A novel co-segregating *DCTN1* splice site variant in a family with Bipolar Disorder may hold the key to understanding the etiology

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Contributions

AH initiated this research project, was responsible for experimental design, and performed all analyses. AH and AJLC wrote and edited the manuscript.

Abstract

A novel co-segregating splice site variant in the *Dynactin-1* (*DCTN1*) gene was discovered by Next Generation Sequencing (NGS) in a family with a history of bipolar disorder (BD) and major depressive diagnosis (MDD). Psychiatric illness in this family follows an autosomal dominant pattern. *DCTN1* codes for the largest dynactin subunit, namely p150^{Glued}. This protein plays an essential role in retrograde axonal transport and in neuronal autophagy. Neuronal autophagy deficits are implied in BD as prophylactic medications, as well as most antipsychotics and antidepressants, have been shown to enhance it. A GT→TT transversion in the *DCTN1* gene, uncovered in the present work, is predicted to disrupt the invariant canonical splice donor site IVS22+1G>T and result in intron retention and a premature termination codon (PTC). Thus, this splice site variant is predicted to trigger RNA nonsense-mediated decay (NMD) and/or result in a C-terminal truncated p150^{Glued} protein (ct-p150^{Glued}), thereby negatively impacting retrograde axonal transport and neuronal autophagy. This variant is analogous to the dominant-negative *GLUED Gl^{1}* mutation in *Drosophila*. The newly identified variant may reflect an autosomal dominant cause of psychiatric pathology in this affected family. Factors that affect alternative splicing of the *DCTN1* gene, leading to NMD and/or ct-p150^{Glued}, may be of fundamental importance in contributing to our understanding of the etiology of BD as well as MDD.

Abbreviations: AD, Alzheimer disease; aPKC, atypical PKC; BDI, bipolar disorder I; BDII, bipolar disorder II; BDIII, bipolar disorder III or cyclothymic disorder; ct-p150^{Glued}, C-terminal truncated p150^{Glued} protein; DCTN1, dynactin-1; IP_3, myo-inositol-1,4,5-trisphosphate; MDD, major depressive diagnosis; MND, motor neuron disease; NMD, RNA nonsense-mediated decay; NGS, next generation sequencing; PD, Parkinson disease; PKC, protein kinase C; PS, Perry syndrome; PTC, premature termination codon; RC, related control; WES, whole exome sequencing; WGS, whole genome sequencing.

Introduction

Bipolar disorder (BD) is a chronic and severe psychiatric disorder. In most cases, it is a recurrent psychiatric disorder characterized by oscillations between mania and major depressive episodes, although some patients may only experience mania. Mania in affected patients may or may not involve psychosis. BD is subdivided into two main subtypes, notably type I (BDI) and type II (BDII).

BDI is generally regarded as more severe than BDII due to the presence of mania in BDI patients compared to an absence or decreased severity of mania in BDII affected individuals[1]. Cyclothymic disorder or bipolar disorder III (BDIII) is regarded as part of the BD spectrum of illnesses, sharing similar characteristics with other BDs. However, BDIII is rarely diagnosed because symptoms are generally below the threshold required for a definitive diagnosis of BD. Nevertheless, in many cases individuals with cyclothymic disorder also go on later to experience episodes of mania and develop BDI[2]. BD often requires repeated hospitalizations and is an expensive illness to treat both for the affected individual and for society, often also resulting in significant unemployment[3]. The disorder is often associated with considerable morbidity and a substantial increased risk of suicide (10-20%)[4]. BD is considered a complex illness, often suspected to involve interactions between genetic and environmental factors such as stressful circumstances as well as seasonal changes[5,6].

