

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

André Hallen¹ and Arthur J.L. Cooper²

¹André Hallen, Ryde, Sydney, NSW 2112, Australia.

²Arthur J. L. Cooper, Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, New York 10595, USA.

Corresponding authors

André Hallen, andrehallen@yahoo.com.au. Arthur J.L. Cooper, arthur_cooper@nymc.edu.

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* *G^I* 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; IP₃, 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 individuals¹. 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 BDI². 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 unemployment³. 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}.

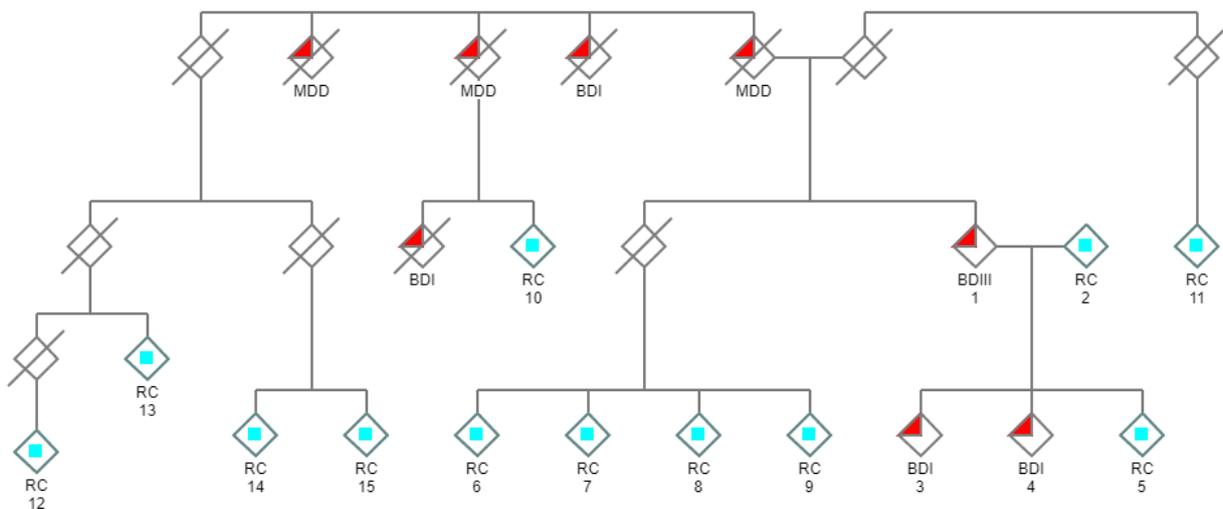


Figure 1. Pedigree of the family under investigation. Members of the core family (#1-5) were each sequenced more than once using whole genome sequencing (WGS) and/or whole exome sequencing (WES) as opposed to the remainder of the related living controls who were only sequenced once with WES. Individuals 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 families¹⁰. The age of onset of mania in BDI patients shows a peak in early adulthood in a 35 year UK study¹¹. 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 life¹². Similarly, MDD patients having a family history of MDD, and experiencing recurrent depressive episodes, also exhibit a peak for age of onset in early adulthood¹³.

Many genes and chromosomal regions have been implicated in BD. However, to this date no single gene defect alone has been shown to be causative¹⁴. 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 database¹⁷ (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 states¹⁸. 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 dystrophy²⁷. 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 Gl¹* mutations result in a neurodegenerative rough-eye phenotype and cause deleterious central nervous system effects in *Drosophila*, disrupting synapse formation and maturation²⁸. This finding is consistent with research demonstrating that the dynactin complex is known to stabilize synapses³⁶. 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 NMD³⁷. 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 brain³⁸. 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 event³⁹. Conversely, BD patients have a significantly higher risk of developing dementia later in life⁴⁰. As a relationship between glutamate excitotoxicity and AD has been widely reported⁴¹, 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 relationship³⁸. 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 autophagy⁴². As glutamate excitotoxicity is coupled with increased intracellular calcium concentrations⁴³ 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 NMD⁴⁴. Similarly, and with reference to this current research, disturbances of excitatory glutamatergic signaling have also been implicated in affective disorders⁴⁵. Indeed, many prophylactic drugs used to treat BD patients are known to modulate glutamatergic signaling⁴⁶. 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 autophagy⁴⁷.

