Developmental Biology: Frontiers for Clinical Genetics

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An emerging role for Wnt and GSK3 signaling pathways in schizophrenia


Schizophrenia is a disabling illness with limited treatment options. The underlying pathophysiology remains unknown, partially due to its heterogeneous nature, and a lack of understanding of the biological functions of genetic risk factors. Several signaling pathways have been implicated, however, with the varying degrees of support. In this article, I will focus on the converging evidence supporting a prominent role for Wnt and glycogen synthase kinase 3 (GSK3) signaling in the biological bases of schizophrenia. This includes current pharmacological therapies that target GSK3, animal model and cell-based studies, and recent human genetic findings that implicate Wnt and GSK3 signaling.

Conflict of interest
The author declares no conflicts of interest.

Wnt signaling is a highly conserved pathway that has many roles in organism development. This signaling pathway also plays a prominent role in the nervous system, from neural development to adult neural circuit function. Because of the wide range of Wnt signaling roles, it is no surprise that this pathway has been implicated in various neuropsychiatric and neurodegenerative disorders, as well as in brain and spinal cord injury paradigms (1). For the purpose of this article, I will focus on the evidence supporting a role for Wnt signaling in schizophrenia, as there have been exciting new developments in the past few years that to an important role of this pathway in the etiology of disease. Furthermore, interested readers are directed to a forthcoming book chapter on Wnt signaling in neuropsychiatric and neurodegenerative disorders, and will include and expand upon the specific topics covered in this article.

Schizophrenia is a brain development disorder
Schizophrenia is a common and chronic psychiatric disorder that is among the leading causes of disability in the world. Current treatments have their major impact on psychotic or positive symptoms (e.g. hallucinations) but do not treat the underlying causal mechanism. One of the key obstacles in developing effective therapeutics is a lack of understanding of the biological basis of the disease. While patients are usually diagnosed in late adolescence and continue to have symptoms well into adulthood, a major hypothesis is that schizophrenia is a disorder of brain development and neural connectivity (2-4). Studies show in utero and early environmental insults such as infection during pregnancy (which can disrupt fetal brain development) increase the risk of schizophrenia (5). Other evidence includes the observation that schizophrenia patients show delays in cognitive milestones during their early childhood.
years (6). In this regard schizophrenia patients are 1.0–2.0 standard deviations below the mean of the general population in cognitive ability, which reflect early deficits in brain function (7–9). Together, these data suggest brain development is likely disrupted in patients, and argues that brain-specific signaling pathways during development, such as Wnt signaling, may be an important factor in disease etiology.

Introduction to Wnt signaling

The canonical pathway consists of signaling initiated by Wnt receptors, and involves β-catenin and the transcription of Wnt-dependent target genes. The canonical Wnt pathway has been described elsewhere in detail (10). In brief, in the absence of Wnt ligand binding to its receptor complex, an intracellular destruction complex exists consisting of glycogen synthase kinase 3 β (GSK3β), Axin, adenomatous polyposis coli (APC), and casein kinase 1 α (CK1α) (Fig. 1). This complex keeps a key player in the pathway, β-catenin, phosphorylated and targets it for β-transducin repeat containing protein (βTRCP)-mediated proteasomal degradation, thus decreasing the concentration of cytosolic β-catenin. However, when Wnt ligands bind their receptor complex [a frizzled (FZ) family member with low density lipoprotein 5/6 (Lrp5/6)], this causes the recruitment of APC and Disheveled (Dvl) to the membrane, facilitating the dissociation of the destruction complex. This results in decreased phosphorylation of β-catenin, an increase in its stability in the cytoplasm, and translocation of β-catenin into the nucleus where it binds other co-factors to initiate T-cell factor/lymphoid enhancing factor (TCF/LEF)-mediated gene transcription.