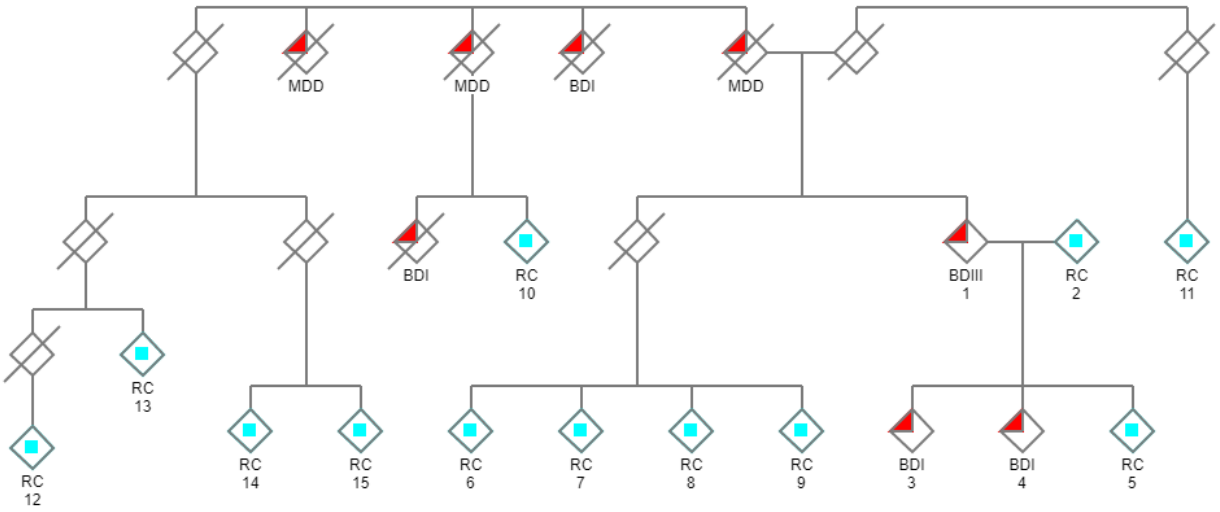

Figure 1. Pedigree of the family under investigation. Members of the core family (#1-5) were each sequenced more than once using whole genome sequencing (WGS) and/or whole exome sequencing (WES) as opposed to the remainder of the related living controls who were only sequenced once with WES. Individuals with a psychiatric history are denoted with filled red triangles. Psychiatric illness in this extended family is inherited in a manner resembling autosomal dominance. NGS was performed, and subtractive analysis was used to discover novel co-segregating candidate gene variants in the three living affected individuals in the core group (#1,3,4), using related unaffected living relatives as negative controls (related controls/RC: light blue squares). (/ = deceased; and males and females are not distinguished for reasons of privacy).

Our present understanding of the underlying molecular basis of BD has thus far been insufficient to explain its etiology and there are no existing biochemical- or genetic-based laboratory tests available to validate a diagnosis. Currently, the diagnoses are based on observations and history taking, and thus are not predictive of illness risk for individuals who may later develop illness. BDI and BDII have a lifetime world-wide prevalence of ~0.6%, and ~0.4% respectively, and the lifetime prevalence of sub-threshold BD has been estimated to be ~1.4%%[7]. Major depressive diagnosis (MDD), on the other hand, is more common with a lifetime world-wide prevalence of ~4-5%[8]. BDI has long been known to be highly heritable (> 80 %)[9]. As major depressive episodes are a notable feature of BD, it is not surprising that MDD is also a common feature in extended families with BD, implying a common etiology in these affected families[10]. The age of onset of mania in BDI patients shows a peak in early adulthood in a 35 year UK study[11]. These findings are corroborated by

a large study in the Netherlands where the authors found two peaks in the age of onset for BD, one likewise in early adulthood (mainly for BDI) and another peak later in life[12]. Similarly, MDD patients having a family history of MDD, and experiencing recurrent depressive episodes, also exhibit a peak for age of onset in early adulthood[13].

Many genes and chromosomal regions have been implicated in BD. However, to this date no single gene defect alone has been shown to be causative[14]. One of the primary difficulties in discovering candidate disease-causing variants is in defining a homogeneous cohort of patients. In this research study we have attempted to address this problem by focusing on a single core family with two siblings diagnosed with BDI, a parent with BDIII and a family pedigree which strongly suggests that psychiatric illness follows an autosomal dominant inheritance pattern (Fig. 1). It is known that there is a high risk of psychiatric disorders among the relatives of patients with BDI and MDD[15,16]. Thus, we did not discriminate among different psychiatric diagnoses in the extended family as the family pedigree historically includes individuals diagnosed with BDI, MDD, or BDIII (Fig. 1). Genomic data from twelve related controls, and one unaffected control, were also used to filter variants to assist in the discovery of the novel co-segregating *DCTN1* splice variant described in this research.