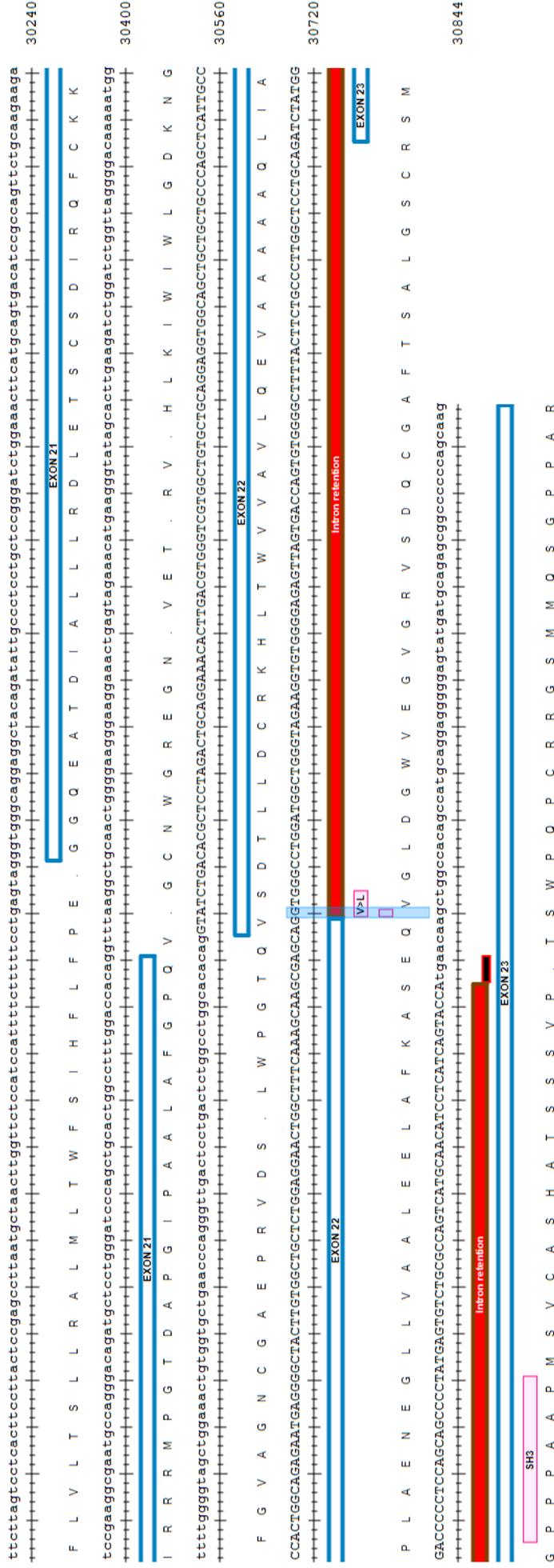
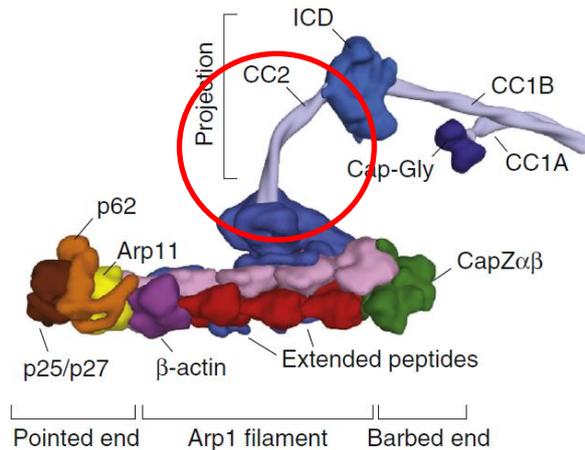


Figure 2. The region of the *DCTN1* gene illustrating the splice site variant (NG_008735.2: g.30630G>T) which is predicted to disrupt the invariant +1 splice donor site resulting in intron retention and a premature termination codon (PTC). The transcript can be degraded by NMD and/or be translated to 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.