There are several non-canonical Wnt signaling pathways that do not involve β-catenin-mediated transcription including the planar cell polarity (PCP) signaling and the Wnt/calcium pathway (Fig. 1). In PCP signaling, FZ functions through G proteins to activate Dvl, and in turn will result in activation of Rho and Rac proteins, which ultimately regulate the cytoskeleton. In the Wnt/Ca2+ signaling pathway, FZ activation results in Dvl-mediated intracellular Ca2+ increase, which will then results in the activation of protein kinase C (PKC) and calcium/calmodulin protein kinase II (CaMKII). Downstream of these proteins are several potential substrates that can have an effect on a wide range of functions in the central nervous system (CNS), including neural circuit formation and synaptic plasticity (11).

Psychiatric pharmaceuticals and Wnt signaling

One of the first lines of therapy for schizophrenia patients are small molecule drugs. There is an unappreciated close relationship between the different classes of drugs currently used to treat psychiatric disorders and Wnt signaling. Wnt signaling seems to be a direct or indirect target of drug treatments, suggesting this signaling network may be of key relevance for the pathogenesis, and potentially etiology, of psychiatric disorders (Fig. 2).

The intersection of dopamine and Wnt signaling: antipsychotics

The dopamine (DA) signaling pathway is one of the main targets of several well-known drugs that have psychiatric-like effects and is also targeted by

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**Fig. 1.** Wnt signaling pathways. There are three main Wnt signaling pathways: (a) canonical Wnt signaling, (b) Wnt-calcium signaling and (c) non-canonical Wnt/planar cell polarity signaling.
pharmaceuticals such as antipsychotics (e.g. clozapine). A novel pathway downstream of DA receptors has recently begun to take shape, whereby DA signaling impinges upon GSK3β (12). Activation of D2 receptors by DA results in the inhibition of the enzyme Akt, which decreases inhibitory phosphorylation of GSK3β, thus rendering GSK3β more active. Chronic administration of different drugs that increase DA levels in the wild-type brain, such as amphetamine, apomorphine, or methamphetamine, also lead to increased inhibitory phosphorylation of Akt and increased activation of GSK3β (13). Furthermore, antipsychotic drugs inhibit DA receptors (14), including the drug haloperidol which is able to increase Akt activation in vivo in mice (15). These data establish a strong relationship between DA levels and Akt/GSK3β signaling, and potentially Wnt signaling. In this regard, the DA D2 receptor has been shown to inhibit Wnt signaling at the level of TCF/LEF-mediated transcription (16). Interestingly, the D2 receptor was found to mediate this effect by directly binding and sequestering β-catenin (16). Additional support for these observations comes from the Rushlow laboratory where it was observed that chronic administration of clozapine and haloperidol into the rat prefrontal cortex and striatum regulates the protein levels of GSK3β, β-catenin, Dvl, and Axin (17). Follow up studies from the same laboratory demonstrated that antipsychotic treatment increases Dvl3 levels, and increases the binding of GSK3β to the D2 receptor (18). These data suggest that one of the effects of antipsychotic medication is to inhibit D2 signaling, and regulate Wnt signaling.

Lithium

Lithium is one of the most widely used treatments for bipolar disorder and is sometimes used in combination with other drugs to treat schizophrenia. One of the main mechanisms of action of lithium is the direct inhibition of GSK3β activity by competing with magnesium, as well as indirectly inhibiting it by increasing the inhibitory phosphorylation on GSK3β serine 9 (19–21). In addition, lithiums ability to inhibit GSK3β can lead to accumulation of β-catenin. As a consequence, lithium application in vivo or to cultured cells, including neural progenitor cells and neurons, results in increased TCF/LEF-mediated gene transcription (22, 23). This direct activation of canonical Wnt signaling is suggestive of a possible role for this pathway in mediating the mood-balancing effects of lithium.