Results and Discussion

Only a single novel co-segregating variant was identified in the affected family described in the current work (Fig. 1). When confounding variants were removed through filtering, a novel transversion splice site variant in the *DCTN1* gene (IVS22+1G>T, GRCh37/hg19: 2:74,593,585C>A) was discovered. It was the only novel variant, in coding or splice regions, that was found to co-segregate with the affected cohort and not be present in any of the controls (Fig. S1; Table S1). This splice site variant is predicted to disrupt the canonical splice donor site at the invariant +1 position and cause retention of intron 22 and introduction of a subsequent PTC (Fig. 2). It is in a highly evolutionary conserved region (Table S2) and is predicted to be deleterious (Table S3). There is a very rare alternative allele noted at this coordinate in the dbSNP database[17] (rs75942278) which contains a C>T transition. However, no population frequency data are available for this allele. Moreover, a transition (where ring structure remains constant) is less likely to impair the spliceosome mechanism and result in donor splice site disruption compared to a transversion (where ring structure changes). Interestingly, a convergent functional genomics study had previously linked increased *DCTN1* gene expression with highly delusional states[18]. How this relates to psychosis associated with BD is currently unknown.

The *DCTN1* gene codes for the p150^{Glued} protein that is highly expressed in mammalian brain and is an essential component of the dynactin complex, which plays a vital role in retrograde axonal transport, microtubule-based transport, cell division, and autophagy[19,20]. In this manuscript we specifically discuss autophagy in the context of neuronal autophagy. *DCTN1* is located on 2p13.1, which is within a region previously found to show significant linkage in autosomal dominant models for BD (2p13-16)[21] and schizophrenia (2p13-14)[22]. *DCTN1* also lies within a region showing suggestive linkage for BD (2p11-q14)[23], as well as significant linkage for MDD (2p11.2 -p13.2)[24]. Rare autosomal-dominant disease-causing variants in *DCTN1* are already known for Perry syndrome (PS)[25], motor neuron disease (MND)[26], and distal spinal and bulbar muscular dystrophy[27]. Psychiatric symptoms, which include severe depression, are a noted feature of PS, which is characterized by onset of atypical Parkinson disease (PD)[25]. These variants result in disruption of retrograde axonal transport and neuronal autophagy. The variants are point mutations affecting the microtubule binding region towards the N-terminus, which contrasts with our research findings in which the three affected probands are shown to possess a novel variant that is predicted to affect the C-terminus (Figs. 2,3). Of note, there is no historical record of PS, MND, any muscular dystrophy, or PD in the extended family under investigation.

The novel splice site variant discovered in the current research is analogous to the heterozygous dominant-negative *Gl*^{1} mutation in the *Drosophila Glued* gene, which is the functional analogue of vertebrate *DCTN1*[28-31]. Homozygous mutations were found to be lethal in *Drosophila*. The *Drosophila* transposon-induced mutation results in a truncated transcript, which in turn results in a truncated p150^{Glued} protein missing the C-terminus (ct-p150^{Glued})[32]. The ensuing ct-p150^{Glued} fails to assemble into the dynactin complex with resulting deficits in retrograde axonal transport and neuronal autophagy[33,34]. Further evidence from recent structural biology work on dynein/dynactin complexes confirms that ct-p150^{Glued} would be unable to assimilate into the dynactin complex as the C-terminus normally anchors p150^{Glued} to the rest of the dynactin complex (Fig. 3)[35]. *Glued Gl*^{1} mutations result in a neurodegenerative rough-eye phenotype and cause deleterious central nervous system effects in *Drosophila*, disrupting synapse formation and maturation[28]. This finding is consistent with research demonstrating that the dynactin complex is known to stabilize synapses[36]. The novel *DCTN1* splice variant discovered in the current research is predicted to result in a PTC and a similar truncated protein missing the C-terminal region (Figs. 2,3). The ct-p150^{Glued} predicted as a result of the splice site variant would also include a predicted benign change V→L (V877L) due to the splice variant (Fig. 2).

