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## ALTERING THE COURSE OF SCHIZOPHRENIA: PROGRESS AND PERSPECTIVES

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## Abstract

Despite a lack of recent progress in the treatment of schizophrenia, our understanding of its genetic and environmental causes has considerably improved, and their relationship to aberrant patterns of neurodevelopment has become clearer. This raises the possibility that “disease-modifying” strategies could modify the course to - and of - this debilitating disorder, rather than simply alleviating symptoms. A promising window for course-altering intervention is around the time of conversion, especially in young people vulnerable to transition. Indeed, studies performed both in subjects at risk and in rodent models for schizophrenia suggest that pre-diagnostic pharmacotherapy and/or psychosocial/cognitive-behavioural interventions can delay and/or moderate the emergence of psychosis. Of particular interest are “hybrid” strategies that both relieve presenting symptoms *and* reduce the risk of transition to schizophrenia - or another psychiatric disorder. This is an opportune moment for a broad-based consideration of the challenges and opportunities inherent in efforts to alter the course of schizophrenia.

**Abbreviations:** APS, Attenuated Psychotic Symptoms; BDNF, Brain-derived Neurotrophic Factor; CBT, Cognitive-Behavioural therapy; CHR, Clinically High Risk; CRF, Corticotrophin Releasing Factor; DISC, Disrupted in Schizophrenia; ERP, Event-Related Potential; FEP, First Episode of Psychosis; fMRI, functional Magnetic Resonance Imaging; HDAC, Histone deacetylase; HPA, Hypothalamo-Pituitary-Adrenal; iPSC, induced pluripotent stem cell; mGluR, metabotropic glutamate receptor; MMN, Mismatch Negativity; NAPLS, North American Prodrome Longitudinal Study; NKCC, Na-K-Cl co-transporter; NMDA, N-Methyl-D-Aspartate; PAM, positive allosteric modulator; PFC, Prefrontal cortex; Poly I:C, Polyribonucleosinic:Polyribocytidylic; PUFA, Polyunsaturated fatty acid; SGA, Second Generation Antipsychotic and VHL, Ventrohippocamal lesion.

**Glossary entries** are indicated as such in text.

## Schizophrenia: clinical features and limitations of current treatment

Schizophrenia is a chronic, heterogeneous, multi-faceted and debilitating disorder triggered by a panoply of interacting genetic, epigenetic, developmental and environmental factors which collectively interfere with normal brain development and maturation<sup>1,2</sup> (Figure 1). It has a prevalence of around 1% and represents a major socio-economic burden, mainly as a result of indirect costs like unemployment and social support, but also due to hospitalisation during crises<sup>1,3,4</sup>. Notwithstanding rare and usually severe cases of early-onset schizophrenia, it is usually diagnosed in the young adult with the “First Episode of Psychosis (FEP)<sup>2-5</sup> (**Box 1**).

Antipsychotics are effective against positive symptoms, yet some patients respond only poorly to treatment. Further, while clozapine remains the most effective agent for “resistant” patients, it has marked haematological and metabolic side-effects, and neither clozapine nor second-generation antipsychotics (SGA) like risperidone, olanzapine and aripiprazole markedly improve negative symptoms, neurocognitive deficits and impaired social processing/cognition. Thus, while the importance of currently-available treatments must be recognised, and there has been considerable progress in moderating their side-effects, there remains a clear unmet need for greater clinical efficacy<sup>6-8</sup>. Recently-evaluated glutamatergic agents have not yet proven sufficiently effective for authorisation<sup>9,10</sup>. Further, numerous mechanisms pursued as “add-ons” have met with limited success, possibly since SGAs undermine their ability to relieve cognitive impairment, and as most studies were undertaken in chronically-ill patients<sup>11,12</sup>. Improving the symptomatic treatment of schizophrenia remains an important goal, but progress will likely require a strategic shift in thinking (Suppl Figure 1).

By definition, symptomatic treatment does not affect the causes, pathophysiology and course of schizophrenia. On the other hand, an improved understanding of the neurobiological substrates of schizophrenia is fostering the notion that its evolution might be modified by novel modes of intervention, especially if instituted at an early stage of the disorder<sup>13-17</sup>. The challenges and opportunities inherent in the realisation of this goal comprise the central themes of this article.

## Course-alteration: general principles and application to schizophrenia

### *Disease-modification as a therapeutic concept*

The notion of early, pre-diagnostic disease-modification (**Figure 2**) has its origins in medical domains like diabetes, oncology and rheumatoid arthritis, with efforts to develop preventative therapies for multiple sclerosis, Alzheimer’s disease and Parkinson disease intensifying over the past decade<sup>18-20</sup>. The more recent application of disease-modification to psychiatric states is highlighted by experimental evidence that developmental anomalies and behavioural deficits characterising (monogenic) autism-related disorders may be, at least partially, correctible<sup>21</sup>. These observations underscore interest in preventive treatment for bipolar depression<sup>22</sup> and schizophrenia, not least since

they are communalities with autism including: symptoms (cognitive and social impairment)<sup>12,21</sup>, genetic risk factors (single genes and Copy Number Variants)<sup>23,24</sup>, epigenetic anomalies (DNA and histone marking)<sup>25,26</sup>, environmental triggers (perinatal infection and inflammation)<sup>27,28</sup> and neurobiological substrates (disruption of synaptic plasticity and cerebral connectivity)<sup>21,29,30</sup>. **That certain core features are shared between schizophrenia and other disorders is significant since it implies that course-altering strategies for blocking the onset of psychosis might be of broader therapeutic utility<sup>1,23,31-33</sup>.**

### *Course-alteration for schizophrenia: core questions*

**Disease-modification** refers to interventions that directly target the pathophysiological processes causing a disorder in a manner that enduringly modifies its progression<sup>19</sup>, but it is important to clarify its operational meaning as applied to schizophrenia. As depicted in Figure 2, it would seem wise to favour a broader and more pragmatic notion of course-alteration as a framework for study and therapeutics. Despite several characteristic neurodevelopmental anomalies, no common upstream trigger for schizophrenia is known<sup>1,2,29,30</sup>. Hence, course-alteration might target a plurality of mechanisms, acting in series and in parallel, and either aggravating or counteracting core pathophysiological substrates. Targetable pathological processes may differ between specific clinical dimensions: for example, avolition *versus* hallucinations *versus* impaired social cognition<sup>1,12-14,16,33</sup>.

The initial diagnosis comes relatively late in the neurodevelopmental trajectory of schizophrenia, usually around 25 years of age, and antipsychotic treatment does not normally begin until this FEP. However, course-altering therapy is most likely to succeed when applied *early*. This is supported by evidence that marked and progressive anomalies in brain structure, neurochemistry and “connectivity” are already present by the time of diagnosis<sup>30,34-36</sup>. Precocious course-altering therapy for schizophrenia also makes sense in terms of potential cost-effectiveness<sup>37</sup>. Hence, despite potential intervention following the FEP (evoked later), the major focus is currently on prevention.

A preventative approach to course-alteration raises several important questions. *First*, what is the optimal strategy for identifying people at risk of developing schizophrenia, and whom should be treated? *Second*, which therapeutic strategies would be the most effective for preventing the onset of schizophrenia and/or interfering with its progression? *Third*, how can the efficacy of course-altering treatments be clinically evaluated and proven? *Fourth*, is it possible to unite the treatment of symptoms seen in clinically-high risk (CHR) subjects with a reduction in the risk of transition? Indeed, might the control of anomalies and dysfunction in CHR patients of itself impede the onset of schizophrenia?

Though currently-used antipsychotics may “stabilise” patients, this is essentially due to symptomatic effects expressed *during* treatment. Hence, a basic tenet of the following discussion is the need to identify *novel* and genuine course-altering therapies. **To realise this objective, a broad suite of strategies is being deployed from cellular studies to therapeutic trials in subjects vulnerable to**

schizophrenia. Accordingly, the discussion is structured around three complementary hierarchies of evidence, commencing with the most advanced and direct: A), clinical trials in high-risk, help-seeking subjects of pharmacotherapeutic and other interventions for preventing transition to psychosis; B), experimental evaluation of pharmacological agents for blocking the adult appearance of a “psychosis-like” phenotype in rodent models for schizophrenia C), cellular and *in vivo* studies of pathophysiological mechanisms implicated in events leading to the onset of psychosis.

## Young people at clinically-high risk of schizophrenia

### *The clinically high-risk state*

Mental health problems are highly prevalent in the young, underpinning efforts to set up nationwide structures for their treatment and detection of subjects at risk of psychosis and other psychiatric disorders (Box 2). Schizophrenia itself strikes the young adult in the wake of exposure to multiple risk factors from conception to adolescence<sup>23,24,27,38</sup> (Figure 1A). Its onset is preceded by a phase (*prodrome*) during which several (sub-diagnostic) features progressively emerge, though with inter-individual differences. In line with the notion of clinical staging<sup>39</sup>, the earliest symptoms may be non-specific and include anxiety, depressed mood, social withdrawal and educational difficulties, followed by the emergence of “basic symptoms” (subtle disturbances of cognition, perception, language and emotion) and reduced stress tolerance/coping<sup>40,41</sup>. Later, more pronounced abnormalities and disorganised speech become apparent, as well as sub-diagnostic positive symptoms. These “Brief Limited Intermittent Psychotic Symptoms” and “Attenuated Psychotic Symptoms” (APS) are less severe than a fully-fledged psychotic episode<sup>35,42</sup> (Box 1). During the prodromal period, both negative symptoms and impairment of social cognition are present: they are often marked and linked to poor functioning and a high likelihood of conversion<sup>8,12,40,43,44</sup>. Neurocognitive deficits are also prominent<sup>12,40,43</sup>.

### *Contrasting fates of CHR subjects at risk of transition to schizophrenia*

The notion of pre-diagnostic disease-modification presupposes the reliable identification of subjects at risk. While the term “prodrome” implies that schizophrenia is ineluctable, this is *not* actually the case. In fact, only a minority of people displaying a CHR state will develop schizophrenia or another psychotic disorder<sup>15,17,34,35,40,45</sup> (Figure 3A). Meta-analyses suggest that the proportion is around 30%, but the percentage varies across samples. This variability reflects contrasting criteria of selection of CHR subjects for study, socio-demographic differences, environmental factors, coping, and varying degrees of clinical/family care and support<sup>14,34,35,38,40,46,47</sup>. The latter point supports the “hybrid” notion that treatment of CHR subjects may reduce the risk of conversion (see below). Nonetheless, complete recovery is rare, certain people persist in a state of impaired overall

functioning, and many develop other psychiatric disorders like depression or anxiety (Figure 3A). Hence, the need for preventive course-altering therapy remains persuasive, and all these individuals require treatment and specialized care<sup>17,32,34,35</sup> (Box 2).

## Neurobiological characteristics of the CHR state: biomarkers of transition

### *Clinical indices of impending conversion*

Although all CHR people may potentially transit to schizophrenia, certain features are associated with a particularly high risk of conversion. These include unusual thought content, marked impairment of verbal learning and memory, suspiciousness and paranoid ideation (especially when enduring and denied), low IQ, substance abuse, migrant status, isolation, poor quality of life and comorbidity with other disorders<sup>32,34,35,40,43,45,48</sup>. Inspection of CHR subjects may also unveil neurological “*soft signs*”: that is, motor and sensory changes provoked by neurodevelopmental abnormalities distributed across widespread cortical regions<sup>49,50</sup>.