Data from the Caron laboratory also demonstrate lithium destabilizes a ternary complex consisting of β-arrestin/Akt/PP2A, which is regulated by D2 receptor signaling (24). Additional studies from the Klein laboratory further show that GSK3β regulates the stability of the β-arrestin-2/Akt/PP2A ternary complex.
through direct interactions, and that chronic lithium treatment at physiologically relevant concentrations disrupts the complex via the inhibition of GSK3 (25). This conclusion was further supported by experiments using direct ATP-competitive GSK3 inhibitors as well as loss-of-function Gsk3+/− heterozygous mice that similarly disrupted the β-arrestin-2/Akt/PP2A ternary complex (26). Conversely, gain-of-function genetic models that overexpress a GSK3β transgene from the brain-specific prion promoter restored β-arrestin-2/Akt/PP2A ternary complex levels following lithium treatment (27). This strongly argues that one of the main actions of lithium is to regulate Wnt signaling directly and impact brain function and behavior.

Metabotropic glutamate receptors

Recently, interest has increased in the targeting of metabotropic glutamate receptors 2/3 (mGlu2/3) as a potential novel therapeutic mechanism for schizophrenia that does not involve the direct modulation of D2 receptors. Rushlow and colleagues reported the treatment with mGlu2/3 agonists that have antipsychotic-like activity results in both the activation of Akt and inhibitory phosphorylation of GSK3 along with accumulation of Dvl2 and Dvl3 and β-catenin following repeated treatment (18). Moreover, they showed that the mGlu2/3 complex physically interacts with Dvl2. These results provide another example of a class of non-dopaminergic pharmacological agents that modulate the Wnt pathway and Akt-GSK3 signaling network.

Psychiatric genetics and Wnt signaling

Psychiatric illnesses are highly complex and heritable disorders and tend to run in families. This argues for a genetic etiological role of psychiatric disorders, and has served as the basis from which human genetic studies have been conducted in recent years. The discovery of risk genes have been highly sought after since they have the potential to reveal the biological basis of schizophrenia and new molecular targets that can be used to develop novel drugs for treatment. Interestingly, several genes that are able to strongly modulate Wnt signaling have been directly implicated.

Single genes associated with Schizophrenia

**DISC1**

One of the first identified and best studied risk genes for schizophrenia is DISC1. DISC1 was first identified in a multi-generation Scottish family that suffered from schizophrenia, bipolar disorder and major depression (28–30). DISC1 was discovered due to a balanced translocation between human chromosomes 1 and 11. The discovery of DISC1 as a risk factor has since been found to occur in one other American family pedigree (31), however, other DISC1 variants have not been found in other genetic studies. The Tsai Laboratory first linked DISC1 to Wnt signaling when it was found that DISC1 itself is able to inhibit the function of GSK3β (32). Furthermore, DISC1 was found to regulate the stability of β-catenin through GSK3β, resulting in increased β-catenin levels that are able to stimulate TCF/LEF-mediated gene transcription. The link between DISC1 and Wnt signaling was made stronger with the observation that a Wnt signaling gene named Dix domain containing 1 (Dixdc1) is a direct interacting partner of DISC1 and together this complex regulates canonical Wnt signaling (33). Overall, the discovery of DISC1 as a regulator of GSK3β and Wnt signaling is quite interesting given that its function is similar to the mood stabilizer lithium, which also inhibits the function of GSK3β and can regulate Wnt signaling. Therefore, the various neurodevelopmental and adult brain functions of DISC1, and its effect on mouse behavior (described below), argue that DISC1 remains important to psychiatric disease.

**Akt**

There is convergent evidence that Akt is a risk factor for schizophrenia. Studies first implicated Akt since the gene is located at human chromosome 14q32, a loci discovered in families with schizophrenia (34). One important finding was the observation that postmortem brain tissue from schizophrenia patients had significantly reduced Akt1 protein levels compared to matched healthy controls (15). Furthermore, the authors also found that application of haloperidol, an antipsychotic drug, dramatically increased the kinase activity of Akt, which led to increased inhibition of GSK3β (15). In support of these findings, Akt was also implicated in schizophrenia in a genome-wide association study (GWAS) meta-analysis performed by the Psychiatric GWAS Consortium (PGC) of 17 independent studies (p < 10^-8) (35). While Akt can phosphorylate GSK3β, it is unclear whether this would directly lead to activation of canonical Wnt signaling. However, a recent study suggests Akt kinase activity is required for lithium’s ability to increase canonical Wnt reporter activity in (22). Additionally, Dvl1 and Wnt1 are able to increase Akt activity leading to β-catenin stabilization (36). Therefore, while general activation of Akt is not sufficient to stimulate Wnt signaling, this suggests under particular circumstances, Akt can participate in and possibly sustain Wnt signaling.

