

## HYPOTHESIS

# Hypothesis review: are clathrin-mediated endocytosis and clathrin-dependent membrane and protein trafficking core pathophysiological processes in schizophrenia and bipolar disorder?

KO Schubert<sup>1</sup>, M Föcking<sup>1</sup>, JHM Prehn<sup>2</sup> and DR Cotter<sup>1</sup>

<sup>1</sup>Department of Psychiatry, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Republic of Ireland and <sup>2</sup>Centre for Human Proteomics and Medical Systems Biology, Royal College of Surgeons in Ireland, Dublin, Republic of Ireland

**Clathrin-mediated endocytosis (CME) is the best-characterized mechanism governing cellular membrane and protein trafficking. In this hypothesis review, we integrate recent evidence implicating CME and related cellular trafficking mechanisms in the pathophysiology of psychotic disorders such as schizophrenia and bipolar disorder. The evidence includes proteomic and genomic findings implicating proteins and genes of the clathrin interactome. Additionally, several important candidate genes for schizophrenia, such as dysbindin, are involved in processes closely linked to CME and membrane trafficking. We discuss that key aspects of psychosis neuropathology such as synaptic dysfunction, white matter changes and aberrant neurodevelopment are all influenced by clathrin-dependent processes, and that other cellular trafficking mechanisms previously linked to psychoses interact with the clathrin interactome in important ways. Furthermore, many antipsychotic drugs have been shown to affect clathrin-interacting proteins. We propose that the targeted pharmacological manipulation of the clathrin interactome may offer fruitful opportunities for novel treatments of schizophrenia.**

*Molecular Psychiatry* advance online publication, 11 October 2011; doi:10.1038/mp.2011.123

**Keywords:** bipolar disorder; clathrin interactome; clathrin-mediated endocytosis; membrane trafficking; protein trafficking; schizophrenia

## Introduction

Our understanding of the core pathophysiology of schizophrenia remains poor. While documented genetic and environmental risk factors have provided clues, they have not identified the precise cellular processes that are responsible for the development of the disorder. What emerges is a somewhat complex picture of a polygenic basis to schizophrenia, which is strongly modulated by environmental factors.<sup>1</sup>

There has been a focus on understanding the pathophysiology of schizophrenia and bipolar disorder in terms of signaling pathways. Thus, pathway analysis of genomic, transcriptomic or proteomic findings is now commonplace and a part of most investigations. One difficulty with such analyses is that they rely on the classic and often rather arbitrary definition of canonical pathways in biology. However, it is increasingly clear that these pathways interact with each other in complex and often in

relatively poorly understood ways. Ascribing biological significance to isolated pathways in complex disorders is therefore bound to overlook possible overlapping functional significance. New approaches are needed in order to account for the complexity of the gene and protein interactions, including the application of systems proteomics and systems biology approaches.<sup>2</sup> In this hypothesis review, we describe the potential relevance of the cellular process of membrane and protein trafficking in psychosis pathophysiology. Based on unbiased systems proteomics and genetic studies, we propose that there is now solid evidence for disturbances of proteins interacting with the membrane vesicle coat protein clathrin. We outline how clathrin-mediated cellular processes such as clathrin-mediated endocytosis (CME) and endosomal protein sorting and trafficking could have a significant influence on many documented aspects of psychosis neuropathology, indicating that they may represent a common functional endpoint of many small molecular ‘lesions’. We suggest that a reevaluation of findings from the available ‘omics’ platforms, seeking convergence across different study domains, may prove a fruitful strategy in the search for causes and therapies for complex psychiatric illnesses.

Correspondence: Professor DR Cotter, Department of Psychiatry, Royal College of Surgeons in Ireland, Smurfit Building, Beaumont Hospital, Beaumont Road, Dublin 9, Republic of Ireland.  
E-mail: drcotter@rcsi.ie

Received 24 May 2011; revised 25 August 2011; accepted 29 August 2011

Endocytic mechanisms control the lipid and protein composition of the plasma membrane, thereby regulating how cells interact with their environments. CME and lipid raft-mediated endocytosis represent the two main endocytic routes. CME is the best-characterized endocytic pathway<sup>3</sup> (see Figure 1). In CME, clathrin-interacting proteins recruit cargo molecules at the bilayer membrane into developing clathrin-coated pits, and subsequently form clathrin-coated vesicles for intracellular trafficking of cargo. The network of proteins involved, and the mechanisms of protein–protein interactions are becoming increasingly well understood. Temporal and spatial network analysis have led to a pathway model of CME,<sup>4,5</sup> in which the central ‘hub’ proteins clathrin and adaptor protein complex 2 (AP-2) interact with a multitude of accessory players, the so-called CME interactome, depending on cargo and other circumstances.

Once endocytosed, uncoating of clathrin occurs and the vesicles form early endosomes. These can either recycle directly back to the plasma membrane, transfer to recycling endosomes or enter the late endosomal/lysosomal pathway for degradation. The route taken is, at least in part, determined by members of the Rab family of GTPases,<sup>6</sup> which are associated with distinct endosomal populations and which are central to ensuring that vesicle cargos find their correct destinations.<sup>7,8</sup>

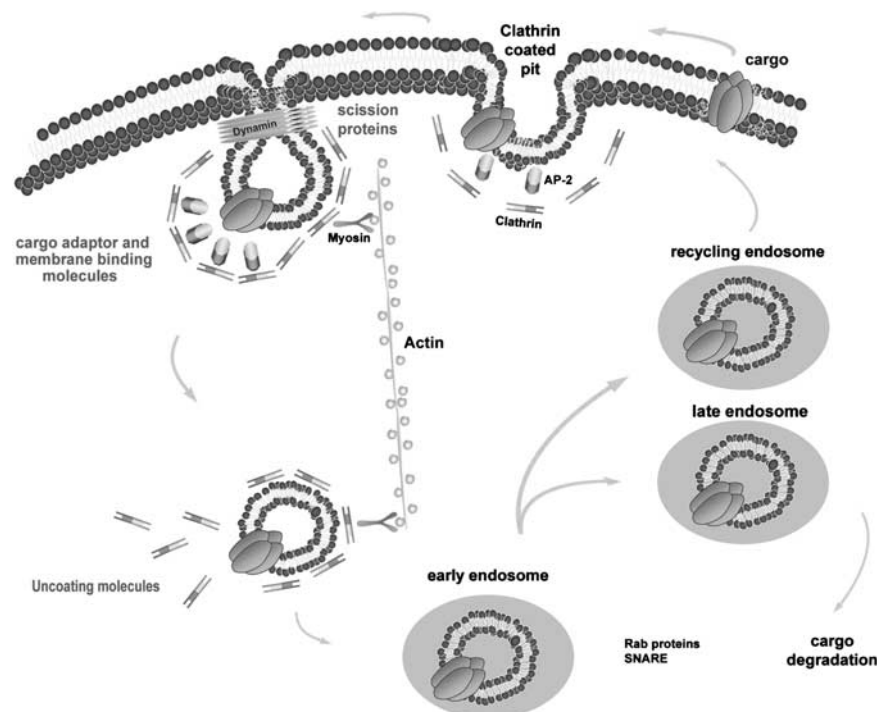
Apart from their role in CME, AP-2- and clathrin-interacting proteins are involved in a wide network of

cellular processes, including endosomal sorting,<sup>9</sup> lysosome biogenesis,<sup>10</sup> mitosis,<sup>11</sup> antigen presentation<sup>12</sup> and cell migration.<sup>13</sup> Members of the CME interactome also have a role in the regulation of many intracellular signaling cascades,<sup>14</sup> and CME is exploited by pathogens to mediate their internalization into cells.<sup>15</sup>

CME proteins and proteins governing cellular trafficking processes have been implicated in proteomic investigations of psychotic disorders, undertaken by our group<sup>16–20</sup> and others.<sup>16,19,21,22</sup> While trafficking changes in schizophrenia have been considered previously,<sup>23–25</sup> a specific role for the clathrin interactome has not been discussed. We therefore reviewed the proteomic and genomic literature for a potential role of CME, and membrane trafficking generally, in psychosis. We further searched for evidence, linking endocytosis with key features of psychosis pathophysiology, namely synaptic function, white matter integrity and neurodevelopment, and studies linking the pharmacology of antipsychotic drugs with endocytic mechanisms.

### Postmortem proteomic investigations find evidence for altered clathrin-interacting proteins in psychotic disorders

Proteomic investigations of postmortem tissue of subjects with psychotic disorders have revealed altered levels of several clathrin- and AP-2-interacting



**Figure 1** Clathrin-mediated endocytosis is characterized by assembly of a coat of clathrin and adaptor proteins such as adaptor protein complex 2 (AP-2) at endocytic membrane sites containing cargo proteins. The sites then form clathrin-coated pits, which are separated from the membrane by scission protein dynamin and its associated machinery. The clathrin-coated vesicle (CCV) is transported intracellularly along actin structures. After uncoating, CCVs merge with early endosomes, and cargo is further processed either for degradation or recycling back to the cell surface.<sup>3</sup>

proteins.<sup>16,19,22</sup> In our own studies, we have identified changes in dynamin-1,<sup>17,18</sup> amphiphysin,<sup>16</sup> AP-2 component alpha adaptin<sup>20</sup> and HSC 70.<sup>17</sup> A review of the literature indicates that at least 25% of the clathrin interactome proteins as defined by Schmid and McMahon<sup>4</sup> have been implicated in postmortem or serum proteomic work to date (see Table 1). We then expanded our search to non-clathrin-interacting proteins, which are involved in the regulation of vesicle scission, actin assembly, vesicle uncoating, tethering of vesicles to early endosomes and vesicle–endosome fusion (for overview see ref. 26), and have included these in our literature review (see Supplementary Table 1).