Such clinical observations are important for assessing vulnerability to psychosis, especially when coupled to information on life-style such as excessive consumption of cannabis<sup>38,51</sup>. Nonetheless, they are insufficient alone for either predicting the likelihood of transition at the *individual* level, or for *stratifying* subgroups of CHR subjects. Accordingly, more refined strategies for estimating the risk of conversion are being developed. Such measures of pathophysiological anomalies also provide insights into potential targets for course-altering medication<sup>13,16,35,48,52</sup> (Figure 3B).

### *Genetic profile: risk genes and gene “networks”*

The genetics of schizophrenia are complex with: 1), a few, very rare variants of large effect vs innumerable variants of very small and collective impact: 2), a role for rare Copy Number Variants encompassing multiple genes and stretches of DNA and 3), both inherited and *de novo* anomalies<sup>23,24</sup> (Suppl Box 1). Nonetheless, ***Neurexin-1* has been consistently linked to schizophrenia, the risk gene *Neuregulin1* was specifically associated with a high risk of transition, and gene clusters, pathways and interactions (“epistasis”) may yield associations more robust than for individual genes<sup>53,54,55</sup>. Thus, no universal genetic biomarker of the risk of transition is currently available, but useful readouts may soon emerge.** Moreover, a family history of psychosis can be readily factored into estimations of risk, as undertaken by the North American Prodrome Longitudinal Studies (NAPLS) Consortium and the European Prediction of Psychosis study<sup>34,43,48</sup>.

### *Biochemical and endocrine measures in the circulation*

Increases in leucocyte levels of Interleukin-6 (a pro-inflammatory cytokine) and decreases in Brain-Derived Neurotrophic Factor (BDNF) are correlated with a reduced volume of the hippocampus in FEP, measures being extended to the CHR state<sup>56</sup>. Further, high plasma levels of pro-inflammatory

cytokines correlated with a steep decline of prefrontal cortex (PFC) grey matter in CHR subjects that progressed to psychosis<sup>57</sup>. **More generally, the risk of transition may be associated with a specific molecular signature in blood involving immune-inflammatory biomarkers<sup>58-60</sup>. Another promising approach focusses on epigenetic readouts, such as patterns of DNA methylation and miRNA profiles in lymphocytes<sup>26,61,62</sup>.**

Reflecting increased exposure and/or sensitivity to psychosocial stress, altered activity of the Hypothalamic-Pituitary-Adrenocorticotrophic (HPA) Axis is common in the CHR state and suggestive of a high risk of conversion: further, HPA axis over-activity is correlated with hippocampal volume loss<sup>63,64</sup>. Though the *specificity* of stress-related HPA hyper-activation to risk for *schizophrenia* is debatable, converters had higher salivary cortisol in the NAPLS study<sup>48,63</sup>. Further, increased circulating cortisol is linked to excessive striatal release of DA and positive symptoms both in CHR subjects and in people with familial high risk<sup>63,65,66</sup>. It is also related to poor stress-coping, anxiety and suspiciousness, psychological factors themselves associated with conversion<sup>34,35,40,67</sup>.

### ***Structural and functional imaging: neurotransmitters and neural circuits***

As quantified by Magnetic Resonance Spectroscopy, schizophrenia is associated with altered glutamatergic transmission in several brain regions, and abnormalities are already evident in CHR subjects<sup>68,69</sup>. Changes include increases in glutamate in the associative striatum, reductions in the thalamus, and an uncoupling of glutamate levels from functional activation of the medial temporal cortex during an episodic memory task<sup>68,69</sup>. Further, a prodromal hyper-metabolic state of the hippocampus, mimicked in an animal model, was attributed to excess glutamate release: it was associated with GABAergic interneurone dysfunction, neuronal atrophy and spreading to the subiculum upon onset of psychosis<sup>70</sup>. In the CHR state, changes in glutamatergic transmission originating in the cortex appear to drive alterations in subcortical dopaminergic transmission<sup>71,72</sup>. **They are exemplified by an increase in the presynaptic synthesis and storage of subcortical DA, especially in the associative striatum, which becomes progressively more pronounced as subjects move to psychosis<sup>72,73</sup>.** DA depletion studies have linked this increased DA availability to greater occupation of postsynaptic D2 receptors<sup>72-74</sup>. On a different tack, reduced levels of the marker of neuronal integrity, N-acetyl-aspartate, in frontocortico-cingulate cortex may be indicative of susceptibility to transition, with decreases intensifying upon diagnosis<sup>75</sup>.

Loss of grey matter volume coupled to increased lateral ventricle size is highly reproducible in schizophrenia and Magnetic Resonance Imaging (MRI) of progressive structural changes in CHR subjects can reveal a risk of transition. Indeed, mirroring grey matter loss in drug-naïve FEP patients, CHR subjects display reductions in grey matter in para-hippocampal territories, the cerebellum, the superior/medial temporal cortex, the insular cortex and the PFC<sup>8,57,76-78</sup>. Progression of CHR subjects through various premorbid stages and conversion correlates with incremental grey matter loss<sup>39,73,79</sup>.



The precise pattern may even predict the type of psychosis since reductions in PFC/parietal cortex and PFC/subgenual cingulate cortex were linked to schizophrenia and affective psychosis, respectively<sup>80</sup>.

Functional MRI (fMRI) of cerebral circuits is likewise instructive<sup>36</sup>. For example, altered activity in cortico-striatal pathways correlated to an APS in the prodromal period<sup>81</sup> and parallels the above-mentioned enhancement of presynaptic dopaminergic activity in the associative striatum<sup>72,73</sup>. Further, suppression of the dorsolateral PFC component of the **Default Mode Network** during a Working Memory task was blunted both in early schizophrenia and in CHR groups<sup>82</sup>. Deficient Working Memory in vulnerable subjects has also been related to aberrant coupling of the PFC to the parietal cortex<sup>83</sup>. Finally, altered activity of fronto-temporo-parietal regions has been linked to negative and social cognitive symptoms<sup>8</sup>.

**To summarize: alterations in dopaminergic, glutamatergic and GABAergic transmission; loss of gray matter in discrete cortico-limbic regions; and disruption of network connectivity are anomalies that parallel the progressive onset of psychosis (Figure 1). Collectively, they provide a broad suite of imaging readouts for estimating the risk of psychosis.**

### ***Electroencephalographical readouts: event-related potentials***

Electroencephalographic probing of large-scale brain networks can reveal anomalies in cerebral connectivity, neural synchrony and the balance of inhibition/excitation. Accordingly, it is potentially useful for assessing the risk of transition<sup>84,85</sup>. In addition, studies of **event-related potentials** (ERPs) have shown that two responses to deviant stimuli, one **positive (P300)** and one **negative (Mismatch Negativity, MMN)** are modified in schizophrenia and in CHR subjects<sup>33,86-88</sup>. Both responses have a strong glutamatergic component and are disrupted by the N-Methyl-D-Aspartate (NMDA) receptor antagonist and pro-psychotic agent, ketamine<sup>87-89</sup>. The attenuation of P300 in CHR subjects that subsequently convert has been related to disruption of grey (and white) matter in temporo-parietal cortex and the frontal gyrus, regions implicated in cognitive deficits, impaired language processing and negative symptoms<sup>8,86,90</sup>. **Importantly, perturbation of duration-deviant MMN is specific to schizophrenia, and its amplitude is reproducibly blunted in the prodrome<sup>87,88,91</sup>. In longitudinal studies, the amplitude of MMN permitted sub-division of patients into higher and lower risk groups<sup>88,91</sup>. Further, meta-analyses suggest that MMN perturbation is the most consistent ERP biomarker of conversion and may be exploitable in a prospective fashion to predict which CHR subjects will convert, with a greater magnitude of reduction correlated to more imminent onset of psychosis<sup>87,88,91</sup>. Another advantage of MMN is its translational dimension inasmuch as neurobiological substrates are common to animals and humans, and disruption can be measured using similar methods<sup>87-89,91</sup>.**

### Towards the stratification of subjects at risk of transition

By analogy to efforts to better classify sub-classes of patient<sup>33</sup>, a major current goal is the sub-categorisation of CHR subjects as defined by individual or suites of biomarkers, preferably linked to a targetable neurobiological anomaly. This should improve the prediction of transition, reduce group heterogeneity and enhance the power and rigour of clinical trials (see below). Despite limitations of genetics (Suppl. Box 1), deletion of the synaptic protein *Neurexin-1* may define a subset of subjects at risk<sup>52,92</sup>, and subgroups may also be revealed by analysis of gene networks<sup>54</sup>. Another potential approach for stratification sub-classifying CHR subjects is a disruption of MMN<sup>88,91</sup> and, when coupled to MMN, deficits, cognitive dysfunction may be particularly informative<sup>93,94</sup>. The pattern of grey matter loss also differs between phenotypically-distinct patient subgroups<sup>80</sup>. Finally, blood-borne palettes of immune and other biochemical markers have been proposed for at-risk subject sub-classification<sup>59</sup>.

### *Multi-modal strategies for more reliable prediction of conversion in individuals*

Irrespective of *group* means, it is imperative to accurately *predict* the risk of transition to schizophrenia in *individuals* with as few false positives (stigma, superfluous treatment, costs etc) and false negatives (failure to protect) as possible. It is unlikely that any *single* readout would be fully reliable, specific and of universal utility. Correspondingly, multi-modal strategies are the focus of several ongoing studies<sup>34,52,83</sup>. For example, the NAPLS Consortium has developed a plasma-based, multi-parametric diagnostic predictive of transition that incorporates excess cortisol secretion, as well as markers of inflammation, oxidative stress and metabolic anomalies<sup>60</sup>. **A similar approach suggested the high predictive performance of an optimised panel of >20 blood protein biomarkers in parallel with patient interviews<sup>59</sup>.** By analogy, a combination of genetic risk, asociality, functional impairment, negative symptoms and/or APS was associated with a particularly high risk of psychosis<sup>48</sup>. Another instance is the coupling of “basic cognitive symptoms” and standard CHR criteria to improve early detection<sup>94</sup>. Patterns of polygenic risk can be linked to fMRI-documented alterations in cerebral activity<sup>95</sup>, while convergent (fMRI) and structural (MRI/grey matter and Diffusion Tensor Imaging/white matter) readouts suggest that superior temporal cortex disruption may be a strong warning sign<sup>8,96</sup>. Finally, ***machine-learning*** and other multivariate techniques for deciphering complex patterns of data are being developed to refine estimations of the risk of transition in single subjects<sup>97,98</sup>.