If ct-p150^{Glued} is indeed produced in humans, it would be contingent on the truncated *DCTN1* transcript escaping NMD. As the PTC lies internally and is >55nt from the last exon junction it should be a very strong candidate to trigger NMD[37]. However, a ct-p150^{Glued} has previously been documented in human specimens, implying that alternative splicing in this region of *DCTN1* may be under dynamic control. Fujiwara et al. discovered substantial amounts of ct-p150^{Glued} in all investigated brain specimens obtained from Alzheimer disease (AD) patients (10-70% of total p150^{Glued})[38]. ct-p150^{Glued} was not readily observable in normal brain[38]. Although their sample size was small (n = 3), the finding of Fujiwara et al. does, nonetheless, importantly confirm that a ct-p150^{Glued} can exist in substantial amounts *in vivo* and may represent an important feature of AD. AD is also strongly associated with psychiatric disorders with psychosis being a common event[39]. Conversely, BD patients have a significantly higher risk of developing dementia later in life[40]. As a relationship between glutamate excitoxicity and AD has been widely reported[41], Fujiwara et al suspected that this may be the cause of the observed accumulation of ct-p150^{Glued} in AD brains. They subsequently demonstrated that glutamate excitotoxicity in primary neuronal cultures similarly resulted in the production of ct-p150^{Glued}, strongly inferring a causal relationship[38]. As was the case for *Drosophila*, this truncated protein was unable to assemble into the dynactin complex and interact with cargoes (β-amyloid protein in the case of human ct-p150^{Glued}), and thus represents a loss of function, disrupting retrograde axonal transport and neuronal autophagy[42]. As glutamate excitotoxicity is coupled with increased intracellular calcium concentrations[43] it is possible that the formation of ct-p150^{Glued} is related to impaired NMD, given that increased intracellular calcium concentrations are an important negative regulator of NMD[44]. Similarly, and with reference to this current research, disturbances of excitatory glutamatergic signaling have also been implicated in affective disorders[45]. Indeed, many prophylactic drugs used to treat BD patients are known to modulate glutamatergic signaling[46]. Our current findings suggest an increased sensitivity to the effects of glutamatergic signaling may occur in the research cohort, which would reinforce alternative splicing in this region of the *DCTN1* gene.

For this variant, and the predicted resulting formation of ct-p150^{Glued} and/or NMD, to be considered with any seriousness as being causally related to BD it would have to satisfy a major requirement: it would need to be strongly correlated with BD prophylactic drugs, antipsychotics, and antidepressants, all of which are commonly used in the treatment of BD. As discussed above, impaired retrograde axonal transport and neuronal autophagy are already known to be associated with ct-p150^{Glued}. Similarly, an increased rate of loss of *DCTN1* mRNA transcripts by NMD would most likely downregulate p150^{Glued} expression with similar autophagic deficits. In this regard, previous ground-breaking research has definitively linked BD prophylactic drugs to neuronal autophagy[47].