### Genetic associations find evidence for altered clathrin-interacting genes in psychotic disorders

Genetic studies show that psychosis candidate genes either encode proteins of the clathrin interactome directly, or proteins that are closely functionally linked to clathrin-dependent processes (see Table 1). Particularly strong evidence implicates the clathrin interactome genes Epsin 4 and Stonin 2. Several clathrin interactome genes are included in the top 1000 variations identified by genome-wide association studies to date.<sup>27</sup>

#### *Clathrin-interacting proteins Stonin 2 and Epsin 4 are implicated by direct genetic association*

Stonin 2 has recently been identified as a susceptibility gene for schizophrenia.<sup>28</sup> It is a member of the clathrin interactome and acts as an endocytic adaptor protein for the retrieval of the surface synaptic vesicle protein synaptotagmin. Genetic association between schizophrenia and the *Epsin 4* gene was demonstrated in independent samples in the UK,<sup>29</sup> China<sup>30</sup> and Latin America.<sup>31</sup> Epsin 4, also known as EpsinR, codes for enthoprotin (CLINT1), which interacts with clathrin, AP-1 and GGA2 during coated vesicle formation at endosomal membranes.<sup>32–34</sup> Additional domains of the enthoprotin molecule allow for complex recognition and differential trafficking of ubiquitinated molecules within the cell.<sup>35</sup> Enthoprotin also interacts with a soluble-N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), which is implicated in schizophrenia.<sup>36–38</sup>

#### *Several psychosis candidate genes are functionally linked to clathrin-dependent processes*

Dysbindin is encoded by the *DTNBP1* gene, one of the best supported candidate genes for schizophrenia.<sup>39,40</sup> Dysbindin is a key member of the biogenesis of lysosome-related organelles complex (BLOC-1), which influences presynaptic membrane trafficking, glutamate exocytosis, vesicle biogenesis and SNARE protein-mediated membrane fusion.<sup>41</sup> Vesicular trafficking of SNARE proteins and BLOC-1 components are clathrin-dependent,<sup>42</sup> and members of the CME interactome appear to mediate their functional properties.<sup>43</sup> Additionally, downregulation of dysbindin

disrupts the BLOC-1/AP-3 complex and causes diversion of dopamine D2 receptor (D2R) trafficking from the lysosomal to the recycling pathway.<sup>41,44,45</sup> Further, upregulation of dysbindin has been shown to lead to reduced cell surface expression of the NR1 subunit and to N-methyl-D-aspartate (NMDA) hypofunction, an effect reversed by pharmacological inhibition of CME, suggesting that dysbindin regulates clathrin-dependent NMDA trafficking.<sup>46</sup>

Another BLOC-1 constituent, Muted, is also associated with increased risk of schizophrenia.<sup>47</sup> Further, schizophrenia candidate gene *DISC1* is a key interactor with the protein product of the mutated gene in Huntington's Disease, huntingtin (htt).<sup>48</sup> Htt acts through clathrin-dependent mechanisms to regulate synaptic vesicle fusion. Calcineurin is also strongly implicated in schizophrenia,<sup>49</sup> and it acts through clathrin-dependent endocytosis to regulate growth cone guidance.<sup>50</sup> Further, the brain and primate specific 3.1 isoform of the KCNH2 potassium channel also shows genetic association in schizophrenia,<sup>51</sup> and is regulated by dynamin-dependent endocytosis.<sup>52</sup> The schizophrenia candidate genes *neuregulin*, *ERBB4* and protein phosphatase *PP2B* have roles in postsynaptic NMDAR trafficking<sup>53</sup> by regulating phosphorylation of NMDAR subunits NR2A and NR2B, and stimulating clathrin-dependent receptor internalization.<sup>54,55</sup> These findings raise the possibility that altered protein trafficking may be one shared functional endpoint directly or indirectly influenced by many candidate genes, which contribute to schizophrenia risk.

### Key aspects of schizophrenia neuropathology are mediated by CME

#### *Altered CME may contribute to synaptic pathology*

In the nervous system, CME is crucially involved in the signaling mechanisms of the synapse. CME is important for both presynaptic and postsynaptic functions, and both functions have been implicated in schizophrenia.<sup>56–59</sup> In presynaptic axon terminals, CME is required for the retrieval of synaptic vesicle proteins following neurotransmitter release, and for the recycling of vesicles back to the reserve pool.<sup>8</sup> Also, in presynaptic terminals, protein kinase C (PKC)-induced internalization of dopamine transporter from the synapse is clathrin- and dynamin-dependent.<sup>60</sup> The dopamine transporter is located in presynaptic terminals and facilitates dopamine reuptake from the synaptic cleft, thereby regulating signal strength (for review see ref. 61). Recent imaging studies provide evidence for dysregulated presynaptic membrane expression of the dopamine transporter in first-episode psychosis.<sup>62</sup>

Postsynaptically, in synaptic spines, CME facilitates endocytosis, trafficking and recycling of various neurotransmitter receptors with suspected roles in psychosis neuropathology. For example, dopamine receptor recycling is largely a CME-dependent process,<sup>63–66</sup> and dopamine D2R surface expression is

**Table 1** List of clathrin-interacting proteins associated with schizophrenia and/or bipolar disorder and their proposed function<sup>4</sup>

<i>Proposed function</i>	<i>Protein</i>	<i>Altered protein levels</i>	<i>Genetic association</i>	<i>GWAS association</i>
Vesicle coat proteins	Clathrin heavy chain Clathrin light chain	Martins-de-Souza <i>et al.</i> (2009) <sup>22</sup> Schubert <i>et al.</i> (2011) <sup>20</sup>		+
Heterotetrameric adaptor protein complex (AP)	AP2			
Protein trafficking at endosomal membranes	Epsin 4 (Epsin R)		Pimm <i>et al.</i> (2005) <sup>29</sup> Tang <i>et al.</i> (2006) <sup>30</sup> Escamilla <i>et al.</i> (2008) <sup>31</sup> (-) Zhou <i>et al.</i> (2010) <sup>160</sup>	+
Membrane binding and bending proteins	AP180 CALM HIP1	Chan <i>et al.</i> (2010) <sup>159</sup> Chan <i>et al.</i> (2010) <sup>159</sup>		+
	Amphiphysin 2 Sorting nexin 9	English <i>et al.</i> (2009) <sup>16</sup> Smalla <i>et al.</i> (2008) <sup>161</sup>		
Clustering molecules	Intersectin 2		Vine <i>et al.</i> (2009) <sup>162</sup>	
Scission molecules	Dynamin 1	Prabakaran <i>et al.</i> (2004) <sup>19</sup> Clark <i>et al.</i> (2006) <sup>163</sup> Pennington <i>et al.</i> (2008) <sup>18</sup> Föcking <i>et al.</i> , (2011) <sup>17</sup> Martins-de-Souza <i>et al.</i> (2009) <sup>164</sup> (-) Scarr <i>et al.</i> (2006) <sup>119</sup> Amar <i>et al.</i> (2008) <sup>165</sup>		+
Alternative cargo adaptors (CLASP's)	β-arrestin 1 β-arrestin 2 Numb		(-) Ikeda <i>et al.</i> (2007) <sup>166</sup> Margolis <i>et al.</i> (1997) <sup>167</sup> Passos Gregorio <i>et al.</i> (2006) <sup>168</sup>	
Potential alternative cargo adaptors	Numb-like Tom1 Stonin 2		Potash <i>et al.</i> (2008) <sup>169</sup> Luan <i>et al.</i> (2011) <sup>28</sup>	+
Uncoating molecules	Synaptojanin  Hsc 70	Föcking <i>et al.</i> (2011) <sup>17</sup> Schubert <i>et al.</i> (2011) <sup>20</sup> Sivagnanasundaram <i>et al.</i> (2007) <sup>172</sup> Martins-de-Souza <i>et al.</i> (2009) <sup>164</sup>	Saito <i>et al.</i> (2001) <sup>170</sup> Stopkova <i>et al.</i> (2004) <sup>171</sup>	+

Published associations between each protein/gene and psychotic disorders were identified by Pubmed searches linking each protein/gene to 'schizophrenia' or 'bipolar disorder'. For the proteomic literature, the search strategy was based upon our recent review of the literature<sup>105</sup> and complemented by reviews of recent literature. Genome-wide association studies (GWAS) associations refers to genes listed among the top 1000 gene associations in the SZGene database.<sup>27</sup> Studies labeled with (-) specifically tested for the gene/protein in question and failed to demonstrate disease-associated changes. Many clathrin- and AP-2 interactome proteins<sup>4</sup> have not yet been tested for associations with either disease. These include AP-1, -3, -4, epsin 1-3, HIP1R, amphiphysin 1, connectin, sorting nexin 9, eps15, eps15R, intersectin 1, HIV-rev interacting protein, dynamin 2, dynamin 3, ARM, Dab2, NECAP-1, AAK, and auxilin.

regulated by CME.<sup>67</sup> Thus, CME directly regulates dopamine receptor numbers at the postsynaptic site, known to be an important determinant of dopamine signal strength. Similarly, NMDA glutamate receptors, which are thought to be functionally impaired in psychosis,<sup>68,69</sup> are endocytosed by clathrin-dependent mechanisms.<sup>53</sup> Other receptors that are implicated in schizophrenia such as AMPA<sup>70,71</sup> and GABA(A)<sup>72,73</sup> are also regulated by clathrin-dependent mechanisms.

