revealed a significant increase in active Abl in httmut/+ versus control individuals. Therefore, we favor a model of Htt acting as an inhibitor of Abl kinase activity during normal MB axonal growth (Fig 7). Abl activity in axons needs to be maintained within rather narrow limits [32,33]. These two recent studies show that either increase or decrease of Abl activity cause disorganization of actin structure in the growth cone and prevent the orderly oscillation of growth cone actin that is the motor for growth cone advance and thus axon extension. Those papers also explain why Abl gain and loss can result in superficially similar mutant axon patterning phenotypes even though the molecular effects of Abl increase versus decrease are opposite.

The HEAT repeat domains of Htt are thought to function as a solenoid-like structure that acts as a scaffold and mediates inter- and intra-molecular interactions [9]. It is therefore tempting to propose that a scaffolding role of Htt could elicit repression of Abl kinase activity. At least in the MBs, a balance seems to exist in the activity of Abl, positively regulated by the membrane complex formed by the core PCP proteins and Appl, and suppressed by Htt and possibly other proteins, as well. On one hand, if Appl is absent, Abl is not optimally activated
leading to defects in MB axon growth. Conversely, decrease of Htt to 50% of wild type levels leads to de-repression of Abl kinase activity, which in turn compensates for its sub-optimal level of activation in the absence of Appl. This unexpected apparent balance of activation and inhibition of Abl by Appl and htt, whose mutant orthologs are central players in human neurological disease, may define a conserved functional interaction to maintain Abl activity in the relatively narrow window to appropriately effect axon outgrowth.

Material and methods

Drosophila stocks

All crosses were performed on standard culture medium at 25°C. Except where otherwise stated, all alleles and transgenes have been described previously (http://flystocks.bio.indiana.edu/). The following alleles were used: Abl\(^{1}\), Abl\(^{2}\), Abl\(^{4}\), Appl\(^{d}\), dsh\(^{1}\), htt\(^{int}\) \([13]\), htt-ko and Df-htt \([12]\). The following transgenes were used: UAS-Abl (Bloomington Stock Center line (BL) #28993), UAS-Abl\(^{K417N}\) (from BL #8566) named here UAS-Abl\(^{KD}\) for kinase dead, UAS-Abl-FRET and UAS-Y\(\rightarrow F\) Abl-FRET \([42]\), UAS-RNAi-htt \([49]\), UAS-mCD8GFP, UAS-mito-GFP, UAS-FRT-\(\gamma\)-FRT and the genomic transgenic Abl-GFP \([50]\). UAS-htt-fl-CTAP was produced for this study (see Constructs). We used three GAL4 lines: c739-GAL4 and OK107-GAL4 expressed in MB neurons \([51]\) and the pan-neuronal driver elav\(^{155}\)-GAL4 (BL #458). Recombinant chromosomes were obtained by standard genetic procedures and were molecularly verified when required.
Adult and pupal brain dissection and immunostaining

Adult brains were dissected in PBS after fly heads and thoraces had been fixed for 1hr in 3.7% formaldehyde in PBS. They were then treated for immunostaining as previously described [52,53]. Pupal brains were dissected in PBS and fixed for 20min in 3.7% formaldehyde in PBS at 4˚C with gentle rocking. After washing twice in PBS with 0.5% Triton X-100 (PBT) for 15 min at room temperature, they were incubated in PBT and 5% bovine serum albumin (BSA) (blocking solution) at room temperature for 30 min, followed by overnight incubation at 4˚C with primary antibody diluted in blocking solution. Brains were then washed three times in PBS for 20 min, followed by 30 min in the blocking solution, and then addition of the secondary antibody with incubation for 3 hr at 4˚C. Brains were then washed three times in PBS for 20 min and were mounted with Vectashield (Vector Laboratories). Antibody combinations used: anti-Fas2 (mAb 1D4 from DSHB) at 1:50 dilution followed by anti-mouse Cy3 (Jackson ImmunoResearch) at 1:300; rabbit anti-Myc (Cell Signaling) at 1:1000 followed by anti-rabbit Cy5 (Jackson ImmunoResearch) at 1:300; mouse anti-TAP (Santa Cruz Biotechnology) at 1:300 followed by anti-mouse Cy3 (Jackson ImmunoResearch) at 1:300; rabbit anti-Trio (kind gift from Barry Dickson) at 1:1000 followed by anti-rabbit Cy2 (Jackson ImmunoResearch) at 1:300. Anti-Fas2 (mAb 1D4 from DSHB) at 1:10 dilution followed by anti-mouse Alexa 647 (Jackson ImmunoResearch) at 1:300.