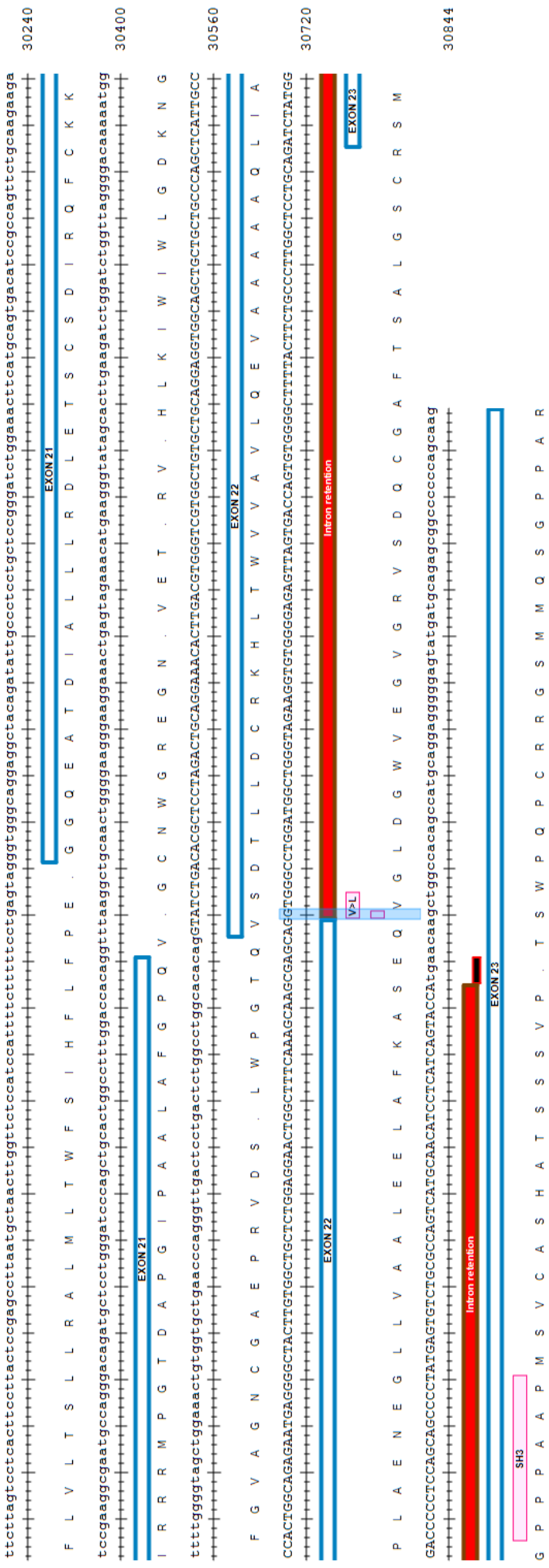

Figure 2. The region of the *DCTN1* gene illustrating the splice site variant (NG_008735.2: g.30630G>T) which is predicted to disrupt the invariant +1 splice donor site resulting in intron retention and a premature termination codon (PTC). The transcript can be degraded by NMD and/or be translated to form a C-truncated p150^{Glued} protein. The exons/introns in this region are short which is characteristic of alternatively spliced regions. The alternatively spliced *DCTN1* transcript variant 7 (NR_033935.1) also codes for a C-truncated protein, with the same PTC, and is sufficiently abundant to be included as a Refseq record. However, the expected protein is not represented, and it is predicted to undergo NMD. The new C-terminus tail includes an amino acid variant with a predicted benign change V>L (V877L) and is characterized by a SH3 polyproline II helix binding motif [PPPxxP] which implies that the C-terminal truncated p150^{Glued} protein may have a physiological role.

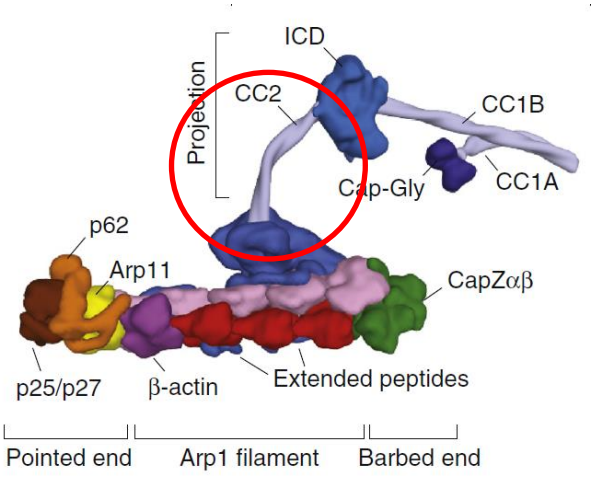

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Figure 3. The structure of dynactin illustrating the region (circled in red) predicted to be missing as a result of the splice site variant. The long projection of p150^{Glued} is anchored to the rest of the dynactin complex by the C-terminal domain. Thus, ct-p150^{Glued} would be unable to assimilate into the dynactin complex. In contrast, disease-causing variants responsible for PS and MND are toward the N-terminal and primarily affect the region around the Cap-Gly microtubule-binding domain.

The BD prophylactic drugs lithium, valproate, and carbamazepine have all been shown to enhance neuronal autophagy and thus would compensate for any deficits that are predicted to result from this variant. They achieve this by a common mechanism, namely via negatively regulating phosphoinositide signaling through depletion of inositol and by reducing myo-inositol-1,4,5-trisphosphate (IP_3)[47,48]. Apart from the aforementioned psychotropic agents, the majority of antipsychotics[49,50] as well as antidepressants[51,52] are also known to enhance neuronal autophagy. In addition, the autophagy-inducers rapamycin and trehalose have both been shown to have therapeutic effects in rodent models of affective disorders[53,54]. All the previously mentioned compounds are also under active consideration as therapeutic options for use in neurodegenerative disorders, by their ability to enhance retrograde axonal transport and neuronal autophagy, and to enhance clearance of aggregated and toxic protein[50,55].