**Overall, multi-modal strategies seem promising both for: 1), improving predictions at the individual level and 2), characterising sub-populations of patient at differential risk of transition and possessing contrasting neurobiological substrates for intervention.**

## Clinical studies of course-altering interventions in CHR subjects

### *Therapeutic trials for preventing transition*

Despite the challenges, and a lack of formal guidelines, several controlled, clinical trials in CHR subjects have addressed the issue of whether the rate and time of conversion can be influenced by potential course-altering therapy<sup>15,16,34</sup> (Table 1). Studies focussing on transition have been performed with: Omega-3 *poly-unsaturated fatty acids* (PUFA)<sup>99,100</sup>; antipsychotics<sup>101</sup>; antipsychotics in combination with *cognitive-behavioural therapy* (CBT)<sup>102-104</sup>; and various modes of non-pharmacotherapeutic intervention alone<sup>105-109</sup>. Several, independent meta-analyses have concluded that: robust clinical trials in **help-seeking** CHR subjects are feasible; treatment is associated with a significant, overall increase in the time to transition and **a decrease in** the numbers of patients converting<sup>34,110,111</sup>. However, whether transition is merely delayed and/or permanently halted remains to be clarified. **Further, since trials undertaken to date were modest in size, larger-scale, higher-powered studies are required to confirm observations, refine tools for assessment and outcome analysis, and evaluate the significance of additional factors like gender and co-morbidity (see below)**<sup>34,110-113</sup>.

### *Omega-3 and poly-unsaturated fatty acids*

Despite the ambivalent efficacy of Omega-3 in established schizophrenia, levels of PUFAs are reduced both in diagnosed patients and in CHR subjects, supporting interest **in trials of preventative** treatment<sup>114,115</sup>. **In a focussed study**, the decreased conversion in CHR subjects treated with Omega-3 PUFAs (eicosapentaenoic acid, 700 mg/day plus docosahexaenoic acid, 480 mg/day) was maintained for a year despite only 3 months treatment, and tolerance was excellent<sup>99,100,116</sup>. Patient function, together with both positive and negative symptoms, was improved. (Interestingly, those subjects with Borderline Personality Disorder were similarly ameliorated upon treatment with Omega-3<sup>117</sup>). **Longer-term findings have just been reported at 6.7 years with better functioning, a persistent reduction in transition to schizophrenia, and a more general decrease in psychiatric morbidity**<sup>118</sup>. However, this was a modest sample size, and another study of Omega-3 was less positive in its outcome<sup>119</sup> (McGorry, P, pers comm.). Thus, course-altering effects of Omega-3 may depend upon the precise regime and conditions of treatment, and further trials are needed to confirm its potential utility, possibly in distinct subgroups of patient. Moreover, whether preventive effects of Omega-3 are specific to psychosis remains to be established<sup>120</sup>. Finally, for future progress, it will be important to better understand how Omega-3 exerts its effects, since a multiplicity of mechanisms has been implicated including: anti-inflammatory and anti-oxidant properties; improved membrane fluidity, mitochondrial performance and synaptic plasticity; inhibition of Phospholipase A2; normalisation of under/over-active mesocortical/mesolimbic dopaminergic

pathways; promotion of white matter integrity and restoration of adequate levels of nervonic acid, a major component of the myelin sheath<sup>100,121,122</sup>.

### ***Second-generation antipsychotics***

In the “PRIME” study of olanzapine, 11 of 29 subjects converted to psychosis on placebo compared to 5 of 31 on medication, but the difference was not significant<sup>101</sup>. In the olanzapine group, psychosis always occurred in the first four weeks when doses were relatively low, so it may not have had sufficient opportunity to act effectively<sup>101</sup>. Interestingly, by week eight, prodromal symptoms were significantly lower on olanzapine compared to placebo, but weight gain was marked: 8.8 kg vs 0.3 kg<sup>101</sup>. Globally similar observations on transition were made when risperidone was combined with CBT/needs-based interventions or cognitive therapy<sup>102,104,123</sup>. In the “PACE” 1 study, despite reduced conversion at 6 months for risperidone (3/31) vs placebo (10/28), the difference was no longer significant at 12 months. In the “PACE” II study, conversion also did not differ, and all groups demonstrated improvement in symptoms and functioning<sup>103</sup>.

Thus, to date, there is no clear evidence that antipsychotics can prevent transition (Table 1). Further, despite relief of sub-diagnostic psychosis in CHR subjects and the fact that risperidone was relatively well-tolerated, CHR subjects are especially susceptible to side-effects like metabolic perturbation and sedation/fatigue<sup>16,32,34,35,102-104</sup>. In practise, many young people will not accept antipsychotics owing to concerns about adverse effects and stigmatisation, and they are not recommended by many National Guidelines for CHR subjects.<sup>17,32,34,35,110,111,113</sup> **More generally, there is concern about over-prescription of antipsychotics to youth for reasons *other* than the control of psychosis<sup>124</sup>. Nonetheless, additional prevention studies with new agents appear warranted. In an open study of the partial D2 receptor agonist, aripiprazole, it improved prodromal symptoms with good tolerance<sup>125</sup> and a larger-scale, controlled study of its influence on symptoms and conversion is currently underway<sup>126</sup>.**

### ***Cognitive-behavioural and other non-pharmacotherapeutic interventions***

CBT and psychosocial therapy were reported, alone and in association with antipsychotics, to moderate negative, emotional and social symptoms in CHR subjects as well as reducing the risk of later transition<sup>102-109</sup> (Table 1). Carer support promotes the efficacy of CBT, and family-based interventions suggested symptomatic benefits in a sub-set of CHR patients though, owing to small sample size and the specific trial design, no conclusions can be made concerning conversion<sup>127,128</sup>. Likewise, a pilot study of Cognitive Remediation Therapy suggested improved social function, but conversion was not reported<sup>129</sup>.

In a clinical setting, CBT and related therapies are more likely to be accepted by CHR subjects than antipsychotics, **and certain National Guidelines advise CBT for (mainly symptomatic treatment of) CHR patients**<sup>31,34,35</sup>. Nonetheless, high patient commitment and practitioner training is required for success<sup>110,130</sup>. **Thus, further, well-powered studies are required to confirm the efficacy of non-pharmacotherapeutic interventions for diminishing transition**<sup>112,113</sup>. Encouragingly, a recent report underpinned the overall cost-effectiveness of CBT for reducing conversion<sup>130</sup>.

## **Experimental studies of course-alteration in rodent models for schizophrenia**

### ***Studies of adolescent interventions in rodent models for schizophrenia***

Paralleling clinical work, many studies have exploited developmental and genetic models for schizophrenia<sup>131</sup> to determine whether adolescent/early adulthood treatment can *prevent* a schizophrenia-like phenotype in adult rodents (Table 2).

### ***Omega-3 and other clinically-tested agents with anti-inflammatory/anti-oxidant properties***

Administered as a dietary supplement to adolescent rats, Omega-3 blunted the behavioural, cognitive and biochemical disruption provoked by long-term exposure of young adult rats to ketamine<sup>132,133</sup>. **These observations are intriguing, but the data would benefit from extension to more conventional models for schizophrenia. The underlying mechanisms of action also merit further investigation. Nonetheless, Omega-3 possesses anti-inflammatory and anti-oxidant properties**<sup>134-136</sup> **and similar effects may intervene in the experimental actions of several other clinically-tested - in established schizophrenia - agents.**

Adolescent treatment of rats gestationally exposed to the viral mimic Polyriboinosinic:Polyribocytidylic (“Poly I:C”) with the anti-inflammatory Cyclo-Oxygenase-2 inhibitor, Celecoxib, blocked the adult appearance of supersensitivity to the NMDA antagonist, dizocilpine<sup>137</sup>. Celecoxib has shown preliminary evidence of utility as augmentation therapy in schizophrenia, but this awaits confirmation<sup>134,135</sup>. **Minocycline** interacts with microglia and oligodendrocytes to exert a palette of anti-inflammatory, anti-oxidant and neuroprotective actions on white and grey matter<sup>134-136</sup>; **it also promotes NMDA-receptor mediated transmission**<sup>138</sup>. It blocked behavioural deficits and microglial activation in adult rats which had received an intra-hippocampal injection of the pro-inflammatory agent, lipopolysaccharide, just after birth<sup>139</sup>. This is interesting since minocycline, while not without risks, is well-tolerated, **and has been evaluated as an adjunct in established schizophrenia. Currently, its efficacy awaits corroboration**, though it appears to be most active in *early* schizophrenia and against negative symptoms<sup>8,134-136,140</sup>. Encouragingly, 12 months add-on minocycline in recent-onset schizophrenia reduced symptoms and protected from grey matter loss in fronto-temporal cortex<sup>141</sup>. **N-acetyl-Cysteine**, another drug possessing anti-

inflammatory properties, promotes the activity of the anti-oxidant glutathione (deficient in early schizophrenia), enhances mitochondrial integrity, acts at the cysteine-glutamate transporter and (like minocycline) facilitates NMDA signalling<sup>135,136,142</sup>. In small-scale trials of schizophrenia, adjunctive N-Acetyl-Cysteine improved negative symptoms, social functioning and deficits in mismatch negativity, facets characteristic of the prodrome<sup>142,143</sup>. Again, confirmation of efficacy is outstanding. Nonetheless, using a neonatal Ventrohippocampal lesion (VHL) model, adolescent administration of N-Acetyl-Cysteine to rats blocked deficits in Pre-Pulse Inhibition in adults: this action was reproduced by the anti-inflammatory/anti-oxidant agent, ebselen, underscoring the relevance of these components of the activity of N-Acetyl-Cysteine<sup>144</sup>.

### ***Antipsychotics and their potential mechanisms of action***

Upon administration during adolescence, antipsychotics consistently blunt the adult hyperlocomotor response to amphetamine and the disruption of sensorimotor gating. Globally similar findings have been made in mice and rats using VHL and pro-inflammatory developmental models for schizophrenia<sup>145-151</sup>. What is less clear is *how* the effects of antipsychotics are expressed. A role for D2 receptor blockade is supported by clinical data suggesting that subcortical dopaminergic over-activity commences during adolescence (see above). Indeed, D2 receptor stimulation in adolescent mice disrupts dendritic spine morphogenesis and has a long-term deleterious impact on cortico-hippocampal connectivity<sup>152</sup>. Furthermore, the Sandy mouse strain (which lacks the putative schizophrenia risk gene, dysbindin) has abnormally high cell-surface levels of D2 receptors: sustained treatment of adolescent animals with eticlopride prevented both disruption of entorhinal cortex-hippocampal connectivity and impaired working memory in adults<sup>152</sup>. **Nevertheless, the potent prevention by risperidone of amphetamine-supersensitivity in a VHL model in rats suggests a role for its 5-HT2A antagonist properties, likewise implicated in blockade of the actions of PCP in rats**<sup>145,146,149,151,153</sup>. In addition, 5-HT1A agonism may be involved in the effects of aripiprazole (and clozapine), ostensibly *via* neuroprotective/neurorestorative actions<sup>148,151,154</sup>.