Quantitation of the absence of mushroom body lobes

We took particular care in order to ascertain a suppression effect from a penetrance of about 15%.

The absence of lobes was assessed with the anti-Fas2 staining or with the c739-GAL4 UAS-mito-GFP marker visualized with an epi-fluorescence microscope (Leica DM 6000). In order to be certain that we were indeed measuring suppression of the Appl or dsh 15–20% of absence of β lobes and not a mere variation from this rather low phenotypic penetrance (Figs 1, 2, 4, S1B and S1C), we followed a strict protocol. A large number (at least 50) of Appl/ or w dsh / or Appl/ w dsh females were collected, pooled together and crossed in groups of 25 with either y w ; wild-type control or y w ; mutant /Balancer males. In this way, there were always Appl/ w /Y or w dsh /Y or Appl/ w dsh /Y males from the same experiment that show the expected mutant phenotype, and any modifier in the Appl or dsh stocks, if it exists, should be statistically equally present in the control and in the experiment. We could therefore associate the suppression phenotype seen in Appl/ w /Y or w dsh /Y or Appl/ w dsh /Y; httmutant /+ males unequivocally to the presence of the httmutant allele. Noticeably, throughout this study in a total of 26 experiments, MBs from Appl/ c739-GAL4 UAS-mito-GFP/+ males were assessed. Of 3110 MBs, 451 (14.5%) displayed an absence of β lobe phenotype (22/141; 15/106; 16/84; 15/124; 15/105; 18/152; 19/108; 14/100; 14/100; 27/156; 25/164; 12/112; 2/36; 17/102; 21/139; 14/109; 19/158; 19/155; 15/104; 30/146; 14/91; 24/138; 13/124; 13/102; 11/90; 27/164). In addition, throughout this study, we assessed the absence of β lobe phenotype in wild-type controls. In a total of nine different experiments (0/106; 0/102; 0/100; 1/107; 0/100; 0/50; 0/50; 0/37; 1/107), control MBs displayed two MBs with absence of β lobes out of 759 MBs (0.26% showing absence of β lobes and 99.74% of the MBs appearing WT). Thus, wild-type flies almost invariably have intact MB lobes as was previously described [5].

MARCM clonal analysis

The MARCM technique was used to generate clones in the MB [53]. We use the term MARCM clones when homozygous mutant clones were examined in a heterozygous background and visualization MARCM clones when homozygous mutant clones were examined in
a homozygous mutant background. For MARCM neuroblast clones, L1 larvae were heat-shocked at 37°C for 1 hr and adult brains were dissected and stained. For visualization MARCM neuroblast clones, L1 larvae were heat-shocked at 37°C for 1 hr and 48 hAPF brains were dissected and stained. For visualization single and two-cell clones, L3 larvae were heat-shocked at 37°C for 15 min and 48 hAPF brains were dissected and stained.

**Microscopy and image processing**

Images were acquired at room temperature using a Zeiss LSM 780 laser scanning confocal microscope (MRI Platform, Institute of Human Genetics, Montpellier, France) equipped with a 40x PLAN apochromatic 1.3 oil-immersion differential interference contrast objective lens. The immersion oil used was Immersol 518F. The acquisition software used was Zen 2011. Contrast and relative intensities of the green (GFP), red (Cy3) and blue (Cy5) channels were processed with Fiji Software. Quantitation was performed using ImageJ software.

