Molecular Psychiatry (2015) 20, 1212–1218

www.nature.com/mp

ORIGINAL ARTICLE

Disruption of sonic hedgehog signaling in Ellis-van Creveld dwarfism confers protection against bipolar affective disorder

EI Ginns[1], M Galdzicka[2], RC Elston[3], YE Song[3], SM Paul[4] and JA Egeland[5]

Ellis-van Creveld syndrome, an autosomal recessively inherited chondrodysplastic dwarfism, is frequent among Old Order Amish of Pennsylvania. Decades of longitudinal research on bipolar affective disorder (BPAD) revealed cosegregation of high numbers of EvC and Bipolar I (BPI) cases in several large Amish families descending from the same pioneer. Despite the high prevalence of both disorders in these families, no EvC individual has ever been reported with BPI. The proximity of the *EVC* gene to our previously reported chromosome 4p16 BPAD locus with protective alleles, coupled with detailed clinical observations that EvC and BPI do not occur in the same individuals, led us to hypothesize that the genetic defect causing EvC in the Amish confers protection from BPI. This hypothesis is supported by a significant negative association of these two disorders when contrasted with absence of disease ($P = 0.029$, Fisher's exact test, two-sided, verified by permutation to estimate the null distribution of the test statistic). As homozygous Amish *EVC* mutations causing EvC dwarfism do so by disrupting sonic hedgehog (Shh) signaling, our data implicate Shh signaling in the underlying pathophysiology of BPAD. Understanding how disrupted Shh signaling protects against BPI could uncover variants in the Shh pathway that cause or increase risk for this and related mood disorders.

Molecular Psychiatry (2015) **20,** 1212–1218; doi:10.1038/mp.2014.118; published online 14 October 2014

INTRODUCTION

Bipolar affective disorder (BPAD; manic-depressive illness) is a common psychiatric disorder with primary features of recurrence (cyclicity) and swings (polarity) from high to low of both mood and energy. Individuals with BPAD can shift from 'mania to melancholia' or from high affectivity and excitement to the profound low energy and sadness of depression.[1–3] BPAD affects 1–2% of the global population and is associated with a high risk of suicide.[3]

Morbid risk analyses of BPAD demonstrate a high prevalence of affective disorder (especially BPI) among first-degree relatives of bipolar probands in the Amish Study families.[4] Because of the long-term, longitudinal nature of the Amish Study, the unaffected, mentally healthy (well individuals) in these families were also followed, most for a period of years past the age of risk for BPAD. Although sporadic cases of Ellis-van Creveld syndrome occur in many populations, EvC is frequent among the Old Order Amish (OOA) of Pennsylvania.[5] Unexpectedly, no EvC individual has ever been reported with BPI, despite more than 40 years of research documenting the cosegregation of EvC and BPI in the same extended pedigree and descending from the same pioneer.

Twin, family and adoption studies have all provided strong evidence for an important genetic component in the susceptibility to develop BPAD.[6,7] However, unlike most common medical illnesses, objective biological markers have not been identified for BPAD, and genetic studies have had to rely on only clinical diagnoses. Despite compelling clinical-epidemiologic evidence supporting a significant genetic susceptibility to develop BPAD, identification of the genetic variants and an associated underlying molecular mechanism or pathophysiology have remained elusive.[8–14] Genetic heterogeneity, phenocopies, genotyping errors and the complexities of performing and interpreting statistical analyses may have contributed to some of the inconsistences observed in genetic studies.[6,15]

In 1998, we reported the results of a genome-wide search for chromosomal loci linked to mental health wellness in relatives at high risk for BPAD among the OOA.[16] We found strong evidence for a locus in the proximity of the *EvC* gene on chromosome 4p at *D4S2949* (maximum GENEHUNTER-PLUS nonparametric linkage Score = 4.05, $P = 5.22 \times 10^{-4}$; SIBPAL empirical $P < 3 \times 10^{-5}$) and suggestive evidence for a locus on chromosome 4q at *D4S397* (maximum GENEHUNTER-PLUS nonparametric linkage score = 3.29, $P = 2.57 \times 10^{-3}$; SIBPAL empirical $P < 1 \times 10^{-3}$; see Supplementary Figure S1).[16,17] The genes for EVC and hedgehog-interacting protein (Hhip), a Shh antagonist, were subsequently cloned and found to be located within 5 million bases of our chromosome 4p16 (D4S2949) and 4q (D4S397) putative protective or susceptibility loci for BPAD, respectively.

Taken together, these observations led us to postulate that the molecular mechanism underlying EvC is protective against BPI. We now report clinical and statistical evidence that disruption of sonic hedgehog signaling in EvC confers protection from BPI and perhaps more generally against affective disorders.

[1]Departments of Clinical Labs, Neurology, Pediatrics, Pathology and Psychiatry, University of Massachusetts Medical School/UMass Memorial Medical Center, Worcester, MA, USA; [2]Departments of Clinical Labs and Pathology, University of Massachusetts Medical School/UMass Memorial Medical Center, Worcester, MA, USA; [3]Department of Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, Cleveland, OH, USA; [4]Departments of Neuroscience, Psychiatry and Pharmacology, Weill Cornell Medical College of Cornell University, New York, NY, USA and [5]Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, FL, USA. Correspondence: Dr EI Ginns, Departments of Clinical Labs, Neurology, Pediatrics, Pathology and Psychiatry, Reed Rose and Gordon Building, University of Massachusetts Medical School/UMass Memorial Medical Center, 222 Maple Avenue, Shrewsbury, MA 01545, USA.
E-mail: Edward.Ginns@umassmed.edu
Received 19 June 2014; revised 6 August 2014; accepted 21 August 2014; published online 14 October 2014

MATERIALS AND METHODS

Patient samples/genotyping

Blood samples from OOA individuals were obtained with written informed consent approved by the Institutional Review Boards for human subject studies at the University of Miami Miller Medical School, the University of Massachusetts Medical School and the Intramural Research Program at the National Institute of Mental Health (NIMH). Lymphoblast and/or fibroblast cell lines were established at the Coriell Institute for Medical Research, Camden, NJ, USA, the Clinical Neuroscience Branch, Intramural Research Program, National Institute of Mental Health, Bethesda, MD, USA and/or at the University of Massachusetts Medical School. The Coriell NIGMS Human Catalogue of Cell Lines contains updated pedigree and BPAD diagnostic information for selected large families from Amish pedigrees.