Single nucleotide variants derived from exome sequencing

Recent studies have taken an alternative approach to studying schizophrenia by using a direct sequencing method to detect new mutations in genes in schizophrenia families, where the affected individual and two unaffected parents have undergone exome sequencing (37, 38). The hope is to determine if there are de novo (new) mutations in schizophrenia patients (i.e. not present in the parental DNA), which are deleterious
and may account for disease. Excitingly, many variants and potential mutations were reported which support a de novo mutational paradigm for schizophrenia. Interestingly, there are a number of variants that directly implicate Wnt signaling. From the work of Karayiorgou and Rouleau groups, recent relevant genes implicated include low-density lipoprotein 1 (LRP1), DAB2IP, UBR5, VPS35, TRAPP and PIK3CB. Interestingly, many de novo single nucleotide variants in Wnt signaling genes (e.g. CHD7 and CHD8) have recently been discovered in autism, suggesting defects in Wnt signaling may generally underlie neuropsychiatric disorders (Table 1) (39, 40). While all of these genes have the potential to regulate Wnt signaling, however, one major caveat is that there was no functional follow-up of the new variants/mutations to confirm that these mutations are in fact pathogenic and impact Wnt signaling. It is imperative that future studies appropriately address this issue as many putative mutations will be discovered using sequencing.

Copy number variations

Recent large-scale studies have identified highly penetrant and rare risk factors named copy number variations (CNVs) in schizophrenia, including 22q11 (DiGeorge Syndrome region), 1q21.1, 16p13.1, 3q29, and 15q13.3 (41–45), while a 16p11.2 microduplication confer risk at this locus (46). Moreover, a very recent study also showed that de novo CNVs increase risk for schizophrenia and bipolar disorder (47). Surprisingly, many recurrent CNVs are not specific for a single neuropsychiatric disease, which makes it very difficult to determine why CNVs increase the risk of one disease over another (48–50).

Given that CNVs are significant risk factors for schizophrenia, is there any link between the genes that reside within these loci and Wnt signaling? In the 1q21.1 CNV individuals with a microdeletion have a smaller head size, while patients with a microduplication have a larger head size compared to age-matched controls, suggesting this CNV regulates brain size. Alterations in brain size can be controlled by different mechanisms, however, one hypothesis is that the regulation of the neural stem cell pool during brain development plays a critical role. One gene in the 1q21.1 CNV is B-cell lymphoma 9 (BCL9), which stands out because of its critical function in Wnt signaling. BCL9 is required to keep β-catenin in the nucleus, where it facilitates the binding between β-catenin and TCF/LEF to mediate gene transcription (51–53). Although speculative, one hypothesis may be that alterations in BCL9 levels in patients with the 1q21.1 CNV leads to changes in Wnt signaling, resulting in alterations in neural stem cell proliferation and brain size. In support of this hypothesis, mice in which β-catenin levels have been stabilized and overexpressed display a significantly larger brain than control mice, demonstrating that hyperactivation of Wnt signaling through increased TCF/LEF signaling is sufficient to increase brain size (54).