**Constructs**

\textit{pUAS-htt-fl-CTAP:} A \textit{htt} “mini-gene” bearing a dual C-terminal Tandem Affinity Purification tag (Protein G and a streptavidin binding peptide: GS-TAP tag) was constructed. PCR was performed using the \textit{dhtt} “mini-gene” comprising the \textit{dhtt} full length cDNA with intron 10 (as described in [13]) and the following primers: HTT-TAP-FOR: ggtaccATGGACAAATCCAGGTCCAG (KpnI site added) and HTT-TAP-REV: tctagaCAGGCACTGCAACATCCGG (XbaI site added). The resulting PCR product was digested with KpnI/XbaI and subcloned into the \textit{pUAST-CTAP(SG)} vector [54]. To avoid rearrangements due to \textit{dhtt} instability, culturing conditions were used as previously described [13]. \textit{pUAS-htt-fl-CTAP} transgenic flies were generated and balanced using standard procedures and expression of dhtt-SG was assessed using western blots.

**FRET imaging**

Fly brains were dissected in 1X PBS at room temperature and collected in ice-cold PBS before being fixed in 3.6% formaldehyde for 20 min. Brains were rinsed twice in PBST 0.5X for 20 min before being mounted in Vectashield (Vector Laboratories). MBs were imaged on adjacent 0.5 μm confocal planes along the anterior-posterior axis using a LSM780 confocal microscope (Zeiss) at x40 with oil immersion. Cyan fluorescent protein (CFP) was excited at 405 nm and emission recorded between 454 and 500 nm. Yellow fluorescent protein (YFP) was excited at 514 nm and emission recorded between 516 and 571 nm. Fluorescence resonance energy transfer (FRET) was generated at 405 nm. To avoid CFP emission, FRET was recorded out of CFP emission range, between 587 and 624 nm.

**FRET image analysis**

Brains were oriented anterior-posteriorly using the peduncle as an anatomical landmark and aligned according to the first confocal plane where a signal was visible. We ensured that the same number of planes were obtained for each group (\textit{c739\textgreater{}Abl-FRET}: 48 ± 4 planes and \textit{c739\textgreater{}Y\textgreater{}F} Abl-FRET): 46 ± 1.5 planes, Student t-test: \( P = 0.7 \), \textit{c739\textgreater{}Abl-FRET}: 79.3 ± 2.3 planes and \textit{c739; htt\textsuperscript{mut}\textgreater{}Abl-FRET}: 75.4 ± 3.7 planes; Student t-test: \( P = 0.4 \)) indicating that there were no differences due to mounting. Average YFP and FRET signals were computed using the measurement of ‘Mean Grey Value’ and the ‘Plot Z-axis Profile’ functions of ImageJ [55] into a region of interest (ROI) corresponding to the contour of the MB and for each confocal plane. Background was corrected using the ‘Rolling Ball Background Subtraction’
function (50 px radius). For each plane and within the same ROI, FRET signal was expressed relative to YFP to account for variability in Abl-FRET biosensor expression level or differences between preparations. Only groups (i.e. Abl-FRET vs \( Y \rightarrow F \) Abl-FRET and Abl-FRET vs \( \text{htt}^{\text{int}} \); Abl-FRET) crossed, collected, dissected and imaged on the same day were compared. For any given confocal plane, the FRET ratio was averaged between left and right MBs and multiple genetically identical animals.