DNA analysis

Genomic DNA was obtained from peripheral blood samples, immortalized lymphoblastoid cell lines and/or skin fibroblasts. Analysis for the Amish *EVC* gene intron13 (IVS13+5G>T) mutation[18,19] on 358 DNA samples obtained from the Amish pedigree subjects under study was performed using Sanger sequencing and the MassARRAY MALDI-TOF (Sequenom/Agena Biosciences, San Diego, CA, USA) platforms.

EvC ascertainment and diagnosis

The current research on EvC syndrome began 50 years ago.[20] EvC families were ascertained systematically by Amish church districts. EvC subjects were examined and diagnosed at home or at the Johns Hopkins Moore Clinic in Baltimore, MD, USA.[20] Deceased were certified by death records. A pedigree trace was conducted for every EvC sibship. The resulting progenitor charts tested whether all Amish EvC cases descended from a common progenitor. At that time, the single existing OOA genealogy covered only two founders (Fisher and Stoltzfus).[21] It was coupled with archival materials, and subsequent research resulted in a large EvC pedigree that has been previously published.[20,22] Drafting progenitor charts for EvC cases ascertained since the original research has benefited from the publication of a major genealogy that incorporates all present Amish families tracked along 26 different pioneer family lines.[23]

BPI ascertainment and diagnoses

The Amish Study on major affective disorders (1976–2014) has been conducted with annual IRB approval and multiple informed consents.[24] Details regarding ascertainment procedures, documentation of clinical materials and reliability of diagnoses have been published widely, including by the Coriell NIGMS Human Catalogue of Cell Lines.[25,26] Two types of clinical data (psychiatric interviews and medical records) were processed for independent and 'blinded' assessment by a five-member Amish Study Psychiatric Board[26] using Research Diagnostic Criteria (RDC)[27] and Diagnostic Criteria from DSM-III-IV (DSM)[28] to make categorical diagnoses (see Table 1). After consensus diagnosis of BPI cases, their families became the basis of a large, multi-generational pedigree known as Amish Study BPAD PED.110/210/310/410.

RESULTS

EvC samples past and present

The original EvC study reported on 31 EvC sibships yielding 59 dwarfs (41 infant deaths and 18 living to maturity).[20] Currently, 28 of the original 31 EvC sibships have been updated and an additional 39 families recently ascertained. This doubled the original sample to 67 EvC families with 156 dwarfs, 33 surviving to adulthood. Among these 33 individuals with EvC were a number in the 40–70 years of age categories (67% over age 40 years; 52% over age 50 years) and past the window of risk for onset of BPI disorder. Our sample includes 21 nuclear families with one EvC dwarf, 22 with two, 11 with three, 7 with four and 6 families with five EvC dwarfs (total of 156 dwarfs). Data were also assembled regarding EvC features among the 'normal' relatives in the extended EvC families. Relatives were identified with a missing digit, toe, arm, foot or leg, as further evidence of the broad spectrum of skeletal dysplasia in EvC features for Amish families.

Cosegregation of EvC and BPI

Progenitor traces on confirmed BPI cases yielded the same pioneer, Koenig, identified previously as the progenitor for EvC and giving early evidence for significant cosegregation of these two illnesses. The individual progenitor traces for each EvC family determined their precise location on BPAD Master Pedigree 110/210/310/410 and resulted in a new Amish Study pedigree designated as EvC/BPAD Pedigree 800. Although the bipolar pedigree comprises 42 BPI sub-pedigrees, the EvC families were mainly included in four of them. (See Figure 1). Apart from the documented cosegregation of EvC and BPI, case ascertainment spanning four decades did not reveal subjects with comorbidity.

Association analysis

Two types of association analysis were performed on all 358 individuals in the BPAD master pedigree for whom both EvC genotype and BPAD diagnosis were available: a Fisher's exact test, which ignores all relationships, and large sample tests based on a logistic regression that allowed for the familial dependencies. Only one primary hypothesis was tested, that of association between BPI/no disease status and EvC/no disease status; all other tests were exploratory and for these *P*-values are given with no correction for multiple tests. It is important to note that, although individuals were genotyped because of being related to BPAD-diagnosed pedigree members, all individuals who were genotyped were included in the data, regardless of whether they had BPAD or not (BPAD disease/no disease status) when the sample

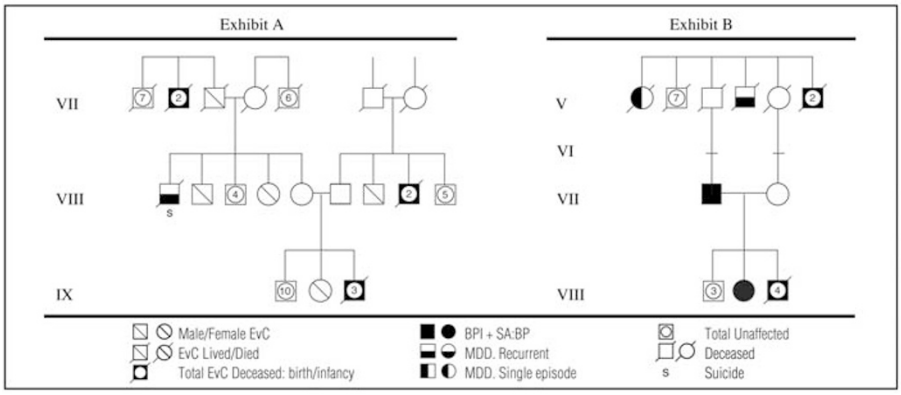
Table 1. Diagnostic Hierarchies for Bipolar Affective Disorder (BPAD)

Phenotypic subcategories for BPAD (the standard DSM subcategory for the Affective Disorders) as developed by the AMISH STUDY Psychiatric Board, according to Research Diagnostic Criteria (RDC) and the DSM-IV guidelines