**Additional studies using a broader range of (translatable) measures are needed to clarify the clinical relevance of these experimental findings.**

### ***Antidepressants, anxiolytics and modulators of neurotransmission***

CHR subjects are frequently treated with antidepressants for relief of anxiety or depression, and data from clinical audits are consistent with (though *not* proof of) a decreased likelihood of developing psychosis<sup>32,34,35</sup>. Hence, it is of note that sustained treatment of Poly I:C mice during adolescence and young adulthood with fluoxetine decreased both amphetamine-hyperlocomotion and disruption of sensorimotor gating: both indirect recruitment of 5-HT1A receptors and modulation of neurosteroids may be involved<sup>148,150</sup>. **However, caution is warranted since fluoxetine disrupted gating in control subjects and failed to prevent the increased sensitivity to dizocilpine.** Another



developmental model for schizophrenia, prenatal exposure to the mitotoxin and disruptor of neurogenesis, “MAM”, is associated with anxiety<sup>155</sup>. The ability of peri-pubertal diazepam to prevent dopaminergic hyperactivity in adult animals was attributed to its stress-alleviating properties<sup>156</sup>. Further studies of antidepressant and anxiolytic agents appear justified in light of their extensive use in CHR subjects.

Metabotropic glutamate receptor (mGluR)-5 and  $\alpha 7$ -nicotinic receptor positive allosteric modulators (PAMs) possess pro-cognitive properties attracting interest for symptomatic treatment of established schizophrenia<sup>12</sup>. Accordingly, mGluR5 PAMs, applied acutely, rescued impaired social cognition in adult rats neonatally exposed to phencyclidine. Of greater significance, however, chronic administration during adolescence enduringly blocked the appearance of cognitive deficits throughout adulthood<sup>157</sup>. Since adult administration was not effective, *prevention* was clearly the underlying mechanism. Several mechanisms are implicated: anti-inflammatory properties at microglia, recruitment of oligodendrocytes to reduce white matter fibre loss, induction of BDNF, neuroprotective and anti-apoptotic properties<sup>157</sup>. These data are underpinned by another study where adolescent treatment with a mGluR5 PAM impeded the adult development of deficits in sensorimotor gating<sup>158</sup>. This action was mimicked by a  $\alpha 7$ -nicotinic agonist that was proposed to act by a different mechanisms, possibly involving receptor desensitization<sup>158</sup>.

### ***Modulation of intracellular protein networks and histone acetylation***

The scaffolding hub protein, Disrupted in Schizophrenia (DISC)1, is a genetic risk factor for psychosis and several other psychiatric disorders, with DISC1 mutant mice displaying developmental anomalies related to schizophrenia<sup>131,159</sup>. DISC1 is enriched post-synaptically at NMDA receptors where it interacts with protein partners like *Kalirin-7* to control synaptic plasticity and dendritogenesis<sup>160</sup>. While direct manipulation of DISC1 may not be feasible, targeting Kalirin-7 - down-regulated in schizophrenia - might counteract deficient synaptic plasticity<sup>159,160</sup>. Other DISC1 protein partners include “Rac1” (a Rho family GTPase). Rac1 modulates p21 Kinase, and p21 Kinase inhibition during adolescence prevented any further loss of dendritic spines in mice subjected to DISC1 knockdown at embryogenesis, possibly by blocking excess synaptic pruning (see below)<sup>161</sup>. These data resemble findings from mouse models of Fragile X where p21 Kinase inhibition similarly rescued dendritic deficits<sup>162</sup> and suggest a way of preventing the *structural* anomalies underlying schizophrenia. Moreover, they **indicate how, via manipulation of interacting proteins, it may be possible to make the transition from anomalous molecular circuits in animal models to tractable targets for development of course-altering medication in CHR people.**

A rather different approach to course-alteration is represented by **cerebrolysin, a neurotrophic cocktail under investigation for the treatment of stroke and vascular dementia**<sup>163</sup>. When given during adolescence, cerebrolysin decreased the onset of structural, neurochemical and

behavioural deficits in adult mice subjected to VHL during the post-natal period<sup>164</sup>. **The (probably multiple) mechanisms of action of cerebrolysin await further elucidation and it is unlikely to be pursued as a clinical option, but these data are of interest in supporting the potential utility of neuroprotective interventions.**

**The ion channel and GABAergic modulator, valproate, is a candidate for potential clinical trials since, despite risks in pregnant females, it is well-tolerated and used in young people as a mood stabiliser<sup>26,33</sup>. Valproate** prevented the emergence of hyperlocomotor activity and sensorimotor gating deficits in a mutant DISC1 mouse line when given to young adults, an action related to inhibition of excessive glial proliferation in hippocampus<sup>165</sup>. **Further, Valproate blocked the disruption of sensorimotor gating and induction of Histone Deacetylase (HDAC)2 in the above-mentioned “mitotoxin” model for schizophrenia<sup>166</sup>.** Valproate is a pan-inhibitor of HDACs<sup>26</sup>. Though a role for HDAC inhibition in its actions remains to be formally demonstrated, adolescent administration of the more selective HDAC inhibitor, sodium butyrate<sup>26</sup> prior to chronic phencyclidine impeded the development of behavioural-cognitive deficits<sup>167</sup>. Sodium butyrate likewise prevented the appearance of cognitive impairment and dopaminergic hypersensitivity when given to adolescent rats that had sustained neonatal inflammatory damage to the hippocampus<sup>168</sup>.

### ***Behavioural and environmental interventions***

Non-pharmacotherapeutic interventions can also be explored in rodent models. Intriguingly, the preventive effects of sodium butyrate were reproduced by environmental enrichment, consistent with a role for epigenetic mechanisms<sup>167</sup>. **While the therapeutic relevance of environmental enrichment is unclear, this is mechanistically interesting and globally supports the notion that non-pharmacotherapeutic interventions can change outcome when applied early.** Furthermore, mimicking CBT in CHR subjects, cognitive training of neonatal VHL rats during adolescence ameliorated both: 1), inter-hippocampal synchrony of field oscillations and 2), cognitive control (ability to prioritise relevant over irrelevant information) in a test of reversal learning that recruits the PFC<sup>169</sup>. The apparently course-altering effects of early cognitive intervention are, then, sustained well into adulthood.

### ***Collective pre-clinical evidence for effectiveness of pre-symptomatic interventions***

In conclusion, there is a surprisingly diverse, convergent and robust body of data showing that early intervention in rodent models for schizophrenia can modify the appearance of “symptoms” in adults. Underlying mechanisms are unlikely to be unitary and only a limited number of agents have been examined. Nonetheless, these observations provide a promising platform for a broader experimental evaluation of novel strategies for altering the course to (and of) schizophrenia.



## Novel, potential mechanisms of course-alteration: from pathophysiology to pharmacotherapy

### *Network-based concepts: multiple hierarchies of intervention*

Network thinking and, more specifically, Graph Theory, provide a useful framework for studying the disruption of cerebral circuits in schizophrenia and for devising approaches for course-alteration<sup>36,84,170</sup>. Indeed, schizophrenia is a disorder of disconnectivity that reflects progressive disruption of intra-neuronal and cerebral *circuits* rather than the loss or overactivity of any single cellular signal, neurotransmitter or brain region. Once disrupted, networks may phase-shift to an alternative steady state which is hard to reverse, underpinning the need to treat as early as possible<sup>30,84,170</sup>. From a network perspective, interneurons like fast-firing GABAergic basket (and chandelier) cells in the PFC can be considered the nodes (“hubs”, if crucial) with longer, inter-connecting projection neurons such as glutamatergic pyramidal cells the edges (vectors)<sup>14,36,170-173</sup>. For an *individual* neuron, the soma might be considered the node and its axon (and dendrites) the edges.

Disruption of either hubs or edges can provoke network failure, and both are legitimate targets for therapy. Though medication interacts with specific protein(s) rather than networks *per se*, pharmacotherapy can target specific hubs at many hierarchical levels to improve the operation of dysfunctional *networks* and hence reduce the risk of schizophrenia (Figure 4). Drug associations, multi-target agents and non-pharmacotherapeutic interventions like brain stimulation and psychosocial training may be particularly appropriate for protecting and restoring degraded neural networks<sup>36,170</sup> (Figure 4).

### *Deregulated cortico-limbic GABA-glutamatergic circuits: the need for resynchronisation*

A highly consistent change **in patients and animal models for schizophrenia** is a down-regulation of fast-spiking frontocortical GABAergic interneurons which reciprocally interact with glutamatergic pathways<sup>14,29,172,173</sup>. The developmental perturbation of GABAergic interneurons is related to several factors including: NMDA receptor hypoactivity; abnormal regulation by astrocytes and neurotrophins (BDNF); disruption of the extracellular matrix; insufficient energy supply and cell-autonomous anomalies<sup>14,29,172-174</sup>. Deregulation of GABAergic interneurons leads to an imbalance of excitatory/inhibitory transmission, desynchronisation of cortical and cortico-subcortical circuits, disruption of neural oscillations and gamma-band synchrony, and impaired cognition and mood<sup>12,14,25,29,84,171-173</sup>. Further, perturbation of GABA-glutamatergic coupling in the PFC in turn disrupts subcortical circuits and dopaminergic transmission and is linked to the emergence of psychosis<sup>29,84,88,153,171,172</sup> (Figure 4). In young CHR subjects, a reduced dynamic range of frontocortical GABA-glutamatergic networks may aggravate cognitive inflexibility, exaggerate sensitivity to stress and increase vulnerability to recreational drugs like cannabis which reciprocally interferes with GABAergic transmission<sup>84,171-173,175</sup>.

In view of these observations, the protection of GABAergic-glutamatergic circuits from disturbance, and their restoration following disruption, provides an instructive and integrative framework for the following discussion of potential course-altering mechanisms for schizophrenia.

### ***Restoring normal patterns of GABAergic transmission***

**Direct modulation of GABAergic signalling:** Activation of GABA-A $\alpha$ 2 receptors on pyramidal cells has been evaluated as an adjunct in chronically-ill subjects for improving cognition, but results were disappointing<sup>12,14,172</sup>. As pointed out below, evaluation *earlier* in the disorder for network-resynchronisation and course-alteration might prove more successful. Another possible strategy would be pregnenolone. This neurosteroid acts as a PAM at NMDA receptors and is metabolised into allopregnenolone, a PAM at GABA-A receptors. Supporting such a study, there are encouraging findings with adjunctive pregnanolone, negative symptoms and functional outcome in newly-diagnosed patients<sup>8,176,177</sup>.

Recent work has unveiled some other, less familiar ways in which aberrant GABAergic transmission might be normalized to interrupt the path to psychosis.