**qRT-PCR**

To quantify Abl expression, RNA was extracted from the brains of L3 males. Brains (~20/sample) were dissected in PBS 1X (Sigma) and kept on ice before homogenized in Trizol reagent (Ambion). Total RNA was treated with DNase to eliminate genomic DNA (Applied Biosystems). RNA was purified using phenol-chloroform extraction and first strand cDNA synthesis was performed using reverse transcriptase (Invitrogen). Primers for Abl RNA amplification were designed on each side of intron 4–5 within exon 4 and 5 respectively. These exons are present in all Abl transcripts. Abl primers were designed using Primer3Plus online software [56]. Abl forward primer sequence is 5'-GCGGCCATCATGA AGGAAATG-3' and reverse primer sequence is 5’-TGCGCCGAACTGGAACTC-3’. Abl RNAs were quantified in real-time during amplification using incorporation of SYBRGreen (Roche) and Light Cycler (Roche). Primers efficacy was first evaluated using a range of cDNA concentrations to ensure linearity of the amplification (E = 1.944). Only a single PCR product with the expected melting temperature was obtained. The amplicon was run on a gel to verify that the size was as expected for the spliced Abl product (107 bp). A control without reverse transcription was done to ensure that Abl amplicon was not obtained. For each sample, a technical triplicate was performed and averaged. Independent biological replicates were prepared for each condition and the fold change was averaged (see Statistics). The biological replicates correspond to independent dissections, extractions, reverse transcriptions and quantifications. In the experimental condition (\( y^67c23/Y;\ \text{htt}^{\text{int}}/+ \)), Abl expression was expressed relative to control flies (\( y^67c23/Y \)) after normalization to internal controls, Rpl9 and Rpl32, and using the \( \Delta \Delta \)CT method [57].

**Western blotting**

Lysates of adult *Drosophila* heads were prepared using RIPA buffer supplemented with protease inhibitors (Sigma #11836170001). Antibodies used for immunoblotting with dilutions were: anti-d\texttt{htt} (3526, rabbit polyclonal, 1:1,000; [13]), anti-dAbl (as above, 1:1,000; [58]), anti-\( \beta \)-Tubulin (mouse, DSHB E7, 1:10,000), anti-mouse and anti-rabbit IRDye secondary antibodies (LI-COR Biosciences, 1:10,000). Lysates were resolved on NuPAGE 3–8% gradient Tris-Acetate gels with Tris-Acetate running buffer (for Htt and Abl) or NuPAGE 4–12% gradient Bis-Tris gels with MOPS running buffer (for \( \beta \)-Tubulin). After transfer to nitrocellulose membranes, blots were processed according to the Odyssey CLx protocol. Median band intensity was quantified using Image Studio.

**Statistics**

Comparisons between two groups expressing a qualitative variable were analyzed for statistical significance using the Chi\(^2\) or the Fisher exact test (BiostaTGV: [http://biostatgv.sentiiweb.fr/?module=tests](http://biostatgv.sentiiweb.fr/?module=tests)). Comparison of two groups expressing a quantitative variable was analyzed using the two-tailed Mann-Whitney \( U \) test or the unpaired \( t \)-test. For FRET quantitation, statistical analyses were performed using Prism 8.0 (GraphPad). For each confocal plane of each group, the normality of the FRET ratio was assessed using D’Agostino & Pearson normality test. Non-parametric Mann-Whitney tests were used to compare groups at each confocal
Supporting information

S1 Fig. The overexpression of Abl rescues the App1Δ and the dsh1 mutant phenotypes. (A) The loss of htt does not produce per se any significant MB developmental defects. (B) Quantitation of the rescue of App1Δ MB phenotype by the genomic construct Abl-GFP. (C) Quantitation of the rescue of dsh1 phenotype by the Abl-GFP genomic construct. n = number of MBs analyzed, * P < 0.05 and ** P < 0.001 (Chi² test). All panels correspond to adult brains. Genotypes: (A) top to bottom: y w67c23 / Y; c739-GAL4 UAS-mito-GFP / +; htt-ko / +, y w67c23 / Y; c739-GAL4 UAS-mito-GFP / +; Df htt / +, y w67c23 / Y; c739-GAL4 UAS-mito-GFP / +; Df htt / htt-ko / +, y w67c23 / Y; httint / +, y w67c23 / Y; httint / httint. y w67c23 / Y; c739-GAL4 UAS-mito-GFP / UAS-RNAi htt, y w67c23 / Y; UAS-mCD8-GFP / UAS-RNAi htt; OK107-GAL4 / +. (B) top to bottom: App1Δ w / Y; c739-GAL4 UAS-mito-GFP / +, Abl-GFP w / Y; c739-GAL4 UAS-mito-GFP / Abl-GFP. (C) top to bottom: w dsh1 / Y, w dsh1 / Y; Abl-GFP / +, (TIF)