Subcategory 1 (definitely affected): Bipolar I (BPI), Manic episodes, Schizoaffective Disorder: BP sub-type.
Subcategory 2 (affected): 1 plus Bipolar II, Atypical Bipolar (**Atyp:BP/BP:NOS**) Major Depressive Disorder, recurrent subtype, tagged for BP **(MDDR.tag BP)**
NOTE: The Amish Study found that the diagnosis of MDDR.tag BP was likely to convert to a BPI over the course of the illness, especially if the patient was a first degree relative of a BPI
Subcategory 3 (probably affected): 1 + 2 plus add diagnoses assumed to be in a bipolar spectrum: Hypomanic, recurrent; Major Depressive Disorder, recurrent (**MDDR**) Atypical Psychosis, **tagged BP**, plus Undiagnosed Psychiatric Disorder: **tagged BP (UnDxBP)**
Subcategory 4 (possibly affected): 1 + 2 + 3 plus the common diagnosis of a Major Depressive Disorder, single episode **(MDDS**)
(NOTE: Given the nature of situational depression, this could be a false positive.)
Subcategory 5 (unknown psychiatric): The Other subcategory: includes Hypomanic Episodes, Minor Depression, Intermittent Minor Depression, Dysthymia, Labile or Cyclothymic Personality, Obsessive Compulsive Disorder, Somatization Disorder, Generalized Anxiety Disorder, and other affective subcategory disorders. It also includes Psychotic Disorder, unspecified (**Psych.UnDx**) – useful for historic cases.
Subcategory 6 (definitely unaffected or mentally well): A critical subcategory for analyses of first degree well siblings with a brother/sister diagnosed with BPI.

PEDIGREE DESIGN: The foundation for EvC/BPI PED 800 relates to two previously published pedigrees. The first is the 1964 original EvC pedigree drawn in schematic form for that publication. The second is the Amish Study BPAD pedigrees, 110/210/310/410, published in various articles and the NIGMS Catalog of Cell Lines, CIMR. The challenge was to create a new pedigree that would show both medical conditions co-segregating. The original EvC (1964) version was not compatible for posting BPAD and our EvC sample had doubled in size. Therefore each individual EvC family progenitor trace, showing pathways back to all possible OOA founders (King as the unique Progenitor) was plotted on 110/210/310/410. It was discovered that dwarfism families were not scattered throughout the 42 BPI sub-pedigrees that comprise 110/210/310/410. Instead, they were concentrated in only 4 of these 42 sub-pedigrees. Our new EvC/BP PED 800 was constructed around these 4 BPAD family lines descending from the pioneer settler. The unique Progenitor Trace for each of the 67 EvC families in our sample determined their placement.

SAMPLE EvC FAMILIES: To ensure anonymity, two "generic" EvC/BP families are drawn below to represent both structure and data of PED 800.



DISTRIBUTION OF 67 EvC FAMILIES AMONG 4 BP SUB-PEDIGREES:

GEN. 1 Pioneer KOENIG (KING) - Arrived 1744 in America. There were 14 children still living at the time of his death (8 sons and 6 daughters).

GEN. 2 Only 1 of 8 sons and 3 of his 6 daughters belong to the 2nd Generation of PED 800. EvC could not be ascertained for descendants of the remaining 10 children.

GEN. 3 The 3rd GEN is defined by the grandchildren of progenitor Koenig.

GEN. 4 Among all possible great-grandchildren, one alone accounts for 16 (24%) of the 67 EvC families. Two other great-grandchildren account for about 14% each.

GEN. 5 Here one large BPAD family is the source for 32 (48%) of our 67 EvC families. This cluster of BPI/EvC relatives funnel almost half of the entire EvC sample back to Koenig. (NOTE: Intermarriages existed where grandparents or great-grandparents were brothers/sisters.)

GEN. 6 The 6th GEN has some EvC families. Since the majority of EvC families belong at birth to the7th- 9th GEN, their parents / grandparents are located the 4th- 6th GEN.

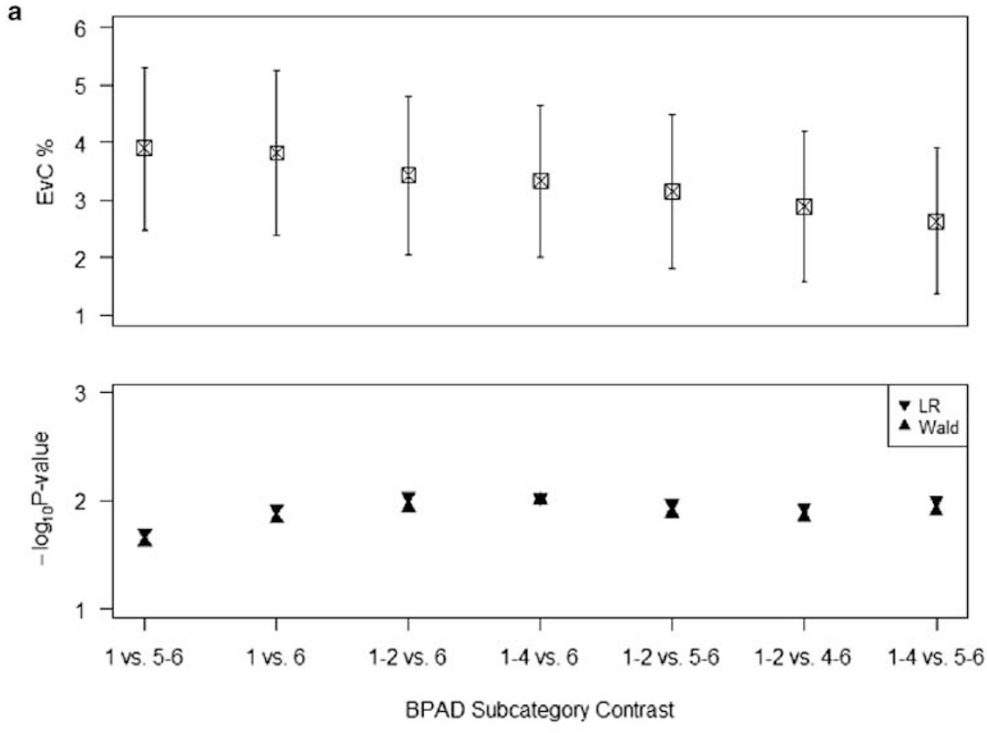
GEN. 7 A number of EvC individuals were ascertained in the 7th generation from Koenig.

GEN. 8 The 8th GEN has the largest concentration of EvC, including many who lived.

GEN. 9 Recent births/deaths for EvC have been recorded (ascertainment incomplete).