The **Na-K-Cl co-transporter (NKCC1)**, which is genetically linked to schizophrenia is involved in expressing the postsynaptic effects of GABA-A receptors, as well as neural circuit formation during brain maturation<sup>178,179</sup>. A developmental decrease in the ratio of NKCC1 to the K-Cl co-transporter explains the gradual transition from depolarising to hyperpolarising actions of GABA<sup>178</sup>. Conversely, an aberrantly *high* level of NKCC1 *vs* K-Cl co-transporters - together with anomalous NKCC1 splicing - is related to network anomalies of developmental disorders<sup>178</sup>. These findings suggest that early inhibition of NKCC1 may restore the balance of excitation/inhibition, promote network synchrony and interrupt the path to psychosis. Underpinning interest in modulation of NKCC1, the antagonist bumetanide improved facial processing in autistic children (3-11 years old)<sup>180</sup>. Further in an experimental model of autism, prenatal treatment of mothers with bumetanide precluded emergence of behavioural deficits in offspring<sup>181</sup>. While bumetanide poorly crosses the blood-brain barrier, more brain-penetrant and selective ligands might be an option for preventative treatment of CHR patients<sup>178</sup>. **One caveat is that most rodent studies of NKCC1 have been undertaken in early post-natal life. Nevertheless, bumetanide enhanced social cognition in adolescent and young adult autists, supporting the relevance of NKCC1 to events occurring around the time of conversion to schizophrenia<sup>182</sup>. Further, polymorphisms in NKCC1 affect PFC function and cognition in adult schizophrenics<sup>179</sup>.**

Like their GABA-A receptor counterparts, pre and post-synaptic GABA-B receptors are involved in the establishment, operation and integration of cortical networks. Their actions are partially mediated *via* BDNF and embrace the control of neural migration, synaptogenesis and neurite growth<sup>183</sup>. Suggesting a link to schizophrenia, activation of GABA-B receptors counters the imbalance

in excitation/inhibition, gamma-asynchrony and deficits in sensorimotor gating, cognition and behaviour seen in: 1), mice with genetic NMDA receptor hypofunction<sup>184</sup> and 2), rodents exposed to psychostimulants: these actions are expressed at least partly in the PFC<sup>185</sup>. In addition, the GABA-B agonist, arbaclofen, improved social behaviour in children with Fragile X<sup>186</sup>. Small-scale clinical trials of baclofen in established schizophrenia were unsuccessful, but GABA-B receptor stimulation would justify evaluation in the CHR phase for reduction of transition.

**Intervening at ion channels on GABAergic interneurons:** Another potential target would be **Kv3 potassium channels** which rapidly repolarise GABAergic interneurons following excitation: this action permits precise patterns of high-frequency firing, coordination of cortical networks, and synchronisation of gamma-oscillations in relation to goal-related behaviour and cognition<sup>29,84,171,172</sup>. Kv3.1b and Kv3.2 expression **peaks during early development but persists through adolescence into adulthood**, and levels of the former were decreased in the PFC of chronic schizophrenics<sup>187</sup>. This decrease was countered by antipsychotics<sup>187</sup>. **Information on Kv3 channel levels in brain are lacking from CHR subjects**, but *direct* Kv3 ligands are an intriguing prospect for symptom relief and prevention of transition in CHR subjects. Indeed, Kv3 channel inducers alleviated cognitive and negative symptoms in a rodent, sub-chronic phencyclidine model of schizophrenia in which Kv3 channel expression was reduced<sup>188,189</sup>. **Whether Kv3 channel inducers, applied during adolescence, impede the appearance of symptoms in animal models for schizophrenia, would be important to determine.**

**The challenge of intracellular targets: Neuregulin-ErbB4 and downstream proteins:** Neuregulin-1/ErbB4 receptor signalling plays an important role in the differentiation and migration of cortical GABAergic interneurons, and in other developmental processes like axon myelination<sup>29,159,190</sup> (Figure 4A). Neuregulin-1 is strongly linked to schizophrenia based on: genetic association; changes in its levels in the brain (and lymphocytes) of patients; and the “schizophrenia-like” phenotype of its manipulation in rodents, including deletion solely in PFC populations of GABAergic interneurons where it is enriched<sup>29,53,159,190</sup>. Furthermore, Neuregulin-1 over-expression in pyramidal neurones triggers synaptic and behavioural anomalies relieved by extinction of its expression, suggesting that effects of aberrant ErbB4 signalling and a schizophrenic phenotype might be reversible *after* emergence<sup>191</sup>. Finally, Neuregulin-1/ErbB4 recruit the DISC-1 partner, Kalarin-7, to influence the plasticity and morphology of dendrites in interneurons<sup>159,160,190</sup>.

**Despite all this, Neuregulin-1 illustrates the challenges of trying to therapeutically manipulate a cellular hub protein<sup>159</sup>.** Early intervention to prevent abnormal neuronal migration is not yet feasible. Increases *and* decreases in Neuregulin-1/ErbB4 signalling have been reported in schizophrenia, and *both* are deleterious in animal models so medication would need to be tightly regulated around a set-point<sup>190</sup>. In addition, Neuregulin-1 has multifarious (and partly sex-specific)

actions across many cells types<sup>159</sup>. Finally, administration of Neuregulin-1 to rodents is not consistently favourable: for example, early-life exposure results in persistent over-stimulation of mesolimbic DA transmission<sup>190,192</sup>. Thus, while focussed manipulation of Neuregulin-1/ErbB4 signalling in GABAergic interneurons might be favourable, this would be hard to specifically realise as a strategy for course-alteration.

One potential solution may be to act at proteins *downstream* of Neuregulin-1, and an ErbB4 “CYT-1” isoform over-expressed in schizophrenia recruits the Phosphoinositide-3/Atypical Kinase signalling cascade<sup>190,193</sup>. One of two constituent proteins (“PIPK3CD”) encodes a p100 $\delta$ -subunit. Pharmacological inhibition of p100 $\delta$  countered anomalies in a VHL model of schizophrenia, so study of inhibitors prior to emergence of symptoms would be warranted<sup>193</sup>. **Finally, it might be possible to act at inhibitory GABAergic synapses via modulation of the risk gene, Neurexin-1 in interaction with its post-synaptic partner, Neuroligin-1<sup>55,53,92</sup>.**

**Thus, there may be openings for manipulating GABAergic, glutamatergic and other modes of transmission via actions at intracellular networks disrupted in schizophrenia, but this currently remains challenging.**

### ***Epigenetic strategies for normalising faulty neurodevelopment leading to schizophrenia***

**Countering anomalous epigenetic control of GABAergic interneurons.** Anomalous control of gene transcription by DNA methylation and histone post-translational marking, together with aberrant regulation of mRNA translation by miRNAs, lies at the interface of genetic and environmental triggers for schizophrenia<sup>25,26,194-195</sup>. Disrupted epigenetic mechanisms may be inherited, provoked by *de novo* CNVs and mutations, and/or triggered by diverse environmental events ranging from perinatal infection to adolescent drug abuse, which increase the risk for schizophrenia<sup>25,26,66,191,195,196</sup>. **Interestingly, the best-documented link between aberrant epigenetic mechanisms and the onset of schizophrenia has been found with GABAergic neurones. Thus,** DNA promoter hypermethylation and aberrant patterns of histone acetylation/methylation in PFC (and hippocampal) populations of GABAergic interneuron lead to reduced synthesis of GABA and ***Reelin***, together with concomitant down-regulation of mGluR2 receptors and BDNF<sup>25,26,196-198</sup>.

As regards strategies for reviving GABAergic interneurons, reducing promoter hypermethylation may be feasible by: interfering with DNA-Methyltransferases, inducing endogenous Demethylases like “Gadd45- $\beta$ ”, or using engineered constructs to mimic their activity and enhance demethylation. However, in all cases, safe and brain-penetrant agents will be required for clinical use<sup>25,26,198,199</sup>. More accessibly, GABA promotor demethylation can be elicited upstream using agonists at mGluR2Rs which rekindle GABA, reelin and BDNF expression *via* induction of the dimethylase, Gadd45- $\beta$ <sup>25,26</sup>. Furthermore, nicotinic  $\alpha 2\beta 4$  agonists harness GABA synthesis by reducing the

expression of DNA-Methyltransferases and disrupting repressive activity of the DNA-binding protein, “MeCP2”<sup>25</sup>.

Approaches other than demethylation might also be used to resuscitate genes repressed in GABAergic interneurons. By interfering with histone deacetylation (aberrant in schizophrenia), Valproate (*vide supra*) re-activates GABA synthesis both directly and *via* recruitment mGluR2 receptors<sup>25,26,200</sup>. While modulators of acetylation affect numerous genes, agents modifying histone methylation (likewise disrupted in schizophrenia) act at a more restricted set: they are well-advanced in oncology and could be experimentally tested for GABA interneuron revival and course-alteration in schizophrenia<sup>26,201</sup>.

Another possibility to promote GABAergic transmission may be *via* miRNAs which fulfill diverse regulatory roles in neurodevelopment and interact with DNA and histone methylation. They are highly dynamic during puberty, impacted by risk factors from CNVs to peri-natal inflammation to adolescent stress, and deregulated in schizophrenia<sup>26 194,202,203</sup>. Interestingly, GABAergic interneurons possess a distinctive complement of miRNAs<sup>204</sup> and their activity is under the control of several miRNAs including miR-137 (genetically linked to schizophrenia), miR-132 (a controller of synaptic plasticity) and miR34a (modulated by NMDA receptors) (Suppl Box 2)<sup>26,194,203</sup>.

**Broader relevance of epigenetic strategies to course-alteration** Epigenetic anomalies in schizophrenia are not restricted to GABAergic interneurons. For example, a broad suite of changes in miRNA affects many mediators like glutamate, D2-receptors, Kalarin/p-21 Kinase and ErbB4<sup>25,26,194,203,205</sup> (Suppl. Box 2). Epigenetic medication for course-alteration would be especially attractive since it acts at the network level to modulate entire clusters of proteins. Further, such agents could have beneficial symptomatic effects in the CHR state on cognition and mood<sup>26</sup>. The major focus is currently on better understanding the functional roles of epigenetic mechanisms, and exploiting them as biomarkers. For therapeutic exploitation, it will be necessary to: 1) establish which epigenetic factors *drive* (*vs* oppose or merely accompany) pathophysiological processes leading to schizophrenia and 3) determine the optimal mode, timing and duration of therapy.

### *Promoting the connectivity of neural circuits*

**Protecting the operation and integrity of cerebral networks.** Though less well-documented than GABAergic mechanisms, there are several other potential approaches for enhancing cerebral connectivity and hence reducing the risk of transition. One of these focusses on cytoskeletal microtubules since their disruption in schizophrenia perturbs the structural support of neurones, impedes axonal transport and compromises synaptic plasticity (Suppl. Box 3). Two further strategies are outlined below, both particularly relevant during the therapeutic window of adolescence and young adulthood.

**Targeting oligodendrocytes to counter aberrant patterns of myelination:** In comparison to extensive subcortical myelination during childhood, intra-cortical and cortico-subcortical myelination intensifies during adolescence and young adulthood: that is, at the time of transition to psychosis<sup>206,207</sup>. Myelin requires continuous renewal in the face of damage by trauma, nutritional deficits, hypoxia, stress and inflammation<sup>206,208,209</sup>. These are all risk factors for schizophrenia and the white matter disruption seen prior to and following diagnosis of psychosis reflects both their impact and faulty oligodendrocyte-controlled programs of myelination<sup>206,208,209</sup>. Cortical white matter (myelin) deficits and oligodendrocyte abnormalities have been related to the onset of network asynchrony, negative symptoms, hallucinations, impaired sensory processing, cognitive decline and perturbed dopaminergic and glutamatergic transmission<sup>204,206,208,210,211</sup>.