At least two different mouse models are known which suggest that deficits in neuronal autophagy may be of critical importance in the etiology of affective disorders. Reduced expression of the lysosomal aspartyl protease cathepsin D (CTSD) is known to impair neuronal autophagy[56]. In this regard, heterozygous CTSD-deficient mice exhibit BD characteristics, such as mania-related behavior and stress-induced depression[57]. Importantly, chronic administration of the BD prophylactic drugs, lithium and valproate, were found to ameliorate their condition and thus strongly suggest a causal relationship. In a similar manner to CTSD-deficient mice, knockdown of *DCTN5* in a murine model also results in abnormal mania-related hyperactivity[58]. *DCTN5* codes for the p25 subunit of dynactin and thus like p150^{Glued} it is intimately involved in retrograde axonal transport and neuronal autophagy. It is also considered a susceptibility gene for BD[59]. Furthermore, deficits in neuronal autophagy are also common to lysosomal storage disorders[60], with psychiatric symptoms noted in many of these disorders[61]. For example, mania and/or psychosis has been noted in the adult forms of metachromatic leukodystrophy[62], Niemann-Pick type C disease[63] and Tay-Sachs disease[64]. Axonal transport, and associated neuronal autophagy, is known to decline with age with two distinct periods of decline, in early adulthood and later in life[65]**.** Moreover, these findings correlate with peaks for the age of onset for BD and MDD alluded to previously, thus further inferring a causal relationship between decreased autophagic flux and the disorders.

No discussion regarding BD could be considered complete without taking into account protein kinase C (PKC) and its possible correlation with the current research. PKC has often been suggested as playing a key role in BD, as both lithium and valproate inhibit PKC signaling[66]. Thus,

inhibitors of PKC have been considered as potential therapeutic agents[67]. In this regard, the selective PKC inhibitor chelerythrine, and the anti-estrogenic PKC inhibitor tamoxifen, have both been shown to have anti-manic effects in a murine model of mania[68]. Tamoxifen has also been shown to be effective in human trials for the treatment of mania[69]. PKCs are also known to suppress autophagy[70], and that includes *Drosophila* atypical PKC (aPKC)[71]. aPKCs differ from other PKC isoforms in that they are not dependent on calcium or diacylglycerol for their activity, but can nonetheless be activated by IP_3[72]. The neurodegenerative phenotype resulting from the *Drosophila GLUED Gl*^{1} mutation (which results in ct-p150^{Glued}) has been shown to be rescued by mutant alleles of aPKC, thus implying that factors that negatively regulate aPKC activation (which includes reduced IP_3 levels) alleviate the mutant phenotype[73]. Given the analogy with the observations of ct-p150^{Glued} in AD brain samples discussed previously, and its predicted formation in the BD research cohort, this finding may provide some indication of the biochemical mechanisms involved in the beneficial effects of therapeutic interventions which negatively regulate IP_3 signaling and PKC.

A *DCTN1* mRNA transcript of very low abundance is known (*DCTN1* transcript variant 7, Refseq ID: NR_033935.1), which reflects low-level alternative splicing. This transcript is predicted to generate ct-p150^{Glued} and/or trigger NMD of the truncated transcript. The *DCTN1* exon 22/intron 22 region has characteristics that are common to many alternatively spliced regions, namely short exons with short flanking introns[74] and G/C-rich regions typical of *cis*-regulatory elements[75] (Fig. 2). Possibly, tissue-specific ct-p150^{Glued} is normally produced and maintained at low levels by NMD, with its formation being dependent on transient neuroexcitatory factors. The ct-p150^{Glued} predicted to occur in the current research contains a new exposed C-terminal sequence, with a typical SH3 polyproline II binding motif (PPPXXP) (Fig. 2). Proline-rich regions such as these play an important role in protein-protein interactions which are central to cellular signaling[76]. This suggests that ct-p150^{Glued} may have a physiological regulatory role, quite apart from the expected deleterious effects related to aberrant neuronal autophagy.