Figure 1. Pedigree 800 sample EvC families.

for analysis was selected. Thus, no ascertainment bias could result from the way the sample was selected. The primary hypothesis attained significance ($P = 0.029$, Fisher's exact test, two-sided), verified by permutation to estimate the null distribution of the test statistic (see Supplementary Materials, especially Supplementary Table S1). Most of the pairs of 285 pedigree members included in this test, as for all the 358 pedigree members, were unrelated at least up to third degree (see Supplementary Table S2 in Supplementary Materials). Mean age of onset, when known, was 20–30 for subcategories 1–4, 40 for subcategory 5 (see Table 1 and Supplementary Table S3 in Supplementary Materials). Tables (2×2) were then formed from individual and groups of BPAD disease/no disease subcategories (as defined in Supplementary Materials) and the two EvC classes of interest, namely carriers of two mutant alleles versus all others, and tested under various mixed effect logistic models as well as by Fisher's exact test. We performed these exploratory logistic regression analyses as implemented in the S.A.G.E program ASSOC (https://code.google.com/p/opensage/), which differentiates the dependence of siblings from that of the parent-offspring relationship, allows for a spousal correlation and performs two different but asymptotically equivalent tests—Wald tests and likelihood ratio (LR) tests. Although the same *P*-value for testing the same null hypothesis does not guarantee that we can rely on the asymptotic test, different *P*-values automatically imply that the sample is not large enough for the asymptotic test to be reliable. The latter assumes no numerical inaccuracies, which might occur owing to computer limitations, but would not be relevant for the small number of significant digits we show in our tables. The regression analyses could thus detect a polygenic component of variance, as well as variance components attributable to effects common to spouses, common to siblings over and above that attributable to a polygenic effect, and/or individual-specific effects. The Wald and LR tests, unlike Fisher's exact test, are strictly valid only for large samples, but can be compared for samples of approximately the same size. Figure 2 summarizes noteworthy features of the numerous analyses performed, showing the percent of the sample variance that can be attributed to EvC status under a full model, and *P*-values that



b

EvC	BPAD Subcategories: 1	5-6	total
Absent	54.00 *50.87*	239.00 *242.13*	293
Present	0.00 *3.13*	18.00 *14.87*	18
total	54	257	311

EvC	BPAD Subcategories: 1	6	total
Absent	54.00 *50.59*	213.00 *216.41*	267
Present	0.00 *3.41*	18.00 *14.59*	18
total	54	231	285

EvC	BPAD Subcategories: 1-2	6	total
Absent	76.00 *71.54*	213.00 *217.46*	289
Present	0.00 *4.46*	18.00 *13.54*	18
total	76	231	307

EvC	BPAD Subcategories: 1-4	6	total
Absent	101.00 *95.52*	213.00 *218.48*	314
Present	0.00 *5.48*	18.00 *12.52*	18
total	101	231	332

EvC	BPAD Subcategories: 1-2	5-6	total
Absent	76.00 *71.89*	239.00 *243.11*	315
Present	0.00 *4.11*	18.00 *13.89*	18
total	76	257	333

EvC	BPAD Subcategories: 1-2	4-6	total
Absent	76.00 *72.01*	249.00 *252.99*	325
Present	0.00 *3.99*	18.00 *14.01*	18
total	76	267	343

EvC	BPAD Subcategories: 1-4	5-6	total
Absent	101.00 *95.92*	239.00 *244.08*	340
Present	0.00 *5.08*	18.00 *12.92*	18
total	101	257	358

EvC	BPAD Subcategories: affected	unaffected	total
Absent	observed *expected*	observed *expected*	
Present	observed *expected*	observed *expected*	
total			

Figure 2. Subcategory contrasts that result in the largest estimates of variance attributable to EvC, ranked by magnitude from left to right. The model allows up to four variance components, in addition the effect of EvC, to be estimated. Subcategories are defined in Table 1: diagnostic subcategories. (**a**) Top panel: variance attributable to EvC, as a percent of the total sample variance, and approximate s.e. bars. Bottom panel: Tests of the EvC effect when included as a covariate in the model; values of $-\log_{10}$P for the Wald and likelihood ratio (LR) tests. (**b**) Fourfold tables showing the observed numbers and expected numbers under independence for each of the subcategory contrasts tested.

make large sample assumptions, for the corresponding association of disease/no disease BPAD subcategories with disease/no disease EvC status (see Supplementary Materials, especially Supplementary Tables S4 and S5). Taken together, these results suggest EvC has a protective effect against all subcategories 1–4 of BPAD. Small *P*-values for association analysis indicate no more than that the null hypothesis of no association is highly unlikely, with no indication of what that association may be due to, as it could be a spurious association due to any number of causes, including an incorrect statistical model. Furthermore, *P*-values are dependent on the sizes of the samples compared. That is why we show in Figure 2 the estimated percent of the total sample variance that can be attributed to EVC segregation for the subcategory contrasts tested. These estimates, but not their s.e., would be expected to reflect the variance components independent of sample size. For our primary hypothesis, when EvC status was included in the model the polygenic component decreased from 37.56 to 28.52%, whereas the individual variance component increased much less, from 62.44 to 67.66%. This strongly suggests that the association found by Fisher's exact test is mostly due to segregation at the EvC locus. No other variance components were detected for this test, but other tests that were performed detected (non-significant) variance components. It is unlikely that EvC is protective against subcategory 5, as the percentage of total variance attributable to EVC segregation is the largest for the comparison of subcategory 1 versus subcategories 5 and 6 combined; here the polygenic variance component decreased from 48.08 to 38.87%, whereas the individual variance component increased from 51.92 to 57.23%, on including EvC in the model (see Supplementary Table S5 in the Supplementary Materials).

DISCUSSION

The cosegregation of EvC and BPAD in our large, multigenerational Amish pedigree provided a rare, informative 'experiment of nature'. Decades of careful longitudinal tracking of EvC and BPAD cases in Amish families (same extended pedigree, descending from the same pioneer) led to our observation that through