Up- and downstream of the synapse, interactions of clathrin-interacting proteins with intracellular trafficking pathways are also well described. In axon terminals, clathrin interactors mediate the incorporation of proteins such as SNARE proteins, into synaptic vesicles, thereby indirectly modulating their functional properties.<sup>43</sup> SNAREs are crucial for synaptic vesicle fusion and exocytosis, and SNARE-related abnormalities are well documented in psychosis.<sup>38,74–76</sup> These functions, as mentioned earlier, are mediated by BLOC-1, another constituent of clathrin-coated vesicles. Postsynaptically, clathrin-interacting proteins have a part in endosomal sorting of cargo molecules through interaction with Rab proteins.<sup>7</sup> Clathrin-dependent processes therefore crucially contribute to the maintenance of synaptic homeostasis, and to modulation of dysbindin, BLOC-1, and SNARE function (Figure 2). Disturbances of the synapse are thought to be central to psychotic disorders,<sup>58,77</sup> with particular emphasis on the dopamine and glutamate systems.<sup>1</sup> We argue that CME and clathrin-dependent protein trafficking may have an important role in synapse pathophysiology.

**Altered CME may contribute to white matter pathology**  
Oligodendroglial cells synthesize the central nervous system myelin sheath by wrapping multiple layers of specialized membrane around the axon. During active myelinogenesis, they exhibit an extraordinarily high production of membrane.<sup>78</sup> This requires a sophisticated membrane-trafficking machinery,<sup>79</sup> which involves oligodendrocytes reacting to signals of the growing neuron and adjusting the relative levels of exocytosis and endocytosis of the major myelin protein proteolipid protein. Recent data reveal that endocytosis of proteolipid protein is clathrin-dependent.<sup>80</sup> White matter disturbances are a widely recognized feature of schizophrenia neuropathology.<sup>81</sup> We speculate that altered CME during myelinogenesis could significantly contribute to these findings.

**Altered CME may contribute to neurodevelopmental disturbances**

Endocytic recycling is important for developmental processes, especially where rapid mobilization of plasma membrane and polarized membrane growth occurs.<sup>82</sup> In polarized neurons, endosomal membrane trafficking is required for activity-induced growth and remodeling of dendritic spines.<sup>83</sup> Neurite outgrowth and neurite extension are regulated by

clathrin-dependent recycling in endosomes and coordinated exocytosis of a specialized endocytic compartment.<sup>84–86</sup> A recent study demonstrated that CME drives repulsive growth cone guidance in developing axons,<sup>87</sup> suggesting that the balance between membrane addition and removal dictates bidirectional axon guidance during brain development. The concept that neurodevelopmental disturbances significantly contribute to psychotic disorders<sup>88</sup> is well supported by epidemiological, genetic, developmental and imaging studies.<sup>89</sup> We argue that disturbed endocytic mechanisms could be one important underlying molecular mechanism.

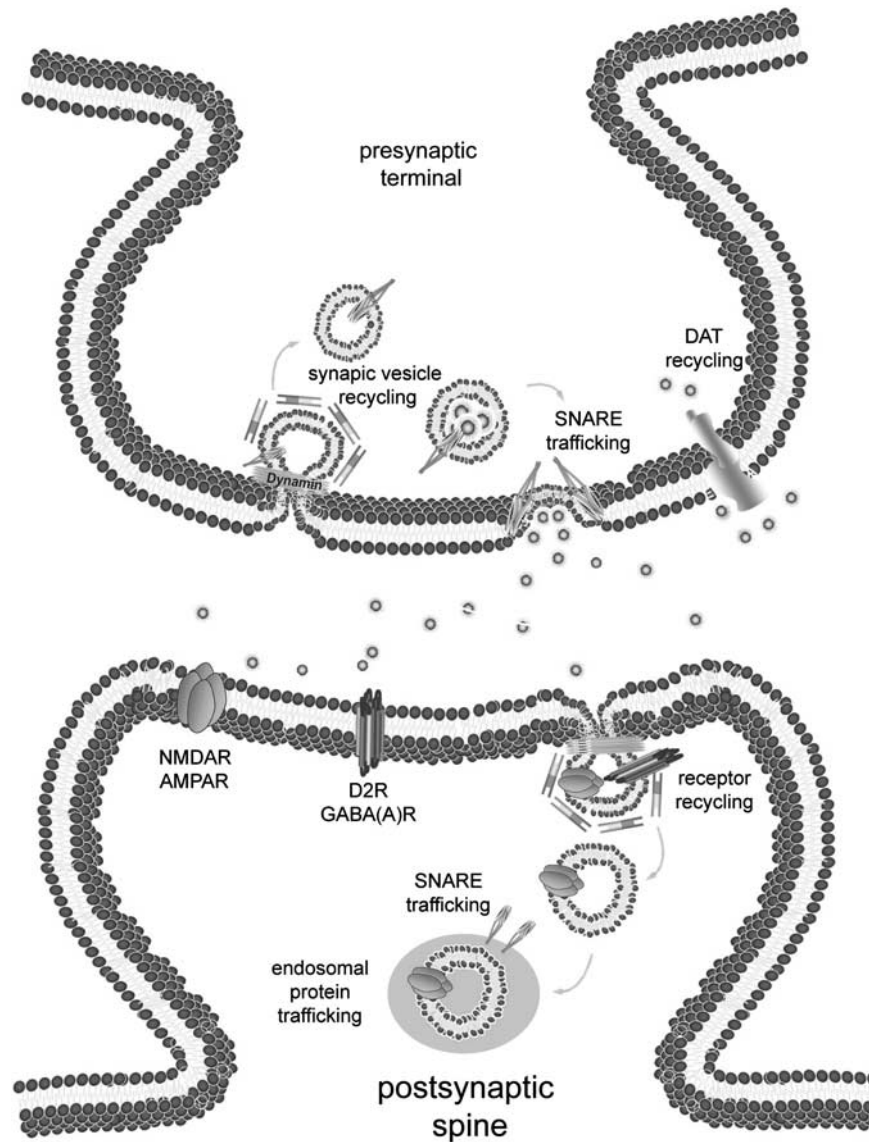
### Antipsychotic medications and lithium directly interact with clathrin-interacting proteins

*Phenothiazines are strong inhibitors of CME*

Phenothiazines such as chlorpromazine and trifluoperazine were the first molecules found to have specific clinical antipsychotic efficacy, and were the cornerstone of psychosis treatment for several decades since their discovery in the 1940s.<sup>90</sup> They strongly inhibit CME by preventing the binding of adaptor protein complexes such as AP-2 to the plasma membrane, thereby effectively abolishing the formation of coated pits.<sup>91</sup> They also appear to relocate clathrin/AP-2 complexes from the plasma membrane to intracellular endosomal structures.<sup>92</sup>

*First- and second-generation antipsychotics and lithium directly interact with clathrin-interacting protein  $\beta$ -arrestin*

Antipsychotic agents of various drug classes differ considerably in their antagonistic properties to D2Rs and their effects on D2R signaling. In search of a common mechanism that may explain their clinical effectiveness, Masri *et al.*<sup>93</sup> recently demonstrated that first- and second-generation antipsychotics strongly antagonize recruitment of the clathrin-interacting protein  $\beta$ -arrestin to D2Rs following stimulation with dopamine or quipirole. The authors further demonstrated that this inhibition effectively prevented  $\beta$ -arrestin-mediated signaling through the Akt/glycogen synthase kinase-3 pathway.<sup>94</sup> It is likely that the observed effects influence receptor endocytosis via CME on some level, given its primary function as an endocytic adaptor. Indeed, inhibition of clathrin-mediated, dopamine-induced D2R internalization has been reported for the typical antipsychotic haloperidol.<sup>95</sup> For other classes of antipsychotics, effects on receptor endocytosis are less clear.<sup>96</sup> The mood stabilizer lithium, which influences many signaling pathways,<sup>97,98</sup> has similar effects on  $\beta$ -arrestin and the Akt/glycogen synthase kinase-3 pathway.<sup>99</sup> Interestingly, clomipramine and its active metabolite desmethylclomipramine have recently been shown to potently inhibit autophagic flux.<sup>100</sup> Autophagy is a key process important for cellular homeostasis and the degradation of cargos ranging



**Figure 2** Pre- and postsynaptic functions associated with psychosis neuropathology are dependent on clathrin-mediated mechanisms. Presynaptic vesicles are recycled from the synapse via clathrin-mediated endocytosis (CME). Surface expression of the dopamine transporter (DAT) is regulated through CME. Postsynaptically, endocytosis and endosomal trafficking of G-protein-coupled receptors such as D2 and GABA(A) as well as heteromers such as *N*-methyl-D-Aspartate (NMDA) and AMPA receptors are CME-dependent. Trafficking of the SNARE protein complex pre-and postsynaptically is CME-mediated.

from proteins to organelles with direct links to the endosome–lysosome pathway.

#### *Polyunsaturated omega-3 fatty acids modulate CME*

Recent data support clinical effectiveness for polyunsaturated omega-3 fatty acids (PUFAs) in preventing progression from pre-psychotic at-risk states to first-episode psychosis.<sup>101</sup> A large epidemiologic investigation of over 33 000 women concluded that high intake of omega-3 fatty acids during pregnancy decreases the incidence of schizophrenia in the offspring.<sup>102</sup> *In vitro*, PUFAs stimulate CME and synaptic vesicle recycling.<sup>103</sup> In cellular membranes, PUFAs act as cone-shaped lipids that induce a

positive curvature of the membrane leaflet,<sup>104</sup> thereby enhancing membrane trafficking.<sup>103</sup> It is thus possible that PUFAs exert at least some of their protective effects through stabilization of disordered membrane endocytosis.

#### **Clathrin-interacting proteins and other neuropsychiatric disorders**

The reported changes of clathrin-interacting proteins apply equally to schizophrenia and bipolar disorder,<sup>105</sup> which echoes growing evidence suggesting that schizophrenia and bipolar disorder share genetic vulnerabilities.<sup>106,107</sup> Clathrin interactome

dysfunction may therefore be a risk factor for psychotic illness generally, rather than for any one specific disorder.