Reproduced from [35] with permission by the authors and Publisher.

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 (IP₃)^{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 autophagy⁵⁶. In this regard, heterozygous CTSD-deficient mice exhibit BD characteristics, such as mania-related behavior and stress-induced depression⁵⁷. 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 hyperactivity⁵⁸. *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 BD⁵⁹. Furthermore, deficits in neuronal autophagy are also common to lysosomal storage disorders⁶⁰, with psychiatric symptoms noted in many of these disorders⁶¹. For example, mania and/or psychosis has been noted in the adult forms of metachromatic leukodystrophy⁶², Niemann-Pick type C disease⁶³ and Tay-Sachs disease⁶⁴. Axonal transport, and associated neuronal autophagy, is known to decline with age with two distinct periods of decline, in early adulthood and later in life⁶⁵. 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 signaling⁶⁶. Thus,

inhibitors of PKC have been considered as potential therapeutic agents⁶⁷. 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 mania⁶⁸. Tamoxifen has also been shown to be effective in human trials for the treatment of mania⁶⁹. PKCs are also known to suppress autophagy⁷⁰, 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 IP₃⁷². The neurodegenerative phenotype resulting from the *Drosophila* *GLUED* *Gl^l* 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 IP₃ levels) alleviate the mutant phenotype⁷³. 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 IP₃ 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 introns⁷⁴ and G/C-rich regions typical of *cis*-regulatory elements⁷⁵ (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 signaling⁷⁶. 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 thrombocythemia⁷⁷, in congenital afibrinogenemia⁷⁸, and mutations in *CHD7* leading to CHARGE syndrome⁷⁹). 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 efficiencies⁸⁰, and also tissue-specific differences⁸¹. 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; LRT⁸⁴, MutationTaster⁸⁴, or SIFT⁸⁵.

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: CADD⁸⁷, DANN⁸⁸, FATHMM-XF⁸⁹, and MutationTaster⁹⁰ (Table S2). Prediction of genomic conservation was determined using GERP++RS⁸⁷, PhastCons100way Vertebrate⁹¹ and PhyloP100way Vertebrate⁹¹ 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).

Funding

This research was partially funded by a small grant from Bioplatforms Australia, partially self-funded by AH, and partially funded through a crowd funding campaign on GoFundMe.

Acknowledgements

In accordance with standard ethical practice all participants who were genetically tested provided their signed informed consent.

Prof. Ole A. Andreassen (Psychosis Research Centre, Oslo University, Norway) and Dr. Srdjan Djurovic (Dept. Medical genetics, Oslo University Hospital, Norway) are thanked for sample collection from unaffected controls, subsequent genomic DNA extraction and assembly of informed consent forms, as well as shipment of the relevant DNA samples for subsequent sequencing.

Andrew Carter (U. Cambridge, UK) is thanked for permission to use his graphic depicting the structure of the dynactin complex.

The following are thanked for providing valuable feedback and/or proof-reading during the preparation of the manuscript: Jenny Vo (Teva Pharmaceuticals, Australia), Jan Fullerton (UNSW, Australia), Steve Ambrose (Ambrose Ecological, Australia), Philip Kuchel (U. Sydney, Australia), Fei Liu (Macquarie U., Australia), Daria Mochly-Rosen (Stanford U., USA), and Andrew Carter (U. Cambridge, UK).

Guy Plunkett III and Eric Ma are thanked for providing DNASTAR Lasergene software technical support (DNASTAR, Madison, Wisconsin, USA).

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.

References

1. de la Vega, D., Pina, A., Peralta, F.J., Kelly, S.A. & Giner, L. A review on the general stability of mood disorder diagnoses along the lifetime. *Curr. Psychiatry Rep.* **20**, 29 (2018).
2. Van Meter, A.R., Youngstrom, E.A. & Findling, R.L. Cyclothymic disorder: a critical review. *Clin. Psychol. Rev.* **32**, 229-243 (2012).
3. Kleine-Budde, K. *et al.* Cost of illness for bipolar disorder: a systematic review of the economic burden. *Bipolar Disord.* **16**, 337-353 (2014).