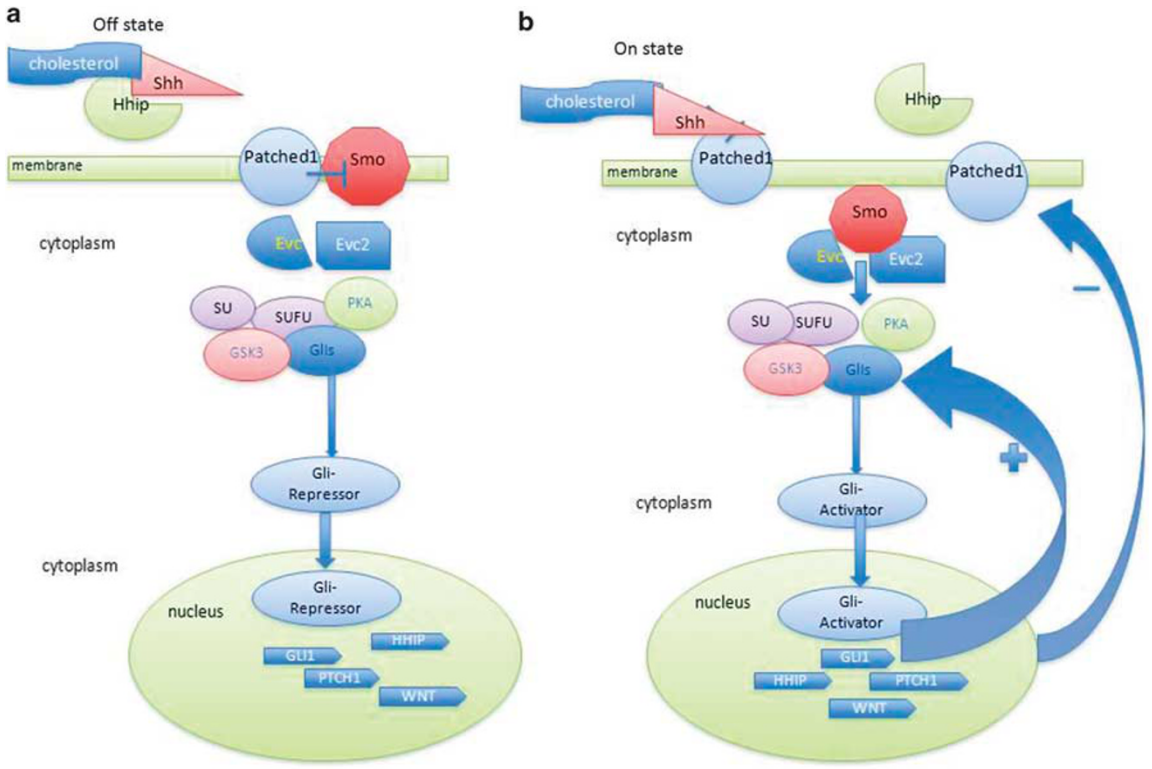


Figure 3. The sonic hedgehog signaling pathway. (**a**) In the off-state, Shh is inhibited by hedgehog-interacting protein (Hhip). In the absence of Shh, Smo is inhibited by Patched 1 (Ptch-1) receptor. Smo bound to Patched 1 is unable to make a complex with Evc, Evc2, Sufu (supressor of fused), Fu (fused) and other proteins. The Gli proteins are phosphorylated by protein kinase (PKA) and form repressors that move to the nucleus and repress the Gli-dependent transcription of targeted genes. (**b**) In the active state, Shh covalently linked to cholesterol moiety (N-Shh) binds to the Patched1/Smo complex and releases Smo. Evc and Evc2 are required for Smo activation and for releasing Gli proteins from their associated cytoplasmic factors. Gli activators translocate into the nucleus where they activate transcription of a variety of genes, including (**a**) GLI1 itself that is responsible for a positive feedback loop, (**b**) genes such as *PTCH-1* and *HHIP* that set up a negative feedback loop and (**c**) other genes coding for proteins involved in the Wnt pathway. Glycogen synthase kinase-3 (GSK-3) negatively regulates the Shh signaling pathway by promoting degradation of GLi1. Lithium blocks the dephosphorylation of GSK3-β causing activation of target proteins including Gli1.

multiple generations no individual with EvC had ever been reported with BPI. Analyses performed to test our primary hypothesis, that is, that of association between absence of BPI and EvC ($P = 0.029$, Fisher's exact test, two-sided; see Supplementary Materials, especially Supplementary Table S1), supported our hypothesis that EvC confers protection (that is, mental health wellness) from BPI, as well as suggesting a more general protection against the spectrum of affective disorders in these families (see Supplementary Materials, especially Supplementary Tables S4 and S5). Attempts to confirm this association can be performed in large GWAS data sets, by restricting analyses to genes comprising or interacting with the Shh pathway. The significant association between absence of BPI and EvC, along with EvC causing disruption of Shh signaling and linkage evidence for protection/susceptibility genes for BPAD at the 4p EvC and 4q Hhip loci suggested the involvement of Shh signaling in mood disorders.

To date, most genetic studies of affective disorders have been limited to identifying genetic variants that increase the risk of disease.[29] By contrast, we have previously provided evidence that, in addition to rare susceptibility alleles, there may be rare alleles that reduce the risk of developing BPAD in a manner similar to that reported for other complex inherited disorders.[16] False-negative genomic study findings could result when individuals inherit disease susceptibility alleles, but are misclassified because they do not manifest the phenotype due to the presence of protective alleles.[16,17] Although the concept that rare protective alleles could modify (or even prevent) a behavioral phenotype like BPAD is relatively novel, several examples for non-psychiatric diseases have been reported. For instance, individuals in Limone sul Garda in Northern Italy were discovered to have Apo A-I_{MILANO}, a rare mutant apolipoprotein that is associated with a reduced risk of atherosclerosis.[30] Among Ecuadorian villagers, the rare, autosomal recessively inherited Laron Syndrome (GHRD) dwarfism appears protective of diabetes and cancer due to reduced levels of insulin-like growth factor 1.[31–33] More recently, a rare mutation in the amyloid precursor protein gene has been shown to protect individuals from developing Alzheimer's disease.[34] Cosegregation of diseases that interact in this way is more likely to occur in a genetic isolate like the Amish, where there are limited numbers of founders.

Our earlier 1998 genome-wide linkage data suggested the presence of two loci with rare alleles protective of BPAD located on chromosome 4, one on 4p16 at D4S2949 and the other on 4q at D4S397, respectively (see Supplementary Figure S1).[16,17] The proximity of the *EVC* gene to our previously reported chromosome 4p16 BPAD protective/susceptability locus, coupled with detailed clinical observations and statistical confirmation that EvC and BPI do not occur in the same Amish individuals, led us to consider that the genetic defect causing EvC in the Amish confers protection from BPI. The discovery that EvC is the result of disrupted Shh signaling focused our attention on the sonic hedgehog (Shh) signaling pathway (Figure 3).[35–39] The presence of a Shh antagonist gene, hedgehog-interacting protein (Hhip), at our

BPAD chromosome 4q protection/susceptibility locus is additionally suggestive of Shh signaling involvement in BPAD.