This point is reinforced by observations in other neuropsychiatric disorders. In Huntington's disease, where psychosis can be prominent at first presentation,<sup>108</sup> the mutant protein interacts with Rab5 to regulate the fusion of clathrin-derived endocytic vesicles.<sup>109</sup> In Alzheimer's disease, clathrin-dependent endocytosis of amyloid precursor protein is believed to be the rate-limiting step in the production of amyloid- $\beta$  peptide whose accumulation is the hallmark of the disorder.<sup>110–112</sup> Further, clathrin was found to interact with two proteins critically involved in the pathogenesis of Parkinson's disease,  $\alpha$ -synuclein and DJ-1.<sup>113</sup>

Rab proteins as a family have roles in membrane trafficking, synaptic function, neurite growth and brain development, and as such they make plausible candidates to be involved in neuropsychiatric disorders (for review see ref. 114). Rab7 and Rab11 act downstream of CME on clathrin-coated vesicles. Rab7 mutations are responsible for Charcot-Marie-Tooth-type 2B neuropathy.<sup>115</sup> Interestingly, Charcot-Marie-Tooth is also associated with another protein with CME activity, Dynamin 2.<sup>116</sup> Rab 39B is believed to be involved in endosomal trafficking and to have roles in neurite arborization and growth cone development.<sup>117</sup> It has been associated with mental retardation, epilepsy and autism.<sup>118</sup> Taken together, these observations show that alterations of proteins even remotely linked to clathrin-dependent processes can lead to significant disturbances of the nervous system. It is intriguing to speculate that perturbation of the clathrin interactome increases the likelihood of developing psychosis as part of the phenotype in any disorder, even if the underlying genetic lesion results in more obvious changes in other pathways. This idea would also resonate with the polygenic concept of schizophrenia and bipolar disorder, where a wide range of genetic alterations of small effect appear to produce similar phenotypes.<sup>1</sup>

### Limitations in evidence for role of the clathrin interactome in psychotic disorders

The involvement of clathrin interactors in several key areas of psychosis is intriguing, but not all lines of evidence support this view. For example, while proteomic studies of psychotic disease individually have identified many members of the clathrin interactome, overlap between the results is incomplete. Among CME core proteins, only dynamin-1 has been found consistently dysregulated across several investigations and brain regions,<sup>105</sup> and postmortem western blot analyses of dynamin 1 expression in several brain areas of subjects with bipolar disorder failed to demonstrate altered protein levels.<sup>119</sup> Further, as mentioned above, mutations of dynamin 2 lead to peripheral neuropathy but not psychotic symptoms or cognitive decline.<sup>120</sup> This raises doubts over the roles

of dynamins, in general, in psychosis pathogenesis, and may be due to their involvement in several non-endocytic pathways.<sup>4</sup> Further, while the effects of most, but not all, antipsychotic medications on  $\beta$ -arrestin and D2R internalization are well documented (see above), the consequences on CME are less clear.<sup>96</sup> Further work is needed to clarify the expression levels of CME and trafficking proteins in schizophrenia brain and tissues, and the effects of antipsychotic drugs on their expression and function.

In pulling together a vast amount of information in an integrative fashion, as was done in our literature search, there undoubtedly remains a range of uncertainty associated with individual aspects. For example, protein-protein interaction data could come from human or nonhuman species, or be based on interactions characterized extensively *in vivo*, or based on yeast two-hybrid screens. The clathrin interactome is likely to include a variety of accessory proteins in different cell types, and alternative isoforms for many CME genes have been described in eukaryotic cells.<sup>4</sup> Clarification of these concerns in relation to psychotic disorders will become important in future investigations.

### From endocytosis to membrane- and protein trafficking: CME may be only one piece of the puzzle

The trafficking of membranes and proteins within cells, and their cycling to and from sites of action are complex processes, which are tightly regulated. Proteins move through several organelles on their journey from synthesis at the nucleus to their final destination. Owing to the interdependence and mutual control of these steps, disturbances at any point of the journey are likely to lead to knock-on effects up- and downstream. The following observations illustrate that the endocytic mechanisms discussed so far must be put into a wider context.

#### Protein-scaffold interactions

Dynamic trafficking of receptor proteins from the endoplasmatic reticulum through the golgi network to the neuronal surface involves interactions with synaptic scaffolding proteins such as SAP-102, PSD-95, PSD-93 and SAP-97. These scaffolds mediate insertion of the receptors into the membrane, and regulate receptor subunit composition at the cell surface (for overview see ref. 53). Transcript analysis in postmortem schizophrenia and bipolar disorder brains has revealed decreased levels of SAP-97, SAP-102 and PSD 95.<sup>24,25,121</sup> The gene *DLG1*, which encodes SAP-97, has been strongly linked to schizophrenia susceptibility.<sup>122</sup> A similar link exists for *DLG4*, which encodes PSD-95.<sup>123</sup>

#### Protein phosphorylation and endocytosis

Clathrin-dependent endocytosis of postsynaptic receptor proteins is mediated primarily by phosphorylation of internalization motifs within receptor molecules. Abnormalities of the phosphorylation

steps during NMDA receptor endocytosis have been demonstrated in psychosis.<sup>124,125</sup> Further, schizophrenia candidate gene neuregulin 1<sup>126</sup> influences phosphorylation of receptor molecules, thus regulating their endocytosis rates.<sup>127</sup>

#### *Proteasomal degradation (and degradation via autophagy)*

Many receptor proteins undergo activity-dependent protein degradation by the ubiquitin–proteasome system.<sup>128</sup> In postmortem schizophrenia and bipolar disorder tissue, several studies found dysregulation of ubiquitin–proteasome-related genes.<sup>129–134</sup> Similarly, recent genetic investigations found evidence for an association of the ubiquitin–proteasome pathway with psychotic disorders, as well as correlations of several ubiquitin–proteasome pathway proteins with clinical dimensions in schizophrenia.<sup>135</sup>

#### *Phosphoinositides and the endocytic pathway*

Phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), a membrane-bound phosphoinositide protein, binds the AP2 complex during the initial stages of CME.<sup>136,137</sup> Depleting PIP<sub>2</sub> in living cells results in AP2 mislocalization and inhibition of endocytosis.<sup>138–142</sup> PIP<sub>2</sub> is important in psychotic disorders, as it is part of the phosphoinositide–protein kinase C (PI–PKC) pathway, which is inhibited by many psychotropic medications. Findings from postmortem studies provide evidence for PI–PKC hyperfunction in psychosis. This hyperfunction may in part be counteracted by the mood stabilizer lithium, which was shown to deplete PIP<sub>2</sub> though reduction of brain inositol.<sup>143</sup> It is possible that phosphoinositide disturbances contribute to the proposed CME alterations in psychosis.

#### *Caveolin-dependent endocytosis and psychiatric disorders*

Disruption of the scaffolding protein Caveolin-1 was recently identified as a rare structural variant associated with schizophrenia.<sup>144</sup> Caveolins are integral parts of lipid raft microdomains in the cellular membrane, which can facilitate ligand-induced, clathrin-independent endocytosis of a range of neurotransmitter receptors.<sup>145</sup> Experiments in Caveolin-1 knockout animals show that disruption of the gene results in behavioral effects in keeping with propychoptic states.<sup>146</sup> This suggests that clathrin-independent mechanisms of receptor endocytosis and trafficking can have a part in generation of the psychosis phenotype.

#### *The role of endosomal trafficking*

As described previously, the consequences of functional disruption of the BLOC-1 complex are well documented, and have attracted much attention from the schizophrenia field due to the genetic association of BLOC components dysbindin and muted with the disorder. BLOC-1 regulates membrane protein targeting to synaptic vesicles, lysosomes and lysosome-related organelles from transferrin-receptor-

positive endosomes.<sup>44,147,148</sup> Taking BLOC-1 as a starting point, Ryder and Faundez have reviewed the genetic literature for evidence supporting endosomal trafficking pathways in schizophrenia,<sup>23</sup> and concluded that an ‘endosomal hypothesis’ accommodates the polygenetic nature of genetic association data as well as neurodevelopmental aspects of the disease. We support this view in part; however, we would stress that endosomal trafficking defects may only represent one piece of a larger, pan-cellular endocytosis and trafficking problem.

### **Conclusion**

The depth and breadth of the findings in support of trafficking and CME abnormalities in psychosis provide support for a new understanding of schizophrenia as a disorder of membrane and receptor trafficking. Compellingly, the data converge from such broad research areas as genetics, postmortem brain proteomics, neuropharmacology, receptor trafficking, synaptic plasticity and developmental neuroscience. The data implicate synaptic vesicle and receptor endocytosis both at presynaptic and postsynaptic sites. Both clathrin-dependent, particularly, and -independent mechanisms are involved.

Any process that contributes in a significant way to the pathophysiology of schizophrenia should explain core aspects of the disease such as neurodevelopmental aspects, synaptic pathology, heritability and neuroleptic response. Thus, the influence of CME on synaptic plasticity,<sup>53</sup> axon growth cone development<sup>84</sup> and myelination<sup>80</sup> is important. The proteomic data were the starting point for this novel hypothesis concerning CME and schizophrenia, and these data implicated ‘core’ proteins of the CME interactome, namely dynamin1, amphiphysin, AP-2 and HSC70. Given the clinical efficacy of antipsychotic medications, it is reasonable to expect that these drugs would influence CME. While this has not yet been specifically assessed, it has been shown that these agents influence core members of the clathrin interactome.<sup>93</sup> Further, lithium and PUFAs also influence CME.<sup>99,103</sup> In terms of heritability, many genes have roles either directly or indirectly in CME or cellular protein trafficking, including Stonin 2, Epsin 4, DTNBP1, muted, neuregulin, ERBB4, PP2B, calcineurin, KCNH2, DLG1 and 4. We consider that this evidence is compelling and points to a need to seriously consider the potential influence of this process in psychosis.