4. Boardman, A.P. & Healy, D. Modelling suicide risk in affective disorders. *Eur. Psychiatry* **16**, 400-405 (2001).
5. Marangoni, C., Hernandez, M. & Faedda, G.L. The role of environmental exposures as risk factors for bipolar disorder: A systematic review of longitudinal studies. *J. Affect. Disord.* **193**, 165-174 (2016).
6. Uher, R. Gene-environment interactions in severe mental illness. *Front. Psychiatry* **5**, 48 (2014).
7. Merikangas, K.R. *et al.* Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Arch. Gen. Psychiatry* **68**, 241-251 (2011).
8. Patten, S.B. *et al.* The prevalence of major depression is not changing. *Can. J. Psychiatry.* **60**, 31-34 (2015).
9. Barnett, J.H. & Smoller, J.W. The genetics of bipolar disorder. *Neuroscience* **164**, 331-343 (2009).
10. Smith, D.J., Harrison, N., Muir, W. & Blackwood, D.H. The high prevalence of bipolar spectrum disorders in young adults with recurrent depression: toward an innovative diagnostic framework. *J. Affect. Disord* **84**, 167-178 (2005).
11. Kennedy, N. *et al.* Incidence and distribution of first-episode mania by age: results from a 35-year study. *Psychol. Med.* **35**, 855-863 (2005).
12. Kroon, J.S. *et al.* Incidence rates and risk factors of bipolar disorder in the general population: a population-based cohort study. *Bipolar Disord.* **15**, 306-313 (2013).
13. Tozzi, F. *et al.* Family history of depression is associated with younger age of onset in patients with recurrent depression. *Psychol. Med.* **38**, 641-649 (2008).
14. Budde, M. *et al.* [Genetics of bipolar disorder]. *Nervenarzt* **88**, 755-759 (2017).
15. Gershon, E.S. *et al.* A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands. *Arch. Gen. Psychiatry* **39**, 1157-1167 (1982).
16. Gershon, E.S. *et al.* A controlled family study of chronic psychoses. Schizophrenia and schizoaffective disorder. *Arch. Gen. Psychiatry* **45**, 328-336 (1988).
17. Sherry, S.T. *et al.* dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* **29**, 308-311 (2001).
18. Kurian, S.M. *et al.* Identification of blood biomarkers for psychosis using convergent functional genomics. *Mol. Psychiatry* **16**, 37-58 (2011).
19. Karki, S. & Holzbaur, E.L. Cytoplasmic dynein and dynactin in cell division and intracellular transport. *Curr. Opin. Cell Biol.* **11**, 45-53 (1999).
20. Melloni, R.H., Jr., Tokito, M.K. & Holzbaur, E.L. Expression of the p150Glued component of the dynactin complex in developing and adult rat brain. *J. Comp. Neurol.* **357**, 15-24 (1995).
21. Liu, J. *et al.* Evidence for a putative bipolar disorder locus on 2p13-16 and other potential loci on 4q31, 7q34, 8q13, 9q31, 10q21-24, 13q32, 14q21 and 17q11-12. *Mol. Psychiatry* **8**, 333-342 (2003).
22. Coon, H. *et al.* Evidence for a chromosome 2p13-14 schizophrenia susceptibility locus in families from Palau, Micronesia. *Mol. Psychiatry* **3**, 521-527 (1998).
23. Goes, F.S. *et al.* Mood-incongruent psychotic features in bipolar disorder: familial aggregation and suggestive linkage to 2p11-q14 and 13q21-33. *Am. J. Psychiatry* **164**, 236-247 (2007).
24. Bulayeva, K. *et al.* [Mapping genes related to early onset major depressive disorder in Dagestan genetic isolates]. *Turk Psikiyatri Derg* **23**, 161-170 (2012).
25. Perry, T.L. *et al.* Hereditary mental depression and Parkinsonism with taurine deficiency. *Arch. Neurol.* **32**, 108-113 (1975).
26. Munch, C. *et al.* Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology* **63**, 724-726 (2004).
27. Puls, I. *et al.* Distal spinal and bulbar muscular atrophy caused by dynactin mutation. *Ann. Neurol.* **57**, 687-694 (2005).