The association of EvC and Shh pathway mutations has been mainly with a heterogeneous group of inherited skeletal disorders,[19] holoprosencephaly and a wide range of tumor growth, progression and metastasis.[40–45] EvC syndrome in the Amish of Pennsylvania is the result of homozygous intron13 (IVS13 +5G > T) EVC gene mutations.[19] The clinical manifestations of EvC are diverse: some patients dying a few days after birth, while others live a long and active life.[18,46] More recently, attention has focused on the importance of EVC and EVC2 in primary cilia signal transduction, structures enriched in key components of the Shh-transduction pathway,[36] controlling the kind, numbers and patterning of cells during development in many tissues, including the nervous system.[47] The correct localization and stoichiometry of EVC and EVC2 proteins as a complex in primary cilia are required for their normal function as positive modulators of the Shh pathway signaling. Lack of this normal EVC/EVC2 protein complex disrupts Shh signaling (Figure 3).[35,37,38] It is likely that the mechanism by which this homozygous mutation acts is by overriding abnormal Shh signaling to protect against appearance of BPAD disorder in these high-risk multigenerational pedigrees. Although hedgehog ligands encode signaling molecules in a wide range of tissues, sonic hedgehog (Shh) is the only hedgehog family member reported to be expressed in the mammalian central nervous system.[47–51]

A growing body of evidence suggests that the state-like and oscillatory interactions of gene products within and extending from the Shh signaling pathway (Figure 3),[49,50,52,53] and modulated by environmental factors, could constitute the basis for the wide range of phenotypic manifestations of BPAD.[54–56] Antidepressant drugs,[57] including lithium,[58] and electroconvulsive therapy[59] have been shown to alter Shh signaling. Glycogen synthase kinase 3 (GSK3; GSK3α/GSK3β) is a target of lithium and has a central role in Shh signaling.[60] In addition to lithium, valproate, selective 5-HT reuptake inhibitors, monoamine oxidase inhibitors and tricyclic antidepressants have been shown to alter GSK3 activity. Studies on interactions between Shh and corticotropin-releasing hormone (CRH) signaling networks have related Shh signaling to neurotransmitter systems underlying anxiety, stress and depressive disorders.[61,62] Cholesterol and palmitoic acid, required for appropriate Shh processing and long range signaling,[63–68] have been associated with suicide, depression and BPAD.[69] Significantly more suicide attempters and completers have been reported among the biological relatives of Smith-Lemli-Opitz syndrome carriers, a population of individuals with reduced activity of 7-dehydrocholesterol reductase (DHCR7), an enzyme required for Shh processing.[70] Altered brain sterol composition, involving cholesterol, 7-dehydrocholesterol and/or 7-dehydrodesmosterol, has also been associated with a greater risk of suicidal behaviors.[70] Variants in EVC have recently been reported to be associated with male completed suicide.[71] Interestingly, the Amish Study ascertained OOA suicides ($n = 26$) for the period 1880–1990 and found that they were heavily clustered in PED 800, almost all were males with BPAD, equally divided between bipolar and major depression.[72] It is possible that BPAD, EvC and male suicide in these Amish Study families could be explained by genetic variants that influence Shh signaling.[73,74]

Our study of the cosegregation of EvC and BPAD in the Old Order Amish further implicates Shh signaling in the pathophysiology of BPAD and suggests that other genes/proteins in the Shh signaling pathway may be involved in protection or susceptibility to developing mood disorders. The increased understanding of the molecular basis of Shh signaling and reactivation occurring in a wide range of cancers has led to the identification of Shh signaling antagonists that are already in human clinical studies.[75] Repurposing of drugs targeting Shh signaling that are already in clinical development for other medical conditions could lead to better treatments for affective disorders in the near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank the essential function of the AMISH STUDY Psychiatric Board since 1976: Drs Abram M Hostetter, John J Schwab, Jon A Shaw, Jean Endicott and the late Drs James N Sussex and David R Offord. We thank Cleona R Allen for pedigree drafting and editing, Bernadette Warman as graphic artist, Mary F Sweger for progenitor charts, Martha P Hansell for computer consultation and Alma Becker, project phlebotomist, for collection of all EvC blood samples. Our deepest respect and appreciation belongs to Old Order Amish patients and families who participated in this research commencing in 1960. We thank Betty and Irving Brudnick, patient families and organizations, and our own families for their encouragement and support. We are grateful for support from the Case Western Reserve University Amasa B. Ford MD chair of Geriatric Medicine (RAE), the Brudnick Neuropsychiatric Research Institute (EIG; MG), Grants MH93415 (SMP) and HD004147 (EIG) the University of Massachusetts Medical School (EIG; MG), and past support from the Intramural Research Program, NIMH (EIG; MG; SMP), Eli Lilly (SMP; JAE Amish Study) and NIMH Grant MH28287 (JAE Amish Study).