CME is central to so many essential cellular processes<sup>3</sup> that the effect of any dysregulation, however subtle, could be widespread and beyond those typically understood to be a part of psychosis. For example, CME has important roles in endocrine function<sup>149</sup> and the possibility that reported endocrine abnormalities in schizophrenia are a consequence of altered CME should be considered. Equally, cytoskeletal proteins are implicated in

schizophrenia,<sup>16,18,19</sup> and of these, actin is particularly influenced by CME.<sup>150</sup> Thus, considering schizophrenia as a disorder of CME and trafficking may allow us to gain new insights into the disorder. Future work will need to clarify whether the CME model relates to risk of illness, or direct causation. As discussed in a recent commentary, there are ‘too many roads not taken’.<sup>151</sup> We propose that discovery proteomics has provided candidates that may allow us to understand the disease pathogenesis from a different angle, that of CME.

The hypothesis that schizophrenia is a disorder of altered trafficking is not a new one and has been considered previously.<sup>23</sup> However, the hypothesis that altered CME is responsible for the pathophysiology of schizophrenia is a novel hypothesis. On balance, we show evidence that both processes are implicated, for the evidence extends from clathrin interactome proteins (Table 1) to clathrin-associated and downstream trafficking proteins that interact with both clathrin-dependent and -independent trafficking processes (Supplementary Table 1). The evidence also supports the view that alterations in CME and membrane trafficking are associated with a neuropsychiatric vulnerability generally,<sup>114</sup> although links are yet to be firmly established.<sup>5</sup>

Future work should consider systemic as well as neurological measures of altered clathrin-dependent and clathrin-independent endocytosis. For example, do serum<sup>152</sup> and brain alterations<sup>16</sup> in the iron-binding protein transferrin represent a biomarker of altered CME in psychosis, and could iron dysregulation,<sup>153</sup> induced by altered CME, lead to abnormal myelin development in schizophrenia? Animal models involving dysregulated CME function and trafficking need to be explored as potential animal models of schizophrenia. For example, exploring the behavioral phenotype of inhibitors of CME such as dynasore<sup>154</sup> may offer insights into psychosis and antipsychotic effects. The central role of CME in viral entry into cells<sup>15</sup> is intriguing and raises the possibility that CME abnormalities may lead to an increased vulnerability to prenatal viral infection, itself a risk factor for schizophrenia.<sup>155,156</sup>

Despite the appreciation of the high cost of schizophrenia to society and the inadequacy of current treatments, drug discovery in schizophrenia is currently at an impasse, and major big pharmaceutical companies are leaving this area of drug development.<sup>157,158</sup> Against this background, we propose that the manipulation of CME offers a genuinely novel ‘pharmacological toolbox’<sup>91</sup> for the treatment of psychosis. Further, the therapeutic effects of the modulation of CME may not be specific to psychosis but apply more generally to other systemic neurological and neuropsychiatric disorders.<sup>5</sup> To our knowledge, the process of CME has not been probed previously for therapeutic effects in psychosis, or relevant animal models, and in our view such work may offer wonderful opportunities in the future.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

We wish to acknowledge technical support by Peter Knief, PhD and Megan Ramsey. KOS is supported by a Molecular Medicine Ireland Clinician Scientist Fellowship. This work was supported by Molecular Medicine Ireland, Science Foundation Ireland, NARSAD, and the Stanley Medical Research Institute.

## References

- 1 van Os J, Kapur S. Schizophrenia. *Lancet* 2009; **374**: 635–645.
- 2 Kleinman JE, Law AJ, Lipska BK, Hyde TM, Ellis JK, Harrison PJ et al. Genetic neuropathology of schizophrenia: new approaches to an old question and new uses for postmortem human brains. *Biol Psychiatry* 2011; **69**: 140–145.
- 3 Doherty GJ, McMahon HT. Mechanisms of endocytosis. *Annu Rev Biochem* 2009; **78**: 857–902.
- 4 Schmid EM, McMahon HT. Integrating molecular and network biology to decode endocytosis. *Nature* 2007; **448**: 883–888.
- 5 McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol* 2011; **12**: 517–533.
- 6 Zerial M, McBride H. Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol* 2001; **2**: 107–117.
- 7 Stenmark H. Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol* 2009; **10**: 513–525.
- 8 Haucke V, Neher E, Sigrist SJ. Protein scaffolds in the coupling of synaptic exocytosis and endocytosis. *Nat Rev Neurosci* 2011; **12**: 127–138.
- 9 Jovic M, Sharma M, Rahajeng J, Caplan S. The early endosome: a busy sorting station for proteins at the crossroads. *Histol Histopathol* 2010; **25**: 99–112.
- 10 Luzio JP, Gray SR, Bright NA. Endosome-lysosome fusion. *Biochem Soc Trans* 2010; **38**: 1413–1416.
- 11 Fu W, Jiang Q, Zhang C. Novel functions of endocytic player clathrin in mitosis. *Cell Res* 2011; doi:10.1038/cr.2011/106 (in press).
- 12 Malhotra S, Kovats S, Zhang W, Coggeshall KM. B cell antigen receptor endocytosis and antigen presentation to T cells require Vav and dynamin. *J Biol Chem* 2009; **284**: 24088–24097.
- 13 Shieh JC, Schaar BT, Srinivasan K, Brodsky FM, McConnell SK. Endocytosis regulates cell soma translocation and the distribution of adhesion proteins in migrating neurons. *PLoS One* 2011; **6**: e17802.
- 14 Hoeller D, Volarevic S, Dikic I. Compartmentalization of growth factor receptor signalling. *Curr Opin Cell Biol* 2005; **17**: 107–111.
- 15 Marsh M, Helenius A. Virus entry: open sesame. *Cell* 2006; **124**: 729–740.
- 16 English JA, Dicker P, Focking M, Dunn MJ, Cotter DR. 2-D DIGE analysis implicates cytoskeletal abnormalities in psychiatric disease. *Proteomics* 2009; **9**: 3368–3382.
- 17 Focking M, Dicker P, English JA, Schubert KO, Dunn MJ, Cotter DR. Common proteomic changes in the hippocampus in schizophrenia and bipolar disorder and particular evidence for involvement of cornu ammonis regions 2 and 3. *Arch Gen Psychiatry* 2011; **68**: 477–488.
- 18 Pennington K, Beasley CL, Dicker P, Fagan A, English J, Pariante CM et al. Prominent synaptic and metabolic abnormalities revealed by proteomic analysis of the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder. *Mol Psychiatry* 2008; **13**: 1102–1117.
- 19 Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry* 2004; **9**: 684–697, 643.
- 20 Schubert KO, Focking M, Dicker P, Dunn MJ, Cotter DR. Proteomic analysis of the basic sub-proteome (pH 6–11) in the