Clearly, then, myelin and oligodendrocytes are potential targets for course-alerting therapy. It has been speculated that antipsychotics (possibly *via* inhibition of Glycogen Synthase Kinase-3 $\beta$ ) exert transient neuroprotective properties for white matter<sup>212</sup>. Further, the anti-inflammatory and anti-oxidant actions of pregnenolone, minocycline and Omega-3 (see above) have a trophic, neuroprotective influence on myelin<sup>100,134-136,175,176</sup> which might also benefit from neutralisation of retroviruses (see below). Several other concepts are emerging for opposing the developmental deregulation of oligodendrocytes and myelination that accelerates the course to schizophrenia<sup>206,213,214</sup>. For example, Valproate and Lithium promote myelination and oligodendrocyte function<sup>214,215</sup> while the anti-parkinson agent, benzotropine, exerts myelin-repairing activity by a mechanism involving M1/M3 muscarinic receptor antagonism<sup>18</sup>. In addition, pro-myelination and neuroprotective “GRE30” estrogen receptors are enriched in oligodendrocytes<sup>216</sup>.

**Countering excessive synaptic pruning during adolescence:** Corticolimbic synaptic density peaks in infancy around 2-4 years followed by the **pruning of excess synapses**, a process occurring most intensely during adolescence and continuing until the third decade. Surviving synapses are stabilized so network connectivity is globally improved<sup>217,218</sup>. **There is evidence that synaptic pruning in the dorsolateral PFC and hippocampus is disproportionate in schizophrenia, which would aggravate the abnormal coupling of glutamatergic-GABAergic neurones and compromise the operation of neural circuits<sup>71,171,217-219</sup>. For example, in interaction with Kalirin-Rac, the “Actin-related protein” 2/3 complex promotes actin polymerization and spine formation: it’s disruption mirrors excess pruning, with a loss of synaptic contact on pyramidal cells leading to over-activity and, *via* a long-range projection to the ventro tegmental area, activation of mesolimbic dopaminergic pathways and psychosis<sup>71</sup>. Further, together with reduced neuronal size, over-pruning may help account for the reduction in cortico-hippocampal spine density/grey matter that anticipates and characterizes schizophrenia<sup>71,76,77,217,219</sup>.**



Several, therapeutically-accessible mechanisms disrupted in schizophrenia might be able to modify the disruption of synaptic pruning and spine dynamics thought to anticipate psychosis, including the DISC1-Kalarin-Rac axis, Neuregulin-ErbB4, estrogen signaling, BDNF and NMDA receptors (see above)<sup>159,161,220-223</sup>. Other opportunities might emerge from the role of cytokines in anomalous astrocytic and microglial sculpting of synapses<sup>218,222</sup> and, based on studies of *defective* synaptic pruning in autism, from “mammalian target of rapamycin”-regulated autophagy, which also controls pruning<sup>223</sup>.

The latter observation underpins the relevance of pruning to developmental disorders in general, and indicates that an optimal “degree” of (neither too much nor too little) is needed for normal development. However, as discussed elsewhere<sup>217</sup>, any *causal* link between aberrant pruning and psychosis remains to be further confirmed, and evidence that normalization of pruning impedes appearance of psychosis in animal models for schizophrenia is awaited.

### *Countering neuro-inflammation, immune deregulation and the impact of infections*

**Anti-inflammatory treatments to counter short and long term repercussions of infection:** Genetic studies have found an association of schizophrenia with the Major Histocompatibility Complex<sup>224</sup> and schizophrenia and CHR subjects display robust evidence for inflammatory and neuroimmune disruption (see above)<sup>134,135,225</sup>. Further, bacterial or viral infection of the mother during pregnancy elicits a immune-inflammatory response (“Maternal Immune Activation”) which deleteriously impacts cerebral development of the foetus, while post-natal infection likewise compromises normal maturation of the brain<sup>27,28,225</sup>. Inflammation is linked to microglial release of pro-inflammatory cytokines like Interleukin-1 $\beta$ /Interleukin-6, Tissue Necrosis Factor- $\alpha$  and kynurenate (a NMDA/ $\alpha$ 7-nicotinic receptor antagonist). Collectively, they exert deleterious effects on neurones, astrocytes and oligodendrocytes leading to anomalous neural proliferation/differentiation, synaptic plasticity and myelin formation and, later in life, disruption of GABA-glutamatergic networks<sup>28,34,135,137,226</sup>. Importantly, then, perinatal inflammatory events provoke *long-lasting* changes in immune status (such as an up-regulation of Interleukin-6 expression) that persist into young adulthood **both in human patients and in animal models for schizophrenia. These enduring changes** render subjects more sensitive to second-wave risk factors like stress, which likewise perturb immune function and unveil latent structuro-functional deficits<sup>28,227-229</sup>. **Of particular interest, early-life immune disruption leads to abnormal PFC levels of inflammatory mediators and anomalous GABAergic transmission in adult mice<sup>230</sup>.**

Treatment could not realistically be instigated at the time of immune disruption/infection if during pregnancy or infancy. Conversely, owing to above-mentioned longer-term repercussions of perinatal infection - and to pro-inflammatory events in adolescence/young adulthood - interventions could be undertaken in CHR subjects. In fact, anti-inflammatory properties may well be implicated in

the clinical influence of Omega-3 upon conversion, and in the preventive actions of Omega-3, minocycline, **N-acetyl-Cysteine and Celecoxib in animal models for schizophrenia (Tables 1 and 2)<sup>100,134,135</sup>**. The latter agents and Aspirin, likewise evaluated for efficacy as an adjunct in schizophrenia<sup>135</sup> merit then consideration for trials of course-alteration. Another candidate would be the **neurosteroid and GABA modulator**, pregnenolone which displays complementary anti-inflammatory properties<sup>176,177</sup>.

These and other agents directly targetting anti-inflammatory mechanisms, like cytokine modulators, warrant experimental exploration in models of course-alteration.

**Neutralising a Retrovirus trigger of neuro-inflammation:** One consequence of infection with bacteria and viruses (such as Herpes Virus-2) and parasites (like *Toxoplasma gondii*) is the revival of dormant **Human Endogeneous Retroviruses (“HERV”)**<sup>231,232</sup>. Neutralisation of their pro-inflammatory protein envelopes by passive immunisation is under investigation for the treatment of multiple sclerosis, and a comparable course-altering strategy might be applicable to course-alteration for schizophrenia<sup>232</sup> (Suppl Box 4).

**Restoring the equilibrium of the intestinal microbiome:** The intestinal microbiome is important for mental (and physical) health and disruption is related to anomalous neurodevelopmental processes leading to disorders like autism and schizophrenia<sup>28,233</sup>. Opportunities for course-altering intervention are suggested by an interrelated suite of developmental anomalies triggered by early-life intestinal infection-inflammation: an abnormal gut microflora; structural damage to the gut; and increased intestinal permeability and excessive penetration of bacteria and their metabolites/toxins into the circulation<sup>234,235</sup>. Potentially benign ways of reducing the risk of psychosis include: pro-biotic, non-pathogenic bacteria like bifidobacteria which affect central GABAergic transmission, and agents that prevent bacterial translocation to the circulation<sup>201,234-236</sup>. As regards antimicrobial agents, it might be asked whether Minocycline exerts its putative actions in schizophrenia at least partially *via* an impact on the gut microbiome. **Nonetheless, it is unclear just how specific “microbiotic” interventions would be for reducing the risk of schizophrenia compared to a broader influence on mental and physical health<sup>234</sup>. Further, rigorous experimental and clinical studies are required to: 1), confirm any causal relationship between an abnormal gut microbiome and pathophysiological changes that ultimately result in schizophrenia and 2), identify potential therapies for their control.**

### ***Protecting young CHR subjects from life-style and environmental risk factors***

The transition to schizophrenia occurs at a time when the adolescent/young adult brain is undergoing a major structural and functional reorganisation. More generally, it is: enduring the onslaught of gender-specific hormones, facing increased energy demands, experiencing shifts in



processes of decision-making, behavioural control and reward mechanisms, and confronting an increasingly complex social, cultural, cognitive and emotional environment<sup>63,237,238</sup>. Accordingly, the brain is especially vulnerable to disruption. However, rather than some inevitable (or stochastic) progression to schizophrenia, conversion is likely triggered by a new wave of risk factors including a triad of mutually-aggravating hits: 1), poor resistance to psychological stress, 2), social isolation and 3), excessive consumption of drugs of abuse, especially cannabis (Suppl Figure 2). While primary prevention (avoidance) and education are desirable, there is considerable interest in psycho-social/cognitive-behavioural and pharmacotherapeutic interventions to counteract these risk factors and decrease conversion (Box 3).

## **Perspectives for accelerating progress towards course-altering therapy**

### **Novel cellular models for probing pathophysiology and identifying new targets**

A major challenge faced in developing course-altering medication is limited understanding of cellular anomalies involved in transition. One way of addressing this knowledge-gap would be induced pluripotent stem cells (iPSC) for: 1), characterisation of perturbed developmental processes underlying schizophrenia; 2), analysis of its genetic, cellular and molecular substrates and 3), identification of novel mechanisms for prevention and rescue<sup>239,240</sup>. iPSCs from patients and CHR subjects could be differentiated into networks of GABAergic, glutamatergic and/or dopaminergic neurones permitting exploration of both intra *and* intercellular communication and the identification of potential course-altering strategies<sup>241</sup>. Compared to monogenic diseases, iPSC models for schizophrenia present a major challenge yet, as outlined in Box 4, considerable progress is now being made.

### **Optimising the use of animal models for schizophrenia**

Despite the availability of many well-characterised animal paradigms for studying schizophrenia, all have limitations and none has, as yet, been specifically designed to study course-alteration<sup>131</sup>. Hence, the important issue of which animal models, procedures and readouts are best-adapted to this goal is considered in Box 4.

### **Improved linking of experimental with clinical studies of course-altering therapies**

To enhance the predictive validity of animal models, it would be instructive to integrate “translational” measures exploitable in clinical investigations. To this end, procedures currently in use for characterising CHR subjects and monitoring the efficacy of treatment should be more systematically applied to studies of prevention in rodents. **Endocrine, immune and biochemical readouts from the circulation are easily accessible, while quantification of GABAergic, glutamatergic and DA transmission, as well as MMN interrogation of sensory processing, are**

likewise readily performable. Recent technical advances now make it feasible to analyse in small animals structuro-functional changes that trigger and signal the onset of psychosis using grey matter MRI, fMRI studies of cerebral connectivity and EEG analyses of neural networks<sup>242-244</sup>.

Mirroring work in animals<sup>131,153</sup>, one promising translational approach in humans is the use of ketamine at sub-psychotic doses in volunteers to mimic the perturbation of glutamatergic and GABAergic transmission seen prior to the FEP<sup>245</sup>. FMRI and cognitive testing can be undertaken in parallel, and this procedure would be useful for early clinical exploration of novel strategies for course-alteration.

### **Biomarkers for tracking and predicting clinical efficacy of course-altering medication**

It is desirable to: 1), link a risk factor to a pathophysiological mechanism favouring onset or progression of schizophrenia; 2), identify a strategy for rectification and 3) develop biomarkers both for identifying subjects to treat and for predicting therapeutic efficacy of the intervention in question<sup>33,52</sup>. **Such biomarkers should serve to track and predict medication efficacy from the inception of treatment since a clinical reduction in course-alteration might only become clear years later. That is, the goal is to find a surrogate biomarker that: 1), directly reflects the pathophysiological mechanism targeted and 2), changes in which are linked with and predictive of an eventual reduction of transition.** Many procedures would be similar to those used to detect and stratify CHR subjects (see above, Figure 3B), but details and application would differ. Further, during medication, biomarkers should be coupled to measures of drug exposure and target engagement.