REFERENCES

1 Winokur G, Clayton PJ, Reich T. *Manic Depressive Illness*. C. V. Mosby, 1969.
2 Shopsin B. *Manic Illness*. Raven Press: New York, 1979.
3 Goodwin FK, Jamison KR. *Manic-Depressive Illness*. Oxford University Press: Oxford, 1991.
4 Pauls DL, Morton LA, Egeland JA. Risks of affective illness among first-degree relatives of bipolar I old-order Amish probands. *Arch Gen Psychiatr* 1992; **49**: 703–708.
5 McKusick VA, Hostetler JA, Egeland JA, Eldridge R. The distribution of certain genes in the Old Order Amish. *Cold Spring Harb Symp Quant Biol* 1964; **29**: 99–114.
6 Craddock N, Sklar P. Genetics of bipolar disorder. *Lancet* 2013; **381**: 1654–1662.
7 Fagnani C, Bellani M, Soares JC, Stazi MA, Brambilla P. Discordant twins as a tool to unravel the aetiology of bipolar disorder. *Epidemiol Psychiatr Sci* 2014; **23**: 137–140.
8 Chen DT, Jiang X, Akula N, Shugart YY, Wendland JR, Steele CJ *et al.* Genome-wide association study meta-analysis of European and Asian-ancestry samples identifies three novel loci associated with bipolar disorder. *Mol Psychiatr* 2013; **18**: 195–205.
9 Georgi B, Craig D, Kember RL, Liu W, Lindquist I, Nasser S *et al.* Genomic view of bipolar disorder revealed by whole genome sequencing in a genetic isolate. *PLoS Genet* 2014; **10**: e1004229.
10 Gershon ES, Alliey-Rodriguez N, Liu C. After GWAS: searching for genetic risk for schizophrenia and bipolar disorder. *Am J Psychiatr* 2011; **168**: 253–256.
11 Moskvina V, Craddock N, Holmans P, Nikolov I, Pahwa JS, Green E *et al.* Gene-wide analyses of genome-wide association data sets: evidence for multiple common risk alleles for schizophrenia and bipolar disorder and for overlap in genetic risk. *Mol Psychiatr* 2009; **14**: 252–260.
12 Nurnberger JI Jr, Koller DL, Jung J, Edenberg HJ, Foroud T, Guella I *et al.* Identification of pathways for bipolar disorder: a meta-analysis. *JAMA Psychiatr* 2014; **71**: 657–664.
13 Serretti A, Mandelli L. The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions. *Molecular Psychiatr* 2008; **13**: 742–771.
14 Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G *et al.* A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular Psychiatr* 2013; **18**: 497–511.
15 Wray NR, Lee SH, Kendler KS. Impact of diagnostic misclassification on estimation of genetic correlations using genome-wide genotypes. *Eur J Hum Genet* 2012; **20**: 668–674.
16 Ginns EI St, Jean P, Philibert RA, Galdzicka M, Damschroder-Williams P, Thiel B *et al.* A genome-wide search for chromosomal loci linked to mental health wellness in relatives at high risk for bipolar affective disorder among the Old Order Amish. *Proc Nati Acad Sci USA* 1998; **95**: 15531–15536.
17 Visscher PM, Haley CS, Ewald H, Mors O, Egeland J, Thiel B *et al.* Joint multi-population analysis for genetic linkage of bipolar disorder or 'wellness' to chromosome 4p. *Am J Med Genet Part B, Neuropsychiatr Genet* 2005; **133B**: 18–24.