- hippocampus in schizophrenia and bipolar affective disorder. *Schizophr Res* 2010; **117**: 372.
- 21 Martins-De-Souza D, Dias-Neto E, Schmitt A, Falkai P, Gormanns P, Maccarrone G *et al.* Proteome analysis of schizophrenia brain tissue. *World J Biol Psychiatry* 2010; **11**: 110–120.
  - 22 Martins-de-Souza D, Gattaz WF, Schmitt A, Rewerts C, Marangoni S, Novello JC *et al.* Alterations in oligodendrocyte proteins, calcium homeostasis and new potential markers in schizophrenia anterior temporal lobe are revealed by shotgun proteome analysis. *J Neural Transm* 2009; **116**: 275–289.
  - 23 Ryder PV, Faundez V. Schizophrenia: the 'BLOC' may be in the endosomes. *Sci Signal* 2009; **2**: pe66.
  - 24 Kristiansen LV, Meador-Woodruff JH. Abnormal striatal expression of transcripts encoding NMDA interacting PSD proteins in schizophrenia, bipolar disorder and major depression. *Schizophr Res* 2005; **78**: 87–93.
  - 25 McCullumsmith RE, Kristiansen LV, Beneyto M, Scarr E, Dean B, Meador-Woodruff JH. Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. *Brain Res* 2007; **1127**: 108–118.
  - 26 Wieffer M, Maritzen T, Haucke V. SnapShot: endocytic trafficking. *Cell* 2009; **137**: 382. e381–383.
  - 27 Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ *et al.* Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet* 2008; **40**: 827–834.
  - 28 Luan Z, Zhang Y, Lu T, Ruan Y, Zhang H, Yan J *et al.* Positive association of the human STON2 gene with schizophrenia. *Neuroreport* 2011; **22**: 288–293.
  - 29 Pimm J, McQuillin A, Thirumalai S, Lawrence J, Quedest D, Bass N *et al.* The Epsin 4 gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. *Am J Hum Genet* 2005; **76**: 902–907.
  - 30 Tang RQ, Zhao XZ, Shi YY, Tang W, Gu NF, Feng GY *et al.* Family-based association study of Epsin 4 and schizophrenia. *Mol Psychiatry* 2006; **11**: 395–399.
  - 31 Escamilla M, Lee BD, Ontiveros A, Raventos H, Nicolini H, Mendoza R *et al.* The epsin 4 gene is associated with psychotic disorders in families of Latin American origin. *Schizophr Res* 2008; **106**: 253–257.
  - 32 Saint-Pol A, Yélamos B, Amessou M, Mills IG, Dugast M, Tenza D *et al.* Clathrin adaptor epsinR is required for retrograde sorting on early endosomal membranes. *Dev Cell* 2004; **6**: 525–538.
  - 33 Wasiak S, Legendre-Guillemain V, Puertollano R, Blondeau F, Girard M, deHeuvel E *et al.* Enthoprotin: a novel clathrin-associated protein identified through subcellular proteomics. *J Cell Biol* 2002; **158**: 855–862.
  - 34 Wasiak S, Denisov AY, Han Z, Leventis PA, de Heuvel E, Boulianne GL *et al.* Characterization of a gamma-adaptin ear-binding motif in enthoprotin. *FEBS Lett* 2003; **555**: 437–442.
  - 35 Chen H, De Camilli P. The association of epsin with ubiquitinated cargo along the endocytic pathway is negatively regulated by its interaction with clathrin. *Proc Natl Acad Sci USA* 2005; **102**: 2766–2771.
  - 36 Antonin W, Holroyd C, Fasshauer D, Pabst S, Von Mollard GF, Jahn R. A SNARE complex mediating fusion of late endosomes defines conserved properties of SNARE structure and function. *EMBO J* 2000; **19**: 6453–6464.
  - 37 Chidambaram S, Müllers N, Wiederhold K, Haucke V, von Mollard GF. Specific interaction between SNAREs and epsin N-terminal homology (ENTH) domains of epsin-related proteins in trans-Golgi network to endosome transport. *J Biol Chem* 2004; **279**: 4175–4179.
  - 38 Honer WG, Falkai P, Bayer TA, Xie J, Hu L, Li HY *et al.* Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. *Cereb Cortex* 2002; **12**: 349–356.
  - 39 Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ *et al.* Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. *J Clin Invest* 2004; **113**: 1353–1363.
  - 40 Weickert CS, Rothmond DA, Hyde TM, Kleinman JE, Straub RE. Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients. *Schizophr Res* 2008; **98**: 105–110.
  - 41 Ji Y, Yang F, Papaleo F, Wang HX, Gao WJ, Weinberger DR *et al.* Role of dysbindin in dopamine receptor trafficking and cortical GABA function. *Proc Natl Acad Sci USA* 2009; **106**: 19593–19598.
  - 42 McPherson PS. Proteomic analysis of clathrin-coated vesicles. *Proteomics* 2010; **10**: 4025–4039.
  - 43 Miller SE, Collins BM, McCoy AJ, Robinson MS, Owen DJ. A SNARE-adaptor interaction is a new mode of cargo recognition in clathrin-coated vesicles. *Nature* 2007; **450**: 570–574.
  - 44 Di Pietro SM, Falcón-Pérez JM, Tenza D, Setty SR, Marks MS, Raposo G *et al.* BLOC-1 interacts with BLOC-2 and the AP-3 complex to facilitate protein trafficking on endosomes. *Mol Biol Cell* 2006; **17**: 4027–4038.
  - 45 Salazar G, Craig B, Styers ML, Newell-Litwa KA, Doucette MM, Wainer BH *et al.* BLOC-1 complex deficiency alters the targeting of adaptor protein complex-3 cargoes. *Mol Biol Cell* 2006; **17**: 4014–4026.
  - 46 Jeans A, Malins R, Padamsey Z, Reinhart M, Emptage N. Increased expression of dysbindin-1A leads to a selective deficit in NMDA receptor signaling in the hippocampus. *Neuropharmacology* 2011; doi:10.1016/j.neuropharm.2011.08.007 (in press).
  - 47 Morris DW, Murphy K, Kenny N, Purcell SM, McGhee KA, Schweiger S *et al.* Dysbindin (DTNBP1) and the biogenesis of lysosome-related organelles complex 1 (BLOC-1): main and epistatic gene effects are potential contributors to schizophrenia susceptibility. *Biol Psychiatry* 2008; **63**: 24–31.
  - 48 Boxall R, Porteous DJ, Thomson PA. DISC1 and Huntington's disease—overlapping pathways of vulnerability to neurological disorder? *PLoS One* 2011; **6**: e16263.
  - 49 Kvaajo M, McKellar H, Gogos JA. Molecules, signaling, and schizophrenia. *Curr Top Behav Neurosci* 2010; **4**: 629–656.
  - 50 Sun T, Wu XS, Xu J, McNeil BD, Pang ZP, Yang W *et al.* The role of calcium/calmodulin-activated calcineurin in rapid and slow endocytosis at central synapses. *J Neurosci* 2010; **30**: 11838–11847.
  - 51 Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V *et al.* A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. *Nat Med* 2009; **15**: 509–518.
  - 52 Ramström C, Chapman H, Viitanen T, Afrasiabi E, Fox H, Kivelä J *et al.* Regulation of HERG (KCNH2) potassium channel surface expression by diacylglycerol. *Cell Mol Life Sci* 2010; **67**: 157–169.
  - 53 Lau CG, Zukin RS. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat Rev Neurosci* 2007; **8**: 413–426.
  - 54 Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY *et al.* Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 2005; **8**: 1051–1058.
  - 55 Hahn CG, Wang HY, Cho DS, Talbot K, Gur RE, Berrettini WH *et al.* Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nat Med* 2006; **12**: 824–828.
  - 56 Sawada K, Barr AM, Nakamura M, Arima K, Young CE, Dwork AJ *et al.* Hippocampal complexin proteins and cognitive dysfunction in schizophrenia. *Arch Gen Psychiatry* 2005; **62**: 263–272.
  - 57 Fung SJ, Sivagnanasundaram S, Weickert CS. Lack of change in markers of presynaptic terminal abundance alongside subtle reductions in markers of presynaptic terminal plasticity in prefrontal cortex of schizophrenia patients. *Biol Psychiatry* 2011; **69**: 71–79.
  - 58 Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* 2005; **10**: 40–68; image 45.
  - 59 Maycox PR, Kelly F, Taylor A, Bates S, Reid J, Logendra R *et al.* Analysis of gene expression in two large schizophrenia cohorts identifies multiple changes associated with nerve terminal function. *Mol Psychiatry* 2009; **14**: 1083–1094.
  - 60 Sorkina T, Hoover BR, Zahniser NR, Sorkin A. Constitutive and protein kinase C-induced internalization of the dopamine transporter is mediated by a clathrin-dependent mechanism. *Traffic* 2005; **6**: 157–170.
  - 61 Meisenzahl EM, Schmitt GJ, Scheuerer J, Möller HJ. The role of dopamine for the pathophysiology of schizophrenia. *Int Rev Psychiatry* 2007; **19**: 337–345.