### Wnt and GSK signaling in schizophrenia

The use of animal models is imperative to the study of genes implicated in schizophrenia. The behavioral phenotypes displayed by animal models (e.g. mice) are unlikely to be the same as in humans; however, these models are still important, as they may reveal underlying defects in brain circuitry, structure, and developmental timing that relate to schizophrenia. Furthermore, there are not specific schizophrenia-related behaviors in animal models, therefore we will discuss Wnt signaling mouse models as they are used to assay a wide variety of behaviors related to psychiatric disorders in general. One of the first animal studies to implicate a core Wnt signaling molecule involved the Dvl1 knockout (KO) mouse (55). These mice display problems with social interaction and prepulse inhibition (PPI) (a defect also observed in patients with schizophrenia), although follow-up studies indicate these deficits are subject to environmental influences (56). However, as Dvl can function in both canonical and non-canonical Wnt signaling pathways, the canonical arm may be of particular importance, as mice in which β-catenin is overexpressed in the adult brain display behavioral phenotypes similar to lithium and antidepressants (57). Furthermore, a recent study showed that APC KO mice display working memory deficits, suggesting core Wnt signaling proteins strongly impact some of the cognitive phenotypes observed in schizophrenia patients (58). Lastly, analysis of mice lacking β-catenin in the forebrain reveals some relatively mild behavior abnormalities related to psychiatric disorders (59).

There are also several mouse models of DISC1 that show a variety of interesting neuropsychiatric phenotypes (60–66). Remarkably, although the models were created using different strategies, there are various common psychiatric-related phenotypes. Many of the models exhibit physical brain abnormalities consistent with those reported in the schizophrenia literature such as increased lateral ventricle size, decreased brain volume, decreased pre-pulse inhibition, and deficits in

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**Table 1. De novo single nucleotide variants (SNVs) in Wnt pathway components identified by exome sequencing in schizophrenia**

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Name</th>
<th>Entrez gene ID</th>
</tr>
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<tbody>
<tr>
<td>DAB2IP</td>
<td>DAB2-interacting protein</td>
<td>51366</td>
</tr>
<tr>
<td>LRP1</td>
<td>Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)</td>
<td>8295</td>
</tr>
<tr>
<td>PIK3CB</td>
<td>Phosphoinositide-3-kinase, catalytic, beta polypeptide</td>
<td>5291</td>
</tr>
<tr>
<td>TRRAP</td>
<td>Transformation/transcription domain-associated protein</td>
<td>4035</td>
</tr>
<tr>
<td>UBR5</td>
<td>Ubiquitin protein ligase E3 component n-recognin 5</td>
<td>4035</td>
</tr>
<tr>
<td>VPS35</td>
<td>Vacuolar protein sorting 35 homolog</td>
<td>55737</td>
</tr>
</tbody>
</table>

**Gene symbol** indicates the name of the gene, **Name** is the official name of the gene, and **Entrez gene ID** is the corresponding ID from the Entrez database.
working memory. However, one of the caveats is that all of the mouse models do not display the same collection of phenotype(s), likely due to the targeting of DISC1 regions in each model, and the different cell populations involved.

Interesting data has also emerged regarding the Akt-GSK3β pathway in the regulation of mouse behavior. Akt1 KO mice are more sensitive to amphetamine-induced disruption of PPI and working memory defects, both observed in schizophrenia patients (15, 67). As Akt phosphorylates and inactivates GSK3α/β, mice that are either heterozygous for Akt, or have targeted inactivating phosphorylation-site mutations in GSK3β, have behavioral phenotypes consistent with Akt’s important role in mediating mood (26, 68, 69). For example, mice overexpressing GSK3β, or knock-in mice carrying mutations at the GSK3α (ser21) or GSK3β (ser9) sites, display a wide-variety of behaviors that are consistent with psychiatric symptoms, such as increased hyperactivity, increased susceptibility to amphetamine-induced hyperactivity, and stress-induced depressive behaviors (27, 70).

Conclusion

Schizophrenia is a complex and multi-factorial disease, making it difficult to study and model in the laboratory, which contributes to the limited progress in developing novel therapeutic agents. In this article, evidence is presented that supports an important role of the Wnt signaling pathway the etiology and treatment of schizophrenia. However, what is unknown is at what time point during development or during adulthood the disruption of Wnt signaling a key risk factor for developing symptoms. With the emerging new genetic findings, together with the development of new animal and human stem cell models, it will be possible to obtain a deeper understanding of the etiology of neuropsychiatric disorders, and how Wnt signaling fits into this fast evolving and complicated puzzle.

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References