S2 Fig. Structure and overexpression of the Abl protein. (A) Molecular scheme of the Abl protein. Abl protein is composed of conserved domains: Src Homology 3 (SH3) domain (blue), Src Homology 2 (SH2) domain (orange), Kinase Domain (red), Poly-Proline PP domain (purple) and F-Actin Binding Domain (FABD) (green). The protein produced by Abl1 mutant allele is truncated between PP and Kinase domains. The protein produced by Abl2 mutant allele is truncated within the Kinase domain. The protein produced by Abl4 mutant allele is truncated within the SH2 domain [47]. (B-E) Anti-Fas2 staining showing the α and β lobes in a WT adult brain (B) and in Abl forced expression by OK107-GAL4 (C-E) with an absence of β lobe (C), an absence of α lobe (D) and an absence of α and β lobes (E). The loss of lobes is emphasized by white dashed lines. Note that panel B is also presented as the left MB in Fig 2A. The scale bar indicates 30 μm. Images are composite stacks. Genotypes: (B) y w67c23 / Y. (C-E) y w67c23 / Y; UAS-Abl / UAS-mCD8-GFP; OK107-GAL4 / +. (F) The expression levels of UAS-Abl and UAS-AblΔ transgenes are similar. UAS-Abl and UAS-AblΔ were expressed using OK107-GAL4. Lysates from adult heads were subjected to western blotting to assess Abl and β-Tubulin (β-Tub) levels. 1, 2, and 3 indicate biological replicates for each line. (G) Quantitation of Abl protein levels in the indicated genotypes was assessed from western blots in (F) relative to β-Tubulin and plotted as relative band intensity. Errors indicate standard deviation. Significance was calculated by one-way ANOVA (P < 0.0001) followed by post-hoc Bonferroni’s multiple comparison correction (n.s. not statistically different and *** P < 0.0001). (H-L””) GFP (green) labelling is showing all the lobes (α and α’ vertically, β and β’ and γ medially), anti-Trio (red) staining is showing the α’ and β’ and γ lobes and anti-Fas2 (blue) staining is showing the α and β and weakly the γ lobes in a WT adult brain (H-H””) and in Abl forced expression by OK107-GAL4 (I-L””) with an absence of β’ lobe (I-I””), an absence of α’ lobe (J-J””) and an absence of α’ and β’ lobes (K-K””). In H’ and H”’ the α’ lobe, indicated by a yellow arrowhead, projects vertically and the β’ lobe, indicated by a pink arrowhead, projects toward the midline. In I’, I’’ and J’, J”’ the present lobes are indicated by arrowheads although the
absent lobes are indicated by empty arrowheads. (L-L’’) is a single confocal section from K-K’’. The loss of lobes is emphasized by white dashed lines. The scale bar indicates 30 μm. Images are composite stacks. Genotypes: (H-H’’) y w67c23 / Y; + / UAS-mCD8-GFP;; OK107-GAL4 / +. (J-L’’) y w67c23 / Y; UAS-Abl / UAS-mCD8-GFP;; OK107-GAL4 / +. (TIF)