28. Allen, M.J. *et al.* Targeted expression of truncated glued disrupts giant fiber synapse formation in *Drosophila*. *J. Neurosci.* **19**, 9374-9384 (1999).
29. Waterman-Storer, C.M. & Holzbaaur, E.L. The product of the *Drosophila* gene, Glued, is the functional homologue of the p150Glued component of the vertebrate dynactin complex. *J. Biol. Chem.* **271**, 1153-1159 (1996).
30. Garen, A., Miller, B.R. & Paco-Larson, M.L. Mutations affecting functions of the *Drosophila* gene glued. *Genetics* **107**, 645-655 (1984).
31. Harte, P.J. & Kankel, D.R. Genetic analysis of mutations at the Glued locus and interacting loci in *Drosophila melanogaster*. *Genetics* **101**, 477-501 (1982).
32. Swaroop, A., Paco-Larson, M.L. & Garen, A. Molecular genetics of a transposon-induced dominant mutation in the *Drosophila* locus Glued. *Proc. Natl. Acad. Sci U S A.* **82**, 1751-1755 (1985).
33. McGrail, M. *et al.* Regulation of cytoplasmic dynein function in vivo by the *Drosophila* Glued complex. *J. Cell. Biol.* **131**, 411-425 (1995).
34. Fan, S.S. & Ready, D.F. Glued participates in distinct microtubule-based activities in *Drosophila* eye development. *Development* **124**, 1497-1507 (1997).
35. Carter, A.P., Diamant, A.G. & Urnavicius, L. How dynein and dynactin transport cargos: a structural perspective. *Curr. Opin. Struct. Biol.* **37**, 62-70 (2016).
36. Eaton, B.A., Fetter, R.D. & Davis, G.W. Dynactin is necessary for synapse stabilization. *Neuron* **34**, 729-741 (2002).
37. Maquat, L.E. Nonsense-mediated mRNA decay in mammals. *J. Cell Sci.* **118**, 1773-1776 (2005).
38. Fujiwara, T., Morimoto, K., Kakita, A. & Takahashi, H. Dynein and dynactin components modulate neurodegeneration induced by excitotoxicity. *J. Neurochem.* **122**, 162-174 (2012).
39. Morgan, S. Psychotic and bipolar disorders: Behavioral disorders in dementia. *FP Essentials* **455**, 18-22 (2017).
40. Diniz, B.S. *et al.* History of bipolar disorder and the risk of dementia: A systematic review and meta-analysis. *Am. J. Geriatr. Psychiatry* **25**, 357-362 (2017).
41. Lewerenz, J. & Maher, P. Chronic glutamate toxicity in neurodegenerative diseases-what is the evidence? *Front. Neurosci.* **9**, 469 (2015).
42. Fujiwara, T. & Morimoto, K. Cooperative effect of p150Glued and microtubule stabilization to suppress excitotoxicity-induced axon degeneration. *Bioch. Biophys. Res. Commun.* **424**, 82-88 (2012).
43. Choi, D.W. Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neurosci. Lett.* **58**, 293-297 (1985).
44. Nickless, A. *et al.* Intracellular calcium regulates nonsense-mediated mRNA decay. *Nat. Med.* **20**, 961-966 (2014).
45. Jun, C. *et al.* Disturbance of the glutamatergic system in mood disorders. *Exp. Neurobiol.* **23**, 28-35 (2014).
46. Rapoport, S.I., Basselin, M., Kim, H.W. & Rao, J.S. Bipolar disorder and mechanisms of action of mood stabilizers. *Brain Res. Rev.* **61**, 185-209 (2009).
47. Sarkar, S. & Rubinsztein, D.C. Inositol and IP3 levels regulate autophagy: biology and therapeutic speculations. *Autophagy* **2**, 132-134 (2006).
48. Tritsarlis, K., Gromada, J., Jorgensen, T.D., Nauntofte, B. & Dissing, S. Reduction in the rate of inositol 1,4,5-trisphosphate synthesis in rat parotid acini by lithium. *Arch. Oral. Biol.* **46**, 365-373 (2001).
49. Li, Y. *et al.* A cell-based quantitative high-throughput image screening identified novel autophagy modulators. *Pharmacol. Res.* **110**, 35-49 (2016).
50. Sarkar, S. & Rubinsztein, D.C. Small molecule enhancers of autophagy for neurodegenerative diseases. *Mol. Biosyst.* **4**, 895-901 (2008).