18 Ruiz-Perez VL, Ide SE, Strom TM, Lorenz B, Wilson D, Woods K *et al.* Mutations in a new gene in Ellis-van Creveld syndrome and Weyers acrodental dysostosis. *Nat Genet* 2000; **24**: 283–286.
19 Galdzicka M, Egeland J, Ginns E. *EVC and EVC2 and the Ellis-van Creveld Syndrome and Weyers Acrofacial Dysostosis*, 2nd edn. Oxford University Press: New York, USA, 2008.
20 McKusick VA, Egeland JA, Eldridge R, Krusen DE. Dwarfism in the Amish I. The Ellis-Van Creveld Syndrome. *Bull Johns Hopkins Hosp* 1964; **115**: 306–336.
21 Fisher JM. *Descendants and history of Christian Fisher family*. Private Publisher: Ronks, PA, USA, 1957; p. 619.
22 McKusick VA, Hostetler JA, Egeland JA. Genetic studies of the Amish, background and potentialities. *Bull Johns Hopkins Hosp* 1964; **115**: 203–222.
23 Egeland JA (ed). *Descendants of Christian Fisher and other Amish-Mennonite Pioneer Families*. Moore Clinic: Baltimore, MD, USA, 1972.
24 Egeland JA, Hostetter AM. Amish Study, I: Affective disorders among the Amish, 1976-1980. *Am J Psychiatr* 1983; **140**: 56–61.
25 Egeland JA, Sussex JN, Endicott J, Hostetter AM. The impact of diagnoses on genetic linkage study for bipolar affective disorders among the Amish. *Psychiatr Genet* 1990; **1**: 5–18.
26 Hostetter AM, Egeland JA, Endicott J. Amish Study, II: Consensus diagnoses and reliability results. *Am J Psychiatr* 1983; **140**: 62–66.
27 Spitzer RL, Endicott J, Robins E. Research diagnostic criteria: rationale and reliability. *Arch Gen Psychiatr* 1978; **35**: 773–782.
28 Association Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 4th edn. American Psychiatric Publishing: Arlington, VA, 2000.
29 Chakravarti A, Clark AG, Mootha VK. Distilling pathophysiology from complex disease genetics. *Cell* 2013; **155**: 21–26.
30 Chiesa G, Sirtori CR. Apolipoprotein A-I(Milano): current perspectives. *Curr Opin Lipidol* 2003; **14**: 159–163.
31 Shevah O, Laron Z. Patients with congenital deficiency of IGF-I seem protected from the development of malignancies: a preliminary report. *Growth Hormone IGF Res* 2007; **17**: 54–57.
32 Steuerman R, Shevah O, Laron Z. Congenital IGF1 deficiency tends to confer protection against post-natal development of malignancies. *Eur J Endocrinol* 2011; **164**: 485–489.
33 Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW *et al.* Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med* 2011; **3**: 70ra13.
34 Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S *et al.* A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 2012; **488**: 96–99.
35 Yang C, Chen W, Chen Y, Jiang J. Smoothened transduces Hedgehog signal by forming a complex with Evc/Evc2. *Cell Res* 2012; **22**: 1593–1604.
36 Ruiz-Perez VL, Goodship JA. Ellis-van Creveld syndrome and Weyers acrodental dysostosis are caused by cilia-mediated diminished response to hedgehog ligands. *Am J Med Genet Part C,* 2009; **151C**: 341–351.
37 Pusapati GV, Hughes CE, Dorn KV, Zhang D, Sugianto P, Aravind L *et al.* EFCAB7 and IQCE regulate hedgehog signaling by tethering the EVC-EVC2 complex to the base of primary cilia. *Dev Cell* 2014; **28**: 483–496.
38 Nakatomi M, Hovorakova M, Gritli-Linde A, Blair HJ, MacArthur K, Peterka M *et al.* Evc regulates a symmetrical response to Shh signaling in molar development. *J Dental Res* 2013; **92**: 222–228.
39 Green JA, Mykytyn K. Neuronal primary cilia: an underappreciated signaling and sensory organelle in the brain. *Neuropsychopharmacology* 2014; **39**: 244–245.
40 Macdonald TJ. Hedgehog pathway in pediatric cancers: they're not just for brain tumors anymore. *Am Soc Clin Oncol Soc* 2012; 605–609.
41 Ming JE, Roessler E, Muenke M. Human developmental disorders and the Sonic hedgehog pathway. *Mol Med Today* 1998; **4**: 343–349.
42 Muenke M, Cohen MM Jr. Genetic approaches to understanding brain development: holoprosencephaly as a model. *Mental Retard Dev Disabl Res Rev* 2000; **6**: 15–21.
43 Oldak M, Grzela T, Lazarczyk M, Malejczyk J, Skopinski P. Clinical aspects of disrupted Hedgehog signaling (Review). *Int J Mol Med* 2001; **8**: 445–452.
44 Roessler E, Muenke M. Holoprosencephaly: a paradigm for the complex genetics of brain development. *J Inherit Metab Dis* 1998; **21**: 481–497.
45 Vaillant C, Monard D. SHH pathway and cerebellar development. *Cerebellum* 2009; **8**: 291–301.
46 Galdzicka M, Patnala S, Hirshman MG, Cai JF, Nitowsky H, Egeland JA *et al.* A new gene, EVC2, is mutated in Ellis-van Creveld syndrome. *Mol Genet Metab* 2002; **77**: 291–295.
47 Ruiz i Altaba A, Palma V, Dahmane N. Hedgehog-Gli signalling and the growth of the brain. *Nat Rev Neurosci* 2002; **3**: 24–33.
48 Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA *et al.* Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* 1993; **75**: 1417–1430.
49 Panovska-Griffiths J, Page KM, Briscoe . A gene regulatory motif that generates oscillatory or multiway switch outputs. *J Royal Soc* 2013; **10**: 20120826.
50 Balaskas N, Ribeiro A, Panovska J, Dessaud E, Sasai N, Page KM *et al.* Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* 2012; **148**: 273–284.
51 Kwon HJ. ATP oscillations mediate inductive action of FGF and Shh signalling on prechondrogenic condensation. *Cell Biochem Funct* 2013; **31**: 75–81.
52 Traiffort E, Angot E, Ruat M. Sonic Hedgehog signaling in the mammalian brain. *J Neurochem* 2010; **113**: 576–590.
53 Ruat M, Angot E, Traiffort E. Shh signal and its functional roles in normal and diseased brain. *Med Sci* 2011; **27**: 979–985.
54 Gradilla AC, Guerrero I. Hedgehog on the move: a precise spatial control of Hedgehog dispersion shapes the gradient. *Curr Opin Genet Devel* 2013; **23**: 363–373.
55 Odenthal J, Haffter P, Vogelsang E, Brand M, van Eeden FJ, Furutani-Seiki M *et al.* Mutations affecting the formation of the notochord in the zebrafish, Danio rerio. *Development* 1996; **123**: 103–115.
56 Bejsovec A, Wieschaus E. Segment polarity gene interactions modulate epidermal patterning in Drosophila embryos. *Development* 1993; **119**: 501–517.
57 Oyabu A, Narita M, Tashiro Y. The effects of prenatal exposure to valproic acid on the initial development of serotonergic neurons. *Int J Devel Neurosci* 2013; **31**: 202–208.
58 Can A, Schulze TG, Gould TD. Molecular actions and clinical pharmacogenetics of lithium therapy. *Pharmacol Biochem Behav* 2014; **123C**: 3–16.
59 Banerjee SB, Rajendran R, Dias BG, Ladiwala U, Tole S, Vaidya VA. Recruitment of the Sonic hedgehog signalling cascade in electroconvulsive seizure-mediated regulation of adult rat hippocampal neurogenesis. *Eur J Neurosci* 2005; **22**: 1570–1580.
60 Watkins CC, Sawa A, Pomper MG. Glia and immune cell signaling in bipolar disorder: insights from neuropharmacology and molecular imaging to clinical application. *Transl Psychiatr* 2014; **4**: e350.
61 Li X, Zhu W, Roh MS, Friedman AB, Rosborough K, Jope RS. In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. *Neuropsychopharmacology* 2004; **29**: 1426–1431.
62 Vila G, Papazoglou M, Stalla J, Theodoropoulou M, Stalla GK, Holsboer F *et al.* Sonic hedgehog regulates CRH signal transduction in the adult pituitary. *FASEB J* 2005; **19**: 281–283.
63 Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. *Gene Dev* 2008; **22**: 2454–2472.
64 Chen MH, Li YJ, Kawakami T, Xu SM, Chuang PT. Palmitoylation is required for the production of a soluble multimeric Hedgehog protein complex and long-range signaling in vertebrates. *Gene Dev* 2004; **18**: 641–659.
65 Ho KS, Scott MP. Sonic hedgehog in the nervous system: functions, modifications and mechanisms. *Curr Opin Neurobiol* 2002; **12**: 57–63.
66 Koide T, Hayata T, Cho KW. Negative regulation of Hedgehog signaling by the cholesterogenic enzyme 7-dehydrocholesterol reductase. *Development* 2006; **133**: 2395–2405.
67 Corcoran RB, Scott MP. Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc Natl Acad Sci USA* 2006; **103**: 8408–8413.
68 Bijlsma MF, Peppelenbosch MP, Spek CA. A dual role for 7-dehydrocholesterol reductase in regulating Hedgehog signalling? *Development* 2006; **133**: 3951.
69 Vuksan-Cusa B, Marcinko D, Nad S, Jakovljevic M. Differences in cholesterol and metabolic syndrome between bipolar disorder men with and without suicide attempts. *Prog Neuro-psychopharmacol Biol Psychiatr* 2009; **33**: 109–112.
70 Lalovic A, Merkens L, Russell L, Arsenault-Lapierre G, Nowaczyk MJ, Porter FD *et al.* Cholesterol metabolism and suicidality in Smith-Lemli-Opitz syndrome carriers. *Am J Psychiatr* 2004; **161**: 2123–2126.
71 Must A, Koks S, Vasar E, Tasa G, Lang A, Maron E *et al.* Common variations in 4p locus are related to male completed suicide. *Neuromol Med* 2009; **11**: 13–19.
72 Egeland JA, Sussex JN. Suicide and family loading for affective disorders. *JAMA* 1985; **254**: 915–918.
73 Hur EM, Zhou FQ. GSK3 signalling in neural development. *Nat Rev Neurosci* 2010; **11**: 539–551.
74 Kim WY, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR *et al.* GSK-3 is a master regulator of neural progenitor homeostasis. *Nat Neurosci* 2009; **12**: 1390–1397.
75 Sheikh A, Alvi AA, Aslam HM, Haseeb A. Hedgehog pathway inhibitors—current status and future prospects. *Infect Agent Cancer* 2012; **7**: 29.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)