S3 Fig. Abl loss of function in MBs. (A-A’) Neuroblast WT αβ neuron MARCM clone in a WT brain (A) associated with anti-Fas2 staining in red (A’). (B-B’) Neuroblast WT-looking αβ neuron clone (B) associated with anti-Fas2 staining in red (B’) in an Abl2/Abtl brain. (C-C’) Neuroblast αβ neuron clone with an absence of α branch (C) associated with anti-Fas2 staining in red (C’) in an Abl2/Abtl brain displaying an absence of α lobe. (D-D’) Neuroblast αβ neuron clone with shorter α and β branches (D) associated with anti-Fas2 staining in red (D’) in an Abl2/Abtl brain displaying an absence of α and β lobes. (E-E’) Multicell WT αβ neuron MARCM clone in a WT brain (E) associated with anti-Fas2 staining in red (E’). (F-F’) Multicell WT-looking αβ neuron clone (F) associated with anti-Fas2 staining in red (F’) in an Abtl2/ Abtl4 brain. (G-G’) Multicell αβ neuron clone with an absence of α branches (G) associated with anti-Fas2 staining in red (G’) in an Abtl2/Abtl4 brain displaying an absence of α lobe. (H-H’) Multicell αβ neuron clone with shorter α and β branches (H) associated with anti-Fas2 staining in red (H’) in an Abtl2/Abtl4 brain displaying an absence of α and β lobes. (A-H’) All panels correspond to 48 hAPF brains. (I-L) Anti-Fas2 staining on wild-type (WT) brain (I) and on Abtl2/Abtl4 brain (J-L) at L3 larval stage. In a wild-type (WT) brain, γ neurons project to vertical and medial lobes. In an Abtl2/Abtl4 brain, 60% of γ neurons are WT (J) whereas, 27% show a loss of the medial lobe (K) and 13% show a loss of both vertical and medial lobes (L). The loss of the vertical and medial lobes is emphasized by white dashed lines. The scale bar in panels A-L indicates 10 μm. Images are composite stacks to allow the visualization of axon trajectories along their entire length. Genotypes: (A and E) w; tubP-GAL80 hs-FLP122 FRT19A / w; sn FRT19A; c739-GAL4 UAS-mCD8-GFP / UAS-mCD8-GFP. (B-D and F-H) w; tubP-GAL80 hs-FLP122 FRT19A / w; sn FRT19A; c739-GAL4 UAS-mCD8-GFP / UAS-mCD8-GFP; Abtl2 / Abtl4. (I-L) y w67c23. (J-L) y w67c23, Abtl2 FRT2A / Abtl4 FRT2A. (TIF)

S4 Fig. htt interaction with Abl. The loss of one copy of htt does not rescue the Abtl2/Abtl4 (A) or Abtl2/Abtl4 (B) mutant phenotype. All panels correspond 48 hAPF brains. n = number of MBs analyzed with P = 0.22 for Abtl2/Abtl4 and P = 0.70 for Abtl2/Abtl4 (Fisher exact test). Genotypes: top to bottom: y w67c23; Abtl2 FRT2A / Abtl4 FRT2A, y w67c23; Abl1 FRT2A, y w67c23; Abtl2 FRT2A, y w67c23. (TIF)

S5 Fig. Detection of the Abl-FRET biosensor in the MBs. (top) Maximum intensity projection of α and β MB lobes in adult flies. (bottom) Maximum intensity projection of vertical and medial MB lobes in stage 3 larvae. The Abl-FRET biosensor is expressed in the MBs using c739-GAL4 and imaged. Maximum intensity projection of confocal stacks corresponding to YFP and FRET signal are shown. These are presented as examples of the kind of image data that goes into the FRET ratio calculation. Scale bar: 10 μm. (TIF)

Acknowledgments

We thank Yoan Arribat for providing htt stocks and discussions in an early development of this work, the Bloomington Drosophila Stock Center for fly stocks, the imaging facility MRI,
member of the national infrastructure France-BioImaging for the FRET imaging. We thank Thierry Gostan and Hervé Seitz respectively at IGMM and IGH, Montpellier for help with the statistics. The 1D4 anti-Fasciclin II hybridoma developed by Corey Goodman and the E7 anti-β-Tubulin monoclonal antibody developed by M. Klymkowsky were obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242.

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