51. Zschocke, J. & Rein, T. Antidepressants encounter autophagy in neural cells. *Autophagy* **7**, 1247-1248 (2011).
52. Zschocke, J. *et al.* Antidepressant drugs diversely affect autophagy pathways in astrocytes and neurons--dissociation from cholesterol homeostasis. *Neuropsychopharmacology* **36**, 1754-1768 (2011).
53. Kara, N.Z. *et al.* Trehalose induced antidepressant-like effects and autophagy enhancement in mice. *Psychopharmacology (Berl)* **229**, 367-375 (2013).
54. Kara, N.Z., Flaisher-Grinberg, S., Anderson, G.W., Agam, G. & Einat, H. Mood-stabilizing effects of rapamycin and its analog temsirolimus: relevance to autophagy. *Behav. Pharmacol.* **29**, 379-384 (2018).
55. Ravikumar, B., Sarkar, S. & Rubinsztein, D.C. Clearance of mutant aggregate-prone proteins by autophagy. *Methods Mol. Biol.* **445**, 195-211 (2008).
56. Sarkar, C. *et al.* Impaired autophagy flux is associated with neuronal cell death after traumatic brain injury. *Autophagy* **10**, 2208-2222 (2014).
57. Zhou, R. *et al.* Mice heterozygous for cathepsin D deficiency exhibit mania-related behavior and stress-induced depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **63**, 110-118 (2015).
58. MacLaren, E.J., Charlesworth, P., Coba, M.P. & Grant, S.G. Knockdown of mental disorder susceptibility genes disrupts neuronal network physiology in vitro. *Mol. Cell. Neurosci.* **47**, 93-99 (2011).
59. Wellcome Trust Case Control, C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661-678 (2007).
60. Settembre, C. *et al.* A block of autophagy in lysosomal storage disorders. *Hum. Mol. Genet.* **17**, 119-129 (2008).
61. Staretz-Chacham, O., Choi, J.H., Wakabayashi, K., Lopez, G. & Sidransky, E. Psychiatric and behavioral manifestations of lysosomal storage disorders. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **153B**, 1253-1265 (2010).
62. Wender, M., Pruchnik-Wolinska, D., Paprzycki, W. & Czartoryska, B. [Familial metachromatic leukodystrophy as a cause of psychotic manifestations in young adults]. *Psychiatr. Pol.* **32**, 113-119 (1998).
63. Maubert, A., Hanon, C. & Metton, J.P. [Adult onset Niemann-Pick type C disease and psychosis: literature review]. *Encephale* **39**, 315-319 (2013).
64. MacQueen, G.M., Rosebush, P.I. & Mazurek, M.F. Neuropsychiatric aspects of the adult variant of Tay-Sachs disease. *J. Neuropsychiatry Clin. Neurosci.* **10**, 10-19 (1998).
65. Milde, S., Adalbert, R., Elaman, M.H. & Coleman, M.P. Axonal transport declines with age in two distinct phases separated by a period of relative stability. *Neurobiol. Aging* **36**, 971-981 (2015).
66. Saxena, A. *et al.* Role of Protein Kinase C in Bipolar Disorder: A Review of the Current Literature. *Mol. Neuropsychiatry* **3**, 108-124 (2017).
67. Zarate, C.A. & Manji, H.K. Protein kinase C inhibitors: rationale for use and potential in the treatment of bipolar disorder. *CNS Drugs* **23**, 569-582 (2009).
68. Abrial, E. *et al.* Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania. *Int. J. Neuropsychopharmacol.* **18** (2014).
69. Yildiz, A., Guleryuz, S., Ankerst, D.P., Ongur, D. & Renshaw, P.F. Protein kinase C inhibition in the treatment of mania: a double-blind, placebo-controlled trial of tamoxifen. *Arch. Gen. Psychiatry* **65**, 255-263 (2008).
70. Jiang, H., Cheng, D., Liu, W., Peng, J. & Feng, J. Protein kinase C inhibits autophagy and phosphorylates LC3. *Biochem. Biophys. Res. Commun.* **395**, 471-476 (2010).
71. Jin, A., Neufeld, T.P. & Choe, J. Kibra and aPKC regulate starvation-induced autophagy in *Drosophila*. *Biochem. Biophys. Res. Commun.* **468**, 1-7 (2015).