The NAPLS study of CHR subjects exemplifies the multi-pronged approach that can be used<sup>48</sup> and surrogate readouts of efficacy include, depending on medication of action: cortisol levels for drugs countering HPA axis overdrive, circulating levels of cytokines for anti-inflammatory agents, changes in lymphocyte patterns of histone marking for epigenetic modulators<sup>26,190</sup>, and GABA/Glutamate levels in PFC for therapies designed to re-coordinate GABA-glutamatergic networks<sup>10,59,61,63,69</sup>. More generalist strategies would be estimation of extracellular DA release/D2 receptor occupation in striatum, MMN measures of neural processing and structural MRI of the ventricles and hippocampus<sup>57,84,88,93</sup>. **As a general rule, it would be important to employ multiple biomarkers both at the initiation of clinical trials and throughout their duration.**

**Finally, in tracking the efficacy of course-altering medication, the influence of several variables not yet factored into clinical trials, like age of onset, potential “placebo” effects of treatment, gender and cultural differences also deserve consideration (Suppl. Box 5)<sup>246-248</sup>.**

### **A “hybrid” concept: symptom relief coupled to a reduction of transition**

Care of CHR subjects necessitates the relief of presenting symptoms - usually of *greater* concern to patients at consultation than an increased risk of conversion. In principle, a separate treatment might

be instituted to impede transition. However, control of symptoms in the CHR state may of itself and *perhaps causally* reduce the risk of progression to psychosis and other disorders (Figure 5). Such a “hybrid” strategy would have advantages compared to pure disease-modification: 1), alleviation of symptoms provides a functionally-relevant and patient/regulator-valued readout of target engagement and potential course-altering efficacy *before* longer-term measures of transition - hence derisking clinical trials of course-altering therapy<sup>16,32,34</sup>; 2), a common treatment for symptom-control and course-alteration would minimize poly-pharmacy; 3), interventions may likewise delimit conversion to *other* disorders like bipolar depression<sup>23,34</sup> and 4), medication would *not* be considered an “antipsychotic,” improving acceptability.

In fact, as discussed above, Omega-3 and psycho-social/cognitive-behavioural interventions may both alleviate symptoms *and* reduce progression in CHR subjects, but it is unclear whether these actions are *mechanistically* linked. Concerning novel strategies, counteracting the adolescent risk factors, psychosocial stress, drug-seeking/cannabis abuse and impaired social cognition/social isolation is particularly attractive (Box 3) (Suppl Figure 3). Pharmacotherapeutic strategies include the pro-social modulator, Oxytocin<sup>249</sup>; CRF1 antagonists for relieving anxiety and moderating stress-induced relapse of drug-seeking behaviour<sup>250</sup>; and dopamine D3 receptor antagonists for countering drug-abuse and promoting cognition<sup>251</sup> (Box 3). 5-HT2A receptor blockade may also be of interest since it is well-tolerated, counters hallucinations and implicated in preventive actions of risperidone in rats<sup>153,252</sup> (see above). As a final example, 5-HT2C receptor blockade is associated with anxiolytic and antidepressant properties and increased frontocortical dopaminergic transmission which may counter negative symptoms<sup>8,253</sup>.

The most effective hybrid treatment may well be the judicious association of psycho-social/cognitive-behavioural strategies with appropriate pharmacotherapy.

### **Re-orienting agents evaluated in chronic schizophrenic for early course-alteration**

Certain classes of agent poorly active in chronic schizophrenia for symptom-relief might profitably be re-evaluated for course-alteration in the CHR state. This option was evoked above for anti-inflammatory agents, but there are several other intriguing possibilities.

GABA-A $\alpha$ 2 agonists disappointed in association with SGAs for amelioration of neurocognitive deficits in chronic patients but, administered to CHR subjects, they might delimit conversion (and improve symptoms) by resynchronising cortico-subcortical glutamatergic circuits<sup>14,29,171-173</sup>. Recruitment of hypoactive NMDA receptors upstream of GABAergic interneurons by agonists and inhibitors of glycine reuptake has yielded chequered results in schizophrenia, but the most compelling hints of efficacy were against negative symptoms which are present in the prodromal phase<sup>8,9,10</sup>. Hence, they might be re-positioned in CHR subjects. Supporting this idea, a pilot 24 week study<sup>254</sup> reported beneficial effects of Glycine in CHR patients, but work on *conversion* remains to be undertaken. Intriguingly, D-Serine rather than Glycine gates dysfunctional NMDA receptors in the

PFC<sup>255</sup> and D-Serine improved negative symptoms in a controlled study of CHR subjects, with larger-scale studies (including measures of conversion) anticipated<sup>256</sup>. D-Amino-Acid Oxidase inhibitors for blocking D-Serine metabolism would be another therapeutic option<sup>257</sup> or drugs blocking generation of the endogenous NDMA antagonist, kynurenate<sup>10,134</sup>. Levels of Kynurenate are elevated by inflammatory states that enhance the risk of schizophrenia<sup>134,135</sup>. Valproate is also of interest as an epigenetic regulator (see above), despite lack of evidence for efficacy in chronic schizophrenia<sup>26</sup>.

There is, then, scope for re-orienting certain agents that were largely unsuccessful in chronic schizophrenia (usually as add-ons) to an earlier time-point in CHR subjects where they may interrupt progress of the disorder and be of symptomatic benefit - alone and/or in association with psychosocial/cognitive-behavioural therapies

### **Evaluation of course-altering therapy after the first episode**

While the above discussion focussed on *preventative* interventions in CHR subjects, an alternative entry-point is just after the FEP. In reality, some “CHR” subjects will already have undergone a “pre-diagnostic” psychotic event (Figure 3) **and treatment is urgent since duration of untreated psychosis correlates with unfavourable short and long-term outcome, a lower chance of remission, more severe symptoms and poor social integration**<sup>34,35,83,258</sup>. In this sense, rapid intervention with antipsychotics might nominally be considered “course-altering”<sup>259</sup>. However, evidence for protective effects of SGAs against *pathophysiological* changes associated with psychosis is limited and of uncertain clinical relevance<sup>154,212,260-264</sup>. Moreover, in certain patients, decreases in brain volume in patients may be *aggravated* by long-term exposure to *high* doses of antipsychotics<sup>76,78</sup>.

Nonetheless, promoting recovery and course-alteration by (*novel*) therapies in the critical phase *after* the FEP would certainly be cost-effective<sup>37</sup>. **One important study is RAISE (Recovery After Initial Schizophrenia Episode) which proposes a broad suite of treatments, both pharmacotherapeutic and other, in order to enhance quality of life, patient function and long-term outcome. Whether this strategy directly engages with neural substrates underlying progression of schizophrenia is unclear, and the lack of a formal “control” group make it hard to be sure of the specificity of treatment effects. Nonetheless, preliminary findings are promising, this study will be of considerable interest to follow, and has led to enhanced Government funding of Early Psychosis programmes**<sup>265-267</sup>.

For use of course-altering agents after the FEP, there is no dilemma of whom to treat compared to CHR subjects who may not convert. Full recovery - sustained functional and symptomatic remission - after the FEP is only 10-15%, remission is often incomplete, and relapse is common (up to 80 % at 5 years)<sup>262,264</sup>. Hence, delayed appearance of a second episode, reduced symptom intensity and recuperation of real-world function would be suggestive of course-alteration if accompanied by structural and other biomarkers of normalised pathophysiology. Success would be favoured by well-tolerated medication with high adherence since relapse after the FEP is coupled to

non-compliance<sup>263</sup>. Substance abuse, negative symptoms and poor social functioning suggest a poor prognosis so agents that also acted on *these* symptoms might, by analogy to “hybrid” CHR agents, particularly improve long-term outcome (Box 3)<sup>8,38,263</sup>.

On the down-side, following the FEP, novel drugs may well have to be given with SGAs so differentiating genuine course-alteration *versus* symptom relief would be difficult<sup>263</sup>. Moreover, once the threshold to psychosis has been transgressed, course-alteration may be harder to achieve owing to a network phase-shift and more pronounced structuro-functional changes in the brain<sup>170</sup>. Finally, though adult rescue of behavioural deficits is under study for monogenic forms of autism, the duration of relief is uncertain and clinical proof awaited<sup>21,26</sup>.

For novel agents, one strategy may be to perform an initial trial with patients in remission after a FEP then to “work back in time” to earlier phases, including *prevention* of conversion in CHR subjects. Dose reduction might also be progressively achieved by association with psychosocial/cognitive-behavioural treatments.

### **Collaborative ventures promoting discovery and development of course-altering therapies**

Governments, Regulators, Academia, Industry and National Associations are collectively searching for solutions to complex and overarching socio-medical challenges like orphan diseases and epidemics<sup>52,268</sup>. One example is the EU Innovative Medicines Initiative “NewMeds” Programme for refining the translational tools needed to validate improved treatments for schizophrenia and depression<sup>268</sup>. Another initiative is “PsyScan” which is developing multi-modal strategies for more reliably predicting transition in individuals, **and which is sufficiently well-powered to deal with factors like gender<sup>52</sup>**. Since preventative medicine and adolescent health are now high on the agenda, such programmes should accelerate progress towards course-alteration for schizophrenia and other psychiatric disorders (Box 2)<sup>1-4,17,40,268</sup>. **Further, prophylactic medicine for cardiovascular and inflammatory disorders is widely-accepted. Nevertheless, in light of costs to health services and the fact that CHR subjects do not yet have a psychotic diagnosis, it will be important to minimize risk and ensure the support of patients, carers, regulators and reimbursers for early interventions to prevent transition to schizophrenia, or other psychiatric disorders (Suppl Box 6). This support should be fostered by: 1), the fact that the great majority of CHR subjects will have enduring psychiatric problems (Figure 4); 2) the current focus is on young people that are actively seeking help and 3), undertaking initial clinical studies of new drugs in patients who have just undergone a FEP (Box 2).**

### **General discussion and concluding comments**

Sustained efforts to improve the symptomatic treatment of schizophrenia have yielded little major progress over the past decades. It is important that this work continues within a revised

framework focussing on novel mechanisms of action and real-world measures of outcome (Suppl Figure 1). However, driven by improved understanding of pathophysiology, there is now scope for a complementary strategy that aims to alter the course to and of schizophrenia.