- 62 Schmitt GJ, la Fougère C, Dresel S, Frodl T, Hahn K, Möller HJ et al. Dual-isotope SPECT imaging of striatal dopamine: first episode, drug naïve schizophrenic patients. *Schizophr Res* 2008; **101**: 133–141.
- 63 Kim OJ, Gardner BR, Williams DB, Marinec PS, Cabrera DM, Peters JD et al. The role of phosphorylation in D1 dopamine receptor desensitization: evidence for a novel mechanism of arrestin association. *J Biol Chem* 2004; **279**: 7999–8010.
- 64 Namkung Y, Dipace C, Urizar E, Javitch JA, Sibley DR. G protein-coupled receptor kinase-2 constitutively regulates D2 dopamine receptor expression and signaling independently of receptor phosphorylation. *J Biol Chem* 2009; **284**: 34103–34115.
- 65 Thompson D, Whistler JL. Trafficking properties of the d5 dopamine receptor. *Traffic* 2011; **12**: 644–656.
- 66 Thompson D, Whistler JL. Dopamine D(3) receptors are down-regulated following heterologous endocytosis by a specific interaction with G protein-coupled receptor-associated sorting protein-1. *J Biol Chem* 2011; **286**: 1598–1608.
- 67 Paspalas CD, Rakic P, Goldman-Rakic PS. Internalization of D2 dopamine receptors is clathrin-dependent and select to dendro-axonic appositions in primate prefrontal cortex. *Eur J Neurosci* 2006; **24**: 1395–1403.
- 68 Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia. *Harv Rev Psychiatry* 1996; **3**: 241–253.
- 69 Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S et al. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* 2008; **31**: 234–242.
- 70 Scarr E, Beneyto M, Meador-Woodruff JH, Dean B. Cortical glutamatergic markers in schizophrenia. *Neuropsychopharmacology* 2005; **30**: 1521–1531.
- 71 Harel A, Mattson MP, Yao PJ. CALM, a clathrin assembly protein, influences cell surface GluR2 abundance. *Neuromolecular Med* 2011; **13**: 88–90.
- 72 Lewis DA. The chandelier neuron in schizophrenia. *Dev Neurobiol* 2011; **71**: 118–127.
- 73 Kittler JT, Delmas P, Jovanovic JN, Brown DA, Smart TG, Moss SJ. Constitutive endocytosis of GABAA receptors by an association with the adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. *J Neurosci* 2000; **20**: 7972–7977.
- 74 Behan AT, Byrne C, Dunn MJ, Cagney G, Cotter DR. Proteomic analysis of membrane microdomain-associated proteins in the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder reveals alterations in LAMP, STXBP1 and BASP1 protein expression. *Mol Psychiatry* 2009; **14**: 601–613.
- 75 Castillo MA, Ghose S, Tamminga CA, Ulerly-Reynolds PG. Deficits in syntaxin 1 phosphorylation in schizophrenia prefrontal cortex. *Biol Psychiatry* 2010; **67**: 208–216.
- 76 Barakauskas VE, Beasley CL, Barr AM, Ypsilanti AR, Li HY, Thornton AE et al. A novel mechanism and treatment target for presynaptic abnormalities in specific striatal regions in schizophrenia. *Neuropsychopharmacology* 2010; **35**: 1226–1238.
- 77 Harrison PJ, Eastwood SL. Preferential involvement of excitatory neurons in medial temporal lobe in schizophrenia. *Lancet* 1998; **352**: 1669–1673.
- 78 Pfeiffer SE, Warrington AE, Bansal R. The oligodendrocyte and its many cellular processes. *Trends Cell Biol* 1993; **3**: 191–197.
- 79 Anitei M, Pfeiffer SE. Myelin biogenesis: sorting out protein trafficking. *Curr Biol* 2006; **16**: R418–R421.
- 80 Winterstein C, Trotter J, Krämer-Albers EM. Distinct endocytic recycling of myelin proteins promotes oligodendroglial membrane remodeling. *J Cell Sci* 2008; **121**(Pt 6): 834–842.
- 81 Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR et al. White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry* 2003; **60**: 443–456.
- 82 Lecuit T, Pilot F. Developmental control of cell morphogenesis: a focus on membrane growth. *Nat Cell Biol* 2003; **5**: 103–108.
- 83 Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, Harris KM et al. Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron* 2006; **52**: 817–830.
- 84 Shirane M, Nakayama KI. Protrudin induces neurite formation by directional membrane trafficking. *Science* 2006; **314**: 818–821.
- 85 Alberts P, Galli T. The cell outgrowth secretory endosome (COSE): a specialized compartment involved in neuronal morphogenesis. *Biol Cell* 2003; **95**: 419–424.
- 86 Arantes RM, Andrews NW. A role for synaptotagmin VII-regulated exocytosis of lysosomes in neurite outgrowth from primary sympathetic neurons. *J Neurosci* 2006; **26**: 4630–4637.
- 87 Tojima T, Itofusa R, Kamiguchi H. Asymmetric clathrin-mediated endocytosis drives repulsive growth cone guidance. *Neuron* 2010; **66**: 370–377.
- 88 Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* 1987; **44**: 660–669.
- 89 Owen MJ, O'Donovan MC, Thapar A, Craddock N. Neurodevelopmental hypothesis of schizophrenia. *Br J Psychiatry* 2011; **198**: 173–175.
- 90 Delay J, Deniker P. 38 cas de psychoses traits par la cure prolongée et continue de 4500 RP. Paper presented at: Rénus du 1ème Congrès des Alienistes et Neurologistes de Langue Française, 1952; Luxembourg, July 21–27.
- 91 Rodemer C, Haucke V. Clathrin/AP-2-dependent endocytosis: a novel playground for the pharmacological toolbox? *Handb Exp Pharmacol* 2008; **186**: 105–122.
- 92 Wang LH, Rothberg KG, Anderson RG. Mis-assembly of clathrin lattices in endosomes reveals a regulatory switch for coated pit formation. *J Cell Biol* 1993; **123**: 1107–1117.
- 93 Masri B, Salahpour A, Didriksen M, Ghisi V, Beaulieu JM, Gainetdinov RR et al. Antagonism of dopamine D2 receptor/beta-arrestin 2 interaction is a common property of clinically effective antipsychotics. *Proc Natl Acad Sci USA* 2008; **105**: 13656–13661.
- 94 Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* 2005; **122**: 261–273.
- 95 Vickery RG, von Zastrow M. Distinct dynamin-dependent and -independent mechanisms target structurally homologous dopamine receptors to different endocytic membranes. *J Cell Biol* 1999; **144**: 31–43.
- 96 Urban JD, Vargas GA, von Zastrow M, Mailman RB. Aripiprazole has functionally selective actions at dopamine D2 receptor-mediated signaling pathways. *Neuropsychopharmacology* 2007; **32**: 67–77.
- 97 Schloesser RJ, Huang J, Klein PS, Manji HK. Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. *Neuropsychopharmacology* 2008; **33**: 110–133.
- 98 Chuang DM, Manji HK. In search of the Holy Grail for the treatment of neurodegenerative disorders: has a simple cation been overlooked? *Biol Psychiatry* 2007; **62**: 4–6.
- 99 Beaulieu JM, Marion S, Rodriguiz RM, Medvedev IO, Sotnikova TD, Ghisi V et al. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell* 2008; **132**: 125–136.
- 100 Rossi M, Munarriz ER, Bartesaghi S, Milanese M, Dinsdale D, Guerra-Martin MA et al. Desmethylclomipramine induces the accumulation of autophagy markers by blocking autophagic flux. *J Cell Sci* 2009; **122**(Pt 18): 3330–3339.
- 101 Amminger GP, Schäfer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM et al. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. *Arch Gen Psychiatry* 2010; **67**: 146–154.
- 102 Hedelin M, Löf M, Olsson M, Lewander T, Nilsson B, Hultman CM et al. Dietary intake of fish, omega-3, omega-6 polyunsaturated fatty acids and vitamin D and the prevalence of psychotic-like symptoms in a cohort of 33 000 women from the general population. *BMC Psychiatry* 2010; **10**: 38.
- 103 Ben Gedalya T, Loeb V, Israeli E, Altschuler Y, Selkoe DJ, Sharon R. Alpha-synuclein and polyunsaturated fatty acids promote clathrin-mediated endocytosis and synaptic vesicle recycling. *Traffic* 2009; **10**: 218–234.
- 104 Chernomordik LV, Leikina E, Frolov V, Bronk P, Zimmerberg J. An early stage of membrane fusion mediated by the low pH conformation of influenza hemagglutinin depends upon membrane lipids. *J Cell Biol* 1997; **136**: 81–93.

- 105 English JA, Pennington K, Dunn MJ, Cotter DR. The neuroproteomics of schizophrenia. *Biol Psychiatry* 2011; **69**: 163–172.
- 106 Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet* 2009; **373**: 234–239.
- 107 Owen MJ, Craddock N, O'Donovan MC. Suggestion of roles for both common and rare risk variants in genome-wide studies of schizophrenia. *Arch Gen Psychiatry* 2010; **67**: 667–673.
- 108 Rosenblatt A, Leroy I. Neuropsychiatry of Huntington's disease and other basal ganglia disorders. *Psychosomatics* 2000; **41**: 24–30.
- 109 Pal A, Severin F, Lommer B, Shevchenko A, Zerial M. Huntingtin-HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease. *J Cell Biol* 2006; **172**: 605–618.
- 110 Wu F, Yao PJ. Clathrin-mediated endocytosis and Alzheimer's disease: an update. *Ageing Res Rev* 2009; **8**: 147–149.
- 111 Kyriazis GA, Wei Z, Vandermeij M, Jo DG, Xin O, Mattson MP et al. Numb endocytic adapter proteins regulate the transport and processing of the amyloid precursor protein in an isoform-dependent manner: implications for Alzheimer disease pathogenesis. *J Biol Chem* 2008; **283**: 25492–25502.
- 112 Schjerve BM, Schnack C, Lambert JC, Lill CM, Kirchheiner J, Tumani H et al. The role of clusterin, complement receptor 1, and phosphatidylinositol binding clathrin assembly protein in Alzheimer disease risk and cerebrospinal fluid biomarker levels. *Arch Gen Psychiatry* 2011; **68**: 207–213.
- 113 Jin J, Li GJ, Davis J, Zhu D, Wang Y, Pan C et al. Identification of novel proteins associated with both alpha-synuclein and DJ-1. *Mol Cell Proteomics* 2007; **6**: 845–859.
- 114 Hutagalung AH, Novick PJ. Role of Rab GTPases in membrane traffic and cell physiology. *Physiol Rev* 2011; **91**: 119–149.
- 115 Verhoeven K, De Jonghe P, Coen K, Verpoorten N, Auer-Grumbach M, Kwon JM et al. Mutations in the small GTP-ase late endosomal protein RAB7 cause Charcot-Marie-Tooth type 2B neuropathy. *Am J Hum Genet* 2003; **72**: 722–727.
- 116 Tanabe K, Takei K. Dynamic instability of microtubules requires dynamin 2 and is impaired in a Charcot-Marie-Tooth mutant. *J Cell Biol* 2009; **185**: 939–948.
- 117 Erez H, Malkinson G, Prager-Khoutorsky M, De Zeeuw CI, Hoogenraad CC, Spira ME. Formation of microtubule-based traps controls the sorting and concentration of vesicles to restricted sites of regenerating neurons after axotomy. *J Cell Biol* 2007; **176**: 497–507.
- 118 Giannandrea M, Bianchi V, Mignogna ML, Sirri A, Carrabino S, D'Elia E et al. Mutations in the small GTPase gene RAB39B are responsible for X-linked mental retardation associated with autism, epilepsy, and macrocephaly. *Am J Hum Genet* 2010; **86**: 185–195.
- 119 Scarr E, Gray L, Keriakous D, Robinson PJ, Dean B. Increased levels of SNAP-25 and synaptophysin in the dorsolateral prefrontal cortex in bipolar I disorder. *Bipolar Disord* 2006; **8**: 133–143.
- 120 Bitoun M, Durieux AC, Prudhon B, Bevilacqua JA, Herledan A, Sakanyan V et al. Dynamin 2 mutations associated with human diseases impair clathrin-mediated receptor endocytosis. *Hum Mutat* 2009; **30**: 1419–1427.
- 121 Toyooka K, Iritani S, Makifuchi T, Shirakawa O, Kitamura N, Maeda K et al. Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia. *J Neurochem* 2002; **83**: 797–806.
- 122 Mulle JG, Dodd AF, McGrath JA, Wolyniec PS, Mitchell AA, Shetty AC et al. Microdeletions of 3q29 confer high risk for schizophrenia. *Am J Hum Genet* 2010; **87**: 229–236.
- 123 Cheng MC, Lu CL, Luu SU, Tsai HM, Hsu SH, Chen TT et al. Genetic and functional analysis of the DLG4 gene encoding the post-synaptic density protein 95 in schizophrenia. *PLoS One* 2010; **5**: e15107.
- 124 Hattori K, Fukuzako H, Hashiguchi T, Hamada S, Murata Y, Isosaka T et al. Decreased expression of Fyn protein and disbalanced alternative splicing patterns in platelets from patients with schizophrenia. *Psychiatry Res* 2009; **168**: 119–128.
- 125 Braithwaite SP, Adkisson M, Leung J, Nava A, Masterson B, Urfer R et al. Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *Eur J Neurosci* 2006; **23**: 2847–2856.
- 126 Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S et al. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 2002; **71**: 877–892.
- 127 Gu Z, Jiang Q, Fu AK, Ip NY, Yan Z. Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *J Neurosci* 2005; **25**: 4974–4984.
- 128 Mabb AM, Ehlers MD. Ubiquitination in postsynaptic function and plasticity. *Annu Rev Cell Dev Biol* 2010; **26**: 179–210.
- 129 Vawter MP, Barrett T, Cheadle C, Sokolov BP, Wood 3rd WH, Donovan DM et al. Application of cDNA microarrays to examine gene expression differences in schizophrenia. *Brain Res Bull* 2001; **55**: 641–650.
- 130 Vawter MP, Thatcher L, Usen N, Hyde TM, Kleinman JE, Freed WJ. Reduction of synapsin in the hippocampus of patients with bipolar disorder and schizophrenia. *Mol Psychiatry* 2002; **7**: 571–578.
- 131 Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P. Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *J Neurosci* 2002; **22**: 2718–2729.
- 132 Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry* 2004; **61**: 300–308.
- 133 Altar CA, Jurata LW, Charles V, Lemire A, Liu P, Bukhman Y et al. Deficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. *Biol Psychiatry* 2005; **58**: 85–96.
- 134 Chu TT, Liu Y, Kemether E. Thalamic transcriptome screening in three psychiatric states. *J Hum Genet* 2009; **54**: 665–675.
- 135 Bousman CA, Chana G, Glatt SJ, Chandler SD, May T, Lohr J et al. Positive symptoms of psychosis correlate with expression of ubiquitin proteasome genes in peripheral blood. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 1336–1341.
- 136 Beck KA, Keen JH. Interaction of phosphoinositide cycle intermediates with the plasma membrane-associated clathrin assembly protein AP-2. *J Biol Chem* 1991; **266**: 4442–4447.
- 137 Haucke V. Phosphoinositide regulation of clathrin-mediated endocytosis. *Biochem Soc Trans* 2005; **33**(Pt 6): 1285–1289.
- 138 Jost M, Simpson F, Kavran JM, Lemmon MA, Schmid SL. Phosphatidylinositol-4,5-bisphosphate is required for endocytic coated vesicle formation. *Curr Biol* 1998; **8**: 1399–1402.
- 139 Krauss M, Kinuta M, Wenk MR, De Camilli P, Takei K, Haucke V. ARF6 stimulates clathrin/AP-2 recruitment to synaptic membranes by activating phosphatidylinositol phosphate kinase type Igamma. *J Cell Biol* 2003; **162**: 113–124.
- 140 Varnai P, Thyagarajan B, Rohacs T, Balla T. Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact living cells. *J Cell Biol* 2006; **175**: 377–382.
- 141 Padrón D, Wang YJ, Yamamoto M, Yin H, Roth MG. Phosphatidylinositol phosphate 5-kinase Ibeta recruits AP-2 to the plasma membrane and regulates rates of constitutive endocytosis. *J Cell Biol* 2003; **162**: 693–701.
- 142 Clague MJ, Urbé S, de Lartigue J. Phosphoinositides and the endocytic pathway. *Exp Cell Res* 2009; **315**: 1627–1631.
- 143 Kim HJ, Thayer SA. Lithium increases synapse formation between hippocampal neurons by depleting phosphoinositides. *Mol Pharmacol* 2009; **75**: 1021–1030.
- 144 Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 2008; **320**: 539–543.
- 145 Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. *Nat Rev Neurosci* 2007; **8**: 128–140.
- 146 Allen JA, Yadav PN, Setola V, Roth BL. The schizophrenia risk gene CAV1 is both pro-psychotic and required for antipsychotic drug activity at 5-HT<sub>2A</sub> serotonin receptors *in vivo*. In: *Advancing Drug Discovery for Schizophrenia, Abstract Book*. The New York Academy of Sciences: New York, 2011: 21.