72. Xiao, H. & Liu, M. Atypical protein kinase C in cell motility. *Cell. Mol. Life Sci.* **70**, 3057-3066 (2013).
73. Ma, L., Johns, L.A. & Allen, M.J. A modifier screen in the Drosophila eye reveals that aPKC interacts with Glued during central synapse formation. *BMC Genet.* **10**, 77 (2009).
74. Sakabe, N.J. & de Souza, S.J. Sequence features responsible for intron retention in human. *BMC Genom.* **8**, 59 (2007).
75. Wang, Z. & Burge, C.B. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. *RNA* **14**, 802-813 (2008).
76. Adzhubei, A.A., Sternberg, M.J. & Makarov, A.A. Polyproline-II helix in proteins: structure and function. *J. Mol. Biol.* **425**, 2100-2132 (2013).
77. Liu, K. *et al.* A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. *Haematologica* **93**, 706-714 (2008).
78. Attanasio, C., de Moerloose, P., Antonarakis, S.E., Morris, M.A. & Neerman-Arbez, M. Activation of multiple cryptic donor splice sites by the common congenital afibrinogenemia mutation, FGA IVS4 + 1 G->T. *Blood* **97**, 1879-1881 (2001).
79. Villate, O. *et al.* Functional analyses of a novel splice variant in the chd7 gene, found by next generation sequencing, confirm its pathogenicity in a Spanish patient and diagnose him with CHARGE syndrome. *Front. Genet.* **9**, 7 (2018).
80. Seoighe, C. & Gehring, C. Heritability in the efficiency of nonsense-mediated mRNA decay in humans. *PLoS One* **5**, e11657 (2010).
81. Zetoune, A.B. *et al.* Comparison of nonsense-mediated mRNA decay efficiency in various murine tissues. *BMC Genet.* **9**, 83 (2008).
82. Cirulli, E.T. & Goldstein, D.B. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nature Rev. Genet.* **11**, 415-425 (2010).
83. Kryukov, G.V., Pennacchio, L.A. & Sunyaev, S.R. Most rare missense alleles are deleterious in humans: implications for complex disease and association studies. *Am. J. Hum. Genet.* **80**, 727-739 (2007).
84. Adzhubei, I.A. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* **7**, 248-249 (2010).
85. Kumar, P., Henikoff, S. & Ng, P.C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat. Protoc.* **4**, 1073-1081 (2009).
86. Lek, M. *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 285-291 (2016).
87. Davydov, E.V. *et al.* Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput. Biol.* **6**, e1001025 (2010).
88. Quang, D., Chen, Y. & Xie, X. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. *Bioinformatics* **31**, 761-763 (2015).
89. Rogers, M.F. *et al.* FATHMM-XF: accurate prediction of pathogenic point mutations via extended features. *Bioinformatics* **34**, 511-513 (2018).
90. Schwarz, J.M., Cooper, D.N., Schuelke, M. & Seelow, D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat. Methods* **11**, 361-362 (2014).
91. Pollard, K.S., Hubisz, M.J., Rosenbloom, K.R. & Siepel, A. Detection of nonneutral substitution rates on mammalian phylogenies. *Genome Res.* **20**, 110-121 (2010).