There is a strong likelihood that a transversion at the invariant +1 position of the splice donor site would disrupt splicing, as evidenced by previous examples of splice donor site transversions resulting in a disease phenotype (e.g. splice site disruption leading to hereditary thrombocythemia[77], in congenital afibrinogenemia[78], and mutations in CHD7 leading to CHARGE syndrome[79]). However, further research is required to validate the exact physiological effects of the splice site variant discovered in the current research cohort, and the relative contributions of NMD and ct-p150^{Glued} formation. Of note is the fact that NMD demonstrates widely differing heritable efficiencies[80], and also tissue-specific differences[81]. This may explain, at least in part, the phenotypical differences noted in the research cohort. The exon/intron 22 region of the *DCTN1* gene, as well as factors that affect splicing efficiency, and alternative splicing, in this region deserve further intense scrutiny in psychiatric disorders. It would also be highly desirable to identify this splice variant in other affected families to further validate its significance and to obtain an estimate of relative frequency. We believe that our findings suggest that a single variant alone (in *DCTN1*), and not cumulative polygenic variants, may be responsible for the etiology of the disease phenotype in the research cohort under investigation. This variant is not only co-segregating, novel, and in a highly evolutionary conserved region, but it is also predicted to be deleterious. Taken together all these qualities strongly infer pathogenicity. Furthermore, the neuronal autophagy deficits that are predicted to result are consistent with established knowledge regarding the therapeutic interventions used in BD and MDD. Previously discovered disease-causing variants (e.g. PS, NMD) in *DCTN1* are inherited in an autosomal-dominant fashion. Disease-causing variants in PS, which results in atypical PD, are already known to also result in severe depression. Thus, there is a strong likelihood that the variant uncovered in the present research may also reflect a primary autosomal dominant cause of psychiatric pathology in the research cohort.

As noted above, a ct-p150^{Glued}, which is predicted to result from the splice site variant discovered in this research, has also been found in AD brain samples as well as in *Drosophila* mutants. In both cases it is related to deficits in neuronal autophagy. Furthermore, deficits in neuronal autophagy are implied as being causal in at least two different murine models of affective disorders. As noted previously, axonal transport and associated neuronal autophagy also slows at ages that are also consistent with onset of illness in both BD as well as recurrent MDD. In addition, lysosomal storage diseases, which are characterized by deficits in neuronal autophagy, are also associated with psychiatric illness. Therefore, this novel co-segregating splice site variant, which is predicted to impair neuronal autophagy, may also hold the key to the understanding of the pathological biochemical mechanisms involved in BD and MDD in general. Neuronal autophagy plays an essential role in maintaining neuronal integrity, and it is thus also likely that secondary genetic compensation would occur as an attempt to maintain it, albeit with perhaps additional psychiatric repercussions.

Methods

Whole exome sequencing (WES) and/or whole genome sequencing (WGS) was used in the present study, which includes genetic analyses of three affected subjects, twelve related unaffected controls (RC) and one unaffected unrelated control (Fig. 1, Table S1). Effective use was made of numerous related controls to narrow the search for candidate genes by filtering out confounding variants present in affected subjects as well as in controls. Apart from the three affected subjects, there were no other living individuals with a psychiatric diagnosis in the extended family. Genomic DNA was extracted from whole blood samples using accepted published protocols and submitted to the relevant sequencing centers for sequencing (Table S1). 100bp Paired-end reads were used. To maximize sequencing coverage, the DNA from the three living core family members under investigation (Fig. 1: #1-5) was sequenced more than once and at different times using WGS/WES (Table S1). The remainder of the DNA samples, which were from negative controls, were only sequenced once using WES (Table S1). For WES the following Exome enrichment kits were used: 1) Illumina Nextera Rapid Capture Expanded Exome (62 Mbp which includes exons, 5'UTRs, 3'UTRs, microRNAs and non-coding RNAs), 2) Agilent SureSelect All Exome V5 (50.4 Mbp), and 3) Agilent SureSelect All Exome V6 (58 Mbp). WGS was performed using Illumina HiSeq 2000 (KNOME Inc. (Cambridge, Massachusetts, USA) and Illumina (San Diego)), and WES performed using Illumina 2000 (Ramaciotti Sequencing Centre, Australia), and Illumina HiSeq 4000, (Macrogen, South Korea). NGS was performed by KNOME Inc. (Cambridge, Massachusetts, USA) and Illumina (San Diego), Ramaciotti Centre for Genomics (UNSW, Sydney, Australia), and Macrogen (Seoul, South Korea).

Analytical strategy:

As the family pedigree suggests autosomal dominant inheritance is most likely (Fig. 1), the strategy used herein was to search for novel co-segregating variants because these variants would be the most likely to be disease-causing. This strategy has consistently been shown to have validity[82,83]. All bioinformatic analyses were performed using the DNASTAR Lasergene Full Suite (DNASTAR, Madison, Wisconsin, USA, v15.1). Raw genomic data was aligned, and variants called and annotated using SeqMan NGen. Comparative analysis was performed using ArrayStar.