- 147 Newell-Litwa K, Salazar G, Smith Y, Faundez V. Roles of BLOC-1 and adaptor protein-3 complexes in cargo sorting to synaptic vesicles. *Mol Biol Cell* 2009; **20**: 1441–1453.
- 148 Setty SR, Tenza D, Truschel ST, Chou E, Sviderskaya EV, Theos AC et al. BLOC-1 is required for cargo-specific sorting from vacuolar early endosomes toward lysosome-related organelles. *Mol Biol Cell* 2007; **18**: 768–780.
- 149 Casas S, Casini P, Piquer S, Altirriba J, Soty M, Cadavez L et al. BACE2 plays a role in the insulin receptor trafficking in pancreatic  $\beta$ -cells. *Am J Physiol Endocrinol Metab* 2010; **299**: E1087–E1095.
- 150 Galletta BJ, Mooren OL, Cooper JA. Actin dynamics and endocytosis in yeast and mammals. *Curr Opin Biotechnol* 2010; **21**: 604–610.
- 151 Edwards AM, Isserlin R, Bader GD, Frye SV, Willson TM, Yu FH. Too many roads not taken. *Nature* 2011; **470**: 163–165.
- 152 Levin Y, Wang L, Schwarz E, Koethe D, Leweke FM, Bahn S. Global proteomic profiling reveals altered proteomic signature in schizophrenia serum. *Mol Psychiatry* 2010; **15**: 1088–1100.
- 153 Garrick MD, Garrick LM. Cellular iron transport. *Biochim Biophys Acta* 2009; **1790**: 309–325.
- 154 Macia E, Ehrlich M, Massol R, Boucrot E, Brunner C, Kirchhausen T. Dynasore, a cell-permeable inhibitor of dynamin. *Dev Cell* 2006; **10**: 839–850.
- 155 Clarke MC, Tanskanen A, Huttunen M, Whittaker JC, Cannon M. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. *Am J Psychiatry* 2009; **166**: 1025–1030.
- 156 O'Callaghan E, Sham P, Takei N, Glover G, Murray RM. Schizophrenia after prenatal exposure to 1957 A2 influenza epidemic. *Lancet* 1991; **337**: 1248–1250.
- 157 Abbott A. Schizophrenia: the drug deadlock. *Nature* 2010; **468**: 158–159.
- 158 The Lancet. Where will new drugs come from? *Lancet* 2011; **377**: 97.
- 159 Chan MK, Tsang TM, Harris LW, Guest PC, Holmes E, Bahn S. Evidence for disease and antipsychotic medication effects in post-mortem brain from schizophrenia patients. *Mol Psychiatry* 2010; doi:10.1038/mp.2010.100 (in press).
- 160 Zhou Y, Wang J, Wang K, Li S, Song X, Ye Y et al. Association analysis between the rs11136000 single nucleotide polymorphism in clusterin gene, rs3851179 single nucleotide polymorphism in clathrin assembly lymphoid myeloid protein gene and the patients with schizophrenia in the Chinese population. *DNA Cell Biol* 2010; **29**: 745–751.
- 161 Smalla KH, Mikhaylova M, Sahin J, Bernstein HG, Bogerts B, Schmitt A et al. A comparison of the synaptic proteome in human chronic schizophrenia and rat ketamine psychosis suggest that prohibitin is involved in the synaptic pathology of schizophrenia. *Mol Psychiatry* 2008; **13**: 878–896.
- 162 Vine AE, McQuillin A, Bass NJ, Pereira A, Kandaswamy R, Robinson M et al. No evidence for excess runs of homozygosity in bipolar disorder. *Psychiatr Genet* 2009; **19**: 165–170.
- 163 Clark D, Dedova I, Cordwell S, Matsumoto I. A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia. *Mol Psychiatry* 2006; **11**: 459–470, 423.
- 164 Martins-de-Souza D, Gattaz WF, Schmitt A, Maccarrone G, Hunyadi-Gulyás E, Eberlin MN et al. Proteomic analysis of dorsolateral prefrontal cortex indicates the involvement of cytoskeleton, oligodendrocyte, energy metabolism and new potential markers in schizophrenia. *J Psychiatr Res* 2009; **43**: 978–986.
- 165 Amar S, Shaltiel G, Mann L, Shamir A, Dean B, Scarr E et al. Possible involvement of post-dopamine D2 receptor signalling components in the pathophysiology of schizophrenia. *Int J Neuropsychopharmacol* 2008; **11**: 197–205.
- 166 Ikeda M, Ozaki N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y et al. Possible association of beta-arrestin 2 gene with methamphetamine use disorder, but not schizophrenia. *Genes Brain Behav* 2007; **6**: 107–112.
- 167 Margolis RL, Abraham MR, Gatchell SB, Li SH, Kidwai AS, Breschel TS et al. cDNAs with long CAG trinucleotide repeats from human brain. *Hum Genet* 1997; **100**: 114–122.
- 168 Passos Gregorio S, Gattaz WF, Tavares H, Kieling C, Timm S, Wang AG et al. Analysis of coding-polymorphisms in NOTCH-related genes reveals NUMBL poly-glutamine repeat to be associated with schizophrenia in Brazilian and Danish subjects. *Schizophr Res* 2006; **88**: 275–282.
- 169 Potash JB, Buervenich S, Cox NJ, Zandi PP, Akula N, Steele J et al. Gene-based SNP mapping of a psychotic bipolar affective disorder linkage region on 22q12.3: association with HMG2L1 and TOM1. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 59–67.
- 170 Saito T, Guan F, Papolos DF, Wolyniec PS, Mitchell AA, Shetty AC et al. Mutation analysis of SYNJ1: a possible candidate gene for chromosome 21q22-linked bipolar disorder. *Mol Psychiatry* 2001; **6**: 387–395.
- 171 Stopkova P, Vevera J, Paclt I, Zukov I, Lachman HM. Analysis of SYNJ1, a candidate gene for 21q22 linked bipolar disorder: a replication study. *Psychiatry Res* 2004; **127**: 157–161.
- 172 Sivagnanasundaram S, Crossett B, Dedova I, Cordwell S, Matsumoto I. Abnormal pathways in the genu of the corpus callosum in schizophrenia pathogenesis: a proteome study. *Proteomics Clin Appl* 2007; **1**: 1291–1305.

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