Genomic alignment: Genomic raw FASTQ data was aligned to the GRCh37.p13-dbSNP150 genome template (pre-configured by DNASTAR). Reads were auto trimmed prior to alignment and a mer size of 21nt and minimum match percentage of 93% was used. The relevant manufacturer targeted region BED files were used for WES. Variants were restricted to coding and splice regions.

As a check for consistency, genomic data were analyzed using two different protocols. Genomic alignment was performed using both "low" and "high" stringency alignment settings. Protocol 1 was

used to maximize true positive calls, whereas protocol 2 was used to minimize the false discovery rate. Both protocols nonetheless resulted in an identical outcome.

1a) ***Protocol 1***. The default "low" stringency alignment settings were used for controls as well as for affected individuals and duplicate reads were combined (minimum alignment length = 35, minimum layout length = 21, maximum gap size = 30). A minimal variant call filter of the probability of not being reference = 90% was applied. Functional prediction filtering was applied where only variants that were predicted to be deleterious in at least one of the following bioinformatic resources included in ArrayStar were retained; LRT[84], MutationTaster[84], or SIFT[85].

1b) ***Protocol 2***. The samples from affected individuals were aligned using the default "high" stringency alignment settings (minimum alignment length = 35, minimum layout length = 50, maximum gap size = 30), and clonal duplicate reads were removed. The default "low" stringency alignment settings were used as above for controls. The following variant call filters were applied: a) the probability of not being reference = 99.9%, b) minimum variant percentage = 15%, c) minimum depth = 20, d) minimum base quality score = 20. No functional prediction filters were used.

2) The variants from the Genome Aggregation Database (gnomAD)[86] were loaded into ArrayStar and all variant calls included in gnomAD were filtered.

3) All variants present in the DNASTAR human variant annotation database (1000 Genomes, dbNSFP, and the Exome Variant Server) were also filtered.

4) Using the Venn diagram feature of ArrayStar only co-segregating variants common to all three affected subjects and not present in any of the controls were retained. This resulted in the discovery of a single novel co-segregating variant in *DCTN1*.

Bioinformatic predictions: prediction of the pathogenicity of the *DCTN1* splice variant was performed using the following bioinformatic resources: CADD[87], DANN[88], FATHMM-XF[89], and MutationTaster[90] (Table S2). Prediction of genomic conservation was determined using GERP++RS[87], PhastCons100way_vertebrate[91] and PhyloP100way_vertebrate[91] from within ArrayStar (Table S3).

Sanger validation: Samples #1-7 were also further validated by Sanger sequencing for the presence/absence of the novel *DCTN1* splicing variant (Fig. S2). These samples covered all the core family under investigation, including all three with a psychiatric diagnosis (Fig. 1, #1,3,4). The following PCR primers were used for Sanger sequencing validation of the *DCTN1* splice variant: Forward-primer 5'-TCATACTCCCCCTCCTGCAT-3'; Reverse-primer 5'-AATGAGGGGCTACTTGTGGC-3'. The forward primer was used in Sanger sequencing. Quality and quantity of the extracted DNA were checked by gel electrophoresis and Qubit dsDNA BR Assay Kit (Life Technologies). To evaluate the probability of DNA degradation, gel electrophoresis was carried out by loading 10 µl (500 ng) of extracted DNA onto a 1% agarose gel. PCR was performed in a 100 µL volume, consisting of: 10 µL Qiagen PCR Buffer (10X), 2 µL of 10 mM dNTPs, primer sets, 0.5 µL of Taq DNA polymerase, and 4 µL (200 ng) of genomic DNA. The amplification program consisted of one initial denaturation at 94°C for 3 min followed by 30 cycles of 30s at 94°C for denaturation, 30s at 65°C for primer annealing, 30s at 72°C for extension and final extension at 72°C for 5 min. Products were separated on a 1% SYBR Safe agarose gel and visualized by Safe Imager™ 2.0 Blue Light Transilluminator (Thermo Fisher).

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Supplementary data

Fig. S1. Sanger sequencing chromatograms

Fig. S2. $p150^{Glued}$ protein sequence illustrating domains and regions

Table S1. Variant counts for the *DCTN1* splice variant

Table S2. Bioinformatic predictions for the *DCTN1* splice variant

Table S3. Genomic conservation data for the *DCTN1* splice variant

The authors have nothing to declare.

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