**Much progress is being made in the identification of CHR subjects, and a substantial body of clinical and (predominantly) preclinical data converge in suggesting that pre-emptive interventions may interrupt (or at least delay) the emergence of a schizophrenia-like phenotype (Tables 1 and 2). Evidence from clinical studies is of especial significance though, as emphasized above, much remains to be done to confirm promising observations, increase the power and size of trials, take account of many complicating variables, and expand studies to novel therapeutic mechanisms. The latter enterprise is being driven by cellular studies and work with rodent models for schizophrenia. Despite their limitations, a strikingly-broad range of interventions administered during adolescence reduces the appearance of schizophrenia-related anomalies in adults. Certain treatments, such as those interacting with GABAergic transmission, inflammatory processes and the response to stress, have a solid conceptual and experimental foundation while others, such as those targeting intracellular proteins, epigenetic marking and synaptic pruning require much further characterisation. In any event, recent progress is encouraging.**

Hybrid strategies appear particularly appealing for co-joint relief of symptoms in CHR subjects and - partly as a consequence - reduction of transition and improved functional outcome. Challenges remain in terms of identifying whom to treat and when best to start treatment, with interventions after the FEP an option for drugs as yet untested in humans. Further, only a handful of potential approaches have been as yet clinically evaluated, so the issue of *how* best to treat remains unresolved. A one-size-fits-all unitary answer appears improbable and the judicious combination of distinct modes of pharmacotherapy and psychosocial/cognitive-behavioural therapy appears the most likely route to success. Finally, a crucial issue is how to rapidly predict/demonstrate efficacy over a reasonable time-scale in the course of clinical trials. These issues are under intense scrutiny and progress should be rapid over the coming years.

Thus, in addition to improved alleviation of the symptoms of schizophrenia, it may eventually become possible to target the underlying pathophysiology and delay, prevent or moderate its progress. In view of the huge personal suffering and socio-economic burden of schizophrenia and other psychoses, this would seem a worthy goal.

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## Glossary

**Psychosis**, a cluster of clinical signs related to, but not identical with, positive symptoms of schizophrenia: detachment from reality; compromised insight; hallucinations and delusions; psychomotor anomalies like catatonia or agitation; disorganised thought, speech and behaviour. Generally used as the operational definition of transition to schizophrenia, despite it's (less systematic) occurrence in other disorders.

**Positive symptoms** include delusional thinking, hallucinations, false beliefs, bizarre thoughts, suspiciousness and paranoia. While usually mild and transient during the prodrome, full-blown positive symptoms during the first episode of psychosis is a diagnostics pillar of schizophrenia. Positive symptoms are reduced in most patients by antipsychotics, but remission may be incomplete, relapses occur and about 20-30% of patients are refractory.

**Negative symptoms** are prominent from the pre-diagnostic to the chronic phase of schizophrenia, poorly controlled by antipsychotics and linked to poor real-world function. There are two sub-clusters: 1), decreased expression (poverty of speech and blunted affect) and 2), avolition (amotivation, asociality and loss of anticipatory pleasure for normally-rewarding activities).

**Neurocognitive symptoms**, deficits in a broad palette of cognitive domains including those dependent on frontocortico-striato and frontocortico-parietal circuits: attention; working memory (on-line handling of information); executive function (planning, decision-making, flexible shifting of goals); speed of processing and procedural memory (learning motor tasks); and verbal learning and memory.

**Social cognition (processing)**: processes used to decode social cues, interpret/predict the mental state, beliefs, desires and intentions of others, and hence to behave in a socially-appropriate, adaptive manner. Signals include facial expression, body posture and hand gestures. Impaired social cognition is seen in the prodrome, interrelated with negative symptoms and linked to poor functional outcome. Deficits are irresponsive to antipsychotics.

**Disease-modification** refers to interventions that directly interrupt, decelerate or even reverse core pathophysiological processes causing a disorder with the goal of preventing or delaying its onset, and/or moderating its severity once it evolves. Effects should, in principal, persist even after treatment has been discontinued, and are not necessarily apparent at the onset of, or even during, administration.

**The prodromal phase** is a high-risk state which precedes by weeks to years the transition to schizophrenia or another psychotic disorder. It is characterised by attenuated and/or brief, self-limiting psychotic symptoms, negative, neurocognitive and social cognitive symptoms, depressed mood and other psychological and behavioural abnormalities. Not all subjects showing a prodrome convert, so the term is somewhat misleading.

**Neurological soft signs** refer to a cluster of minor developmental anomalies in motor and sensory integration, motor coordination and motor sequencing. They are present in high-risk subjects prior to transition to schizophrenia, for which they represent a useful warning sign.

**Neurexin-1 is a presynaptically-localized adhesion molecule that interacts with the post-synaptic protein, Neuroligin-1. Neurexin 1 is abundant in inhibitory GABAergic synapses where it controls transmitter release, as well as synaptic formation and transmission. Neurexin-1 deletions (*de novo* and inherited) are consistently associated with schizophrenia.**

**Default-mode network**: interconnected cerebral regions in humans (including the medial prefrontal cortex, cingulate cortex, inferior parietal cortex and precuneus) that are active in fMRI studies under resting conditions: for example, during introspection and rumination. Conversely, they are *deactivated* by goal-directed actions such as performance of a working memory test. An equivalent network appears to exist in rodents.



**Event-Related Potentials:** Negative (N) and positive (P) shifts in voltage of EEG recordings triggered by external stimuli, usually auditory but also visual. **P300** is a positive deflection peaking about 300 msec after a deviant (“oddball”) stimulus differing in frequency, duration, strength etc to a previous sequence. **Mismatch-Negativity** is a pre-attentional negative response peaking about 100-150 msec after an oddball stimulus.

**Machine Learning** is a mode of multivariate analysis which uses so-called “Support Vector Machine” algorithms to detect otherwise-invisible patterns in large data arrays, and to inter-link the different variables recorded. Its application to combined genetic, imaging, clinical and other readouts from high-risk subjects increases the sensitivity and specificity of prediction of transition to psychosis, even at the individual level.

**Polyunsaturated fatty acids**, major components of cell membranes, are derived from dietary linoleic and alpha-linolenic acid transformed in liver into Omega-6 and Omega-3 derivatives, respectively: numbers refer to location of the first double-bond at the methyl end. They are essential for brain function and development perinatally and in childhood. Precursor intake is often poor in Western diets and a risk factor for schizophrenia.

**Cognitive-Behavioural Therapy** is a form of psychotherapy that helps subjects disengage from negative and self-defeating thoughts about themselves, their lives and environment and hence to think, feel and behave in a more adaptive and positive fashion. It may embrace stress management. Both therapist and computerised modes are available for various psychiatric disorders, including people at high risk of developing schizophrenia.

**Minocycline** is a tetracycline-like antibiotic with anti-inflammatory and anti-oxidant properties which easily enters the brain. It is under investigation for a number of CNS disorders, including adjunctive therapy with antipsychotics for the treatment of schizophrenia, especially negative symptoms soon after diagnosis.

**N-Acetyl-Cysteine** is an analogue of L-Cysteine which is rapidly oxidised into Cystine in the brain. Cystine is a substrate of glial Cystine-Glutamate antiporters, so N-Acetyl-Cysteine elevates extracellular levels of glutamate. In cells, Cystine is reduced into Cysteine, the rate-limiting component of the anti-oxidant, Glutathione. Hence, N-Acetyl-Cysteine enhances Glutathione activity. N-Acetyl-Cysteine also exerts anti-inflammatory properties.

**Kalirin-7** is a member of a family of brain-specific guanine-nucleotide exchange factors for Rac-like GTPases like Rac-1. Kalarin-7 is located in the post-synaptic density, where it interacts with the actin cytoskeleton to regulate the formation, maturation and stability of dendritic spines, and hence influence synaptic plasticity.

A subset of fast-firing ( $\gamma$ -frequency, 40-1,000hz), parvalbumin-positive basket (and chandelier) **GABAergic interneurons** in PFC synchronises the activity of local, intra-cortical and cortico-subcortical networks of glutamatergic pyramidal neurones. Their dysfunction, which leads to a disruption of excitation-inhibition balance, network synchrony, cognition and mood, is implicated in events leading to schizophrenia.

The **extracellular matrix** contains proteins like astrocyte-derived chondroitin sulphate proteoglycans, hyaluronans, Neuregulins and Reelin. It fills the space between cells in the brain, including the synaptic cleft. “Peri-neuronal nets” appear post-natally, are localised peri-synaptically, and stabilize neural connections. Their developmental disruption has been related to abnormal plasticity and the genesis of schizophrenia.

**Graph theory:** mathematical analysis of networks of proteins, cells, brain regions and individuals in social groups. Elements are considered nodes connected by edges (vectors). Brain organisation is modular and non-random incorporating features that enhance integration like highly-connected hubs and short path length between nodes (“small world”). In schizophrenia, this organisation is disrupted.

The **Na-K-Cl co-transporter** transports Chloride ( $\text{Cl}^-$ ) into neurones: two atoms of  $\text{Cl}^-$  plus one each of  $\text{Na}^+$  and  $\text{K}^+$ . Accumulation of excess  $\text{Cl}^-$  is prevented by extrusion *via* the co-transporter “KCC2”. In early life, NKCC1 levels are elevated *vs* KCC2 so internal  $\text{Cl}^-$  increases and GABA-A receptors mediate depolarisation *not* hyperpolarisation. Owing to lack of a developmental switch, this may persist in autism and schizophrenia.

**Reelin** is a developmentally-regulated Glycoprotein that controls neuronal migration, corticogenesis and synaptic plasticity. Reelin is enriched in and secreted by GABAergic interneurons in adult PFC where it is co-regulated with GABA by epigenetic mechanisms. It is a component of the extracellular matrix.

**MicroRNAs** are short, ca 22-24 nucleotide long stretches of protein non-coding RNA that neutralize and destabilize matching mRNAs (up to hundreds of different ones): where the match is perfect, mRNA is degraded.



Hundreds of different species of miRNAs exist in the brain. Many interact with developmentally-important proteins, and cerebral levels of diverse classes of miRNA are altered in schizophrenia.

**Human endogenous retroviruses** encode a protein envelope, reverse transcriptase and other proteins needed for replication. They are relics of ancient infections that survived by integration into the genome where multiple copies occur due to amplification and re-infection. They comprise about 8-10 % of the human genome, mostly in protein non-coding regions. Revival may trigger disorders like multiple sclerosis and, possibly, schizophrenia.

**Synaptic pruning**, processes for developmental elimination of “excess” synaptic connections especially prominent during adolescence. Axo-dendritic synapses not stabilized by neural activity in frontocortical, temporocortical and hippocampal regions are especially impacted. Appropriate synaptic pruning optimizes the operation of neural networks, while both excessive (schizophrenia) and defective (autism) pruning is deleterious.

**RAISE is a National Institute of Mental Health Initiative to alter the course and prognosis of FEP. A well-trained, multidisciplinary team offers (up to two years) pharmacotherapeutic and psychosocial treatments together with family education, resiliency training and supported employment in a patient-centric and real-world fashion to improve function and promote recovery.**

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**Figure 1 Onset and progression of schizophrenia in relation to risk factors and developmental processes impacted by the disorder.**

**Figure 1A.** The diagnosis of schizophrenia operationally corresponds to the first full psychotic episode (“FEP”), is usually made in young adults, but can (rarely) occur in childhood, adolescence or later in life. Diagnosis generally follows a “prodromal”, at-risk phase in which sub-threshold psychotic episodes and other characteristic symptoms are apparent. Once diagnosed, schizophrenia follows a fluctuating course punctuated by acute exacerbation of psychotic crises superimposed upon a background of poorly-controlled negative, neurocognitive and social cognitive symptoms. About 10-15% of patients recover after their FEP, while a similar proportion display a more severe and unremitting form of the disorder. In addition to inheritance (Suppl Box 1), many environmental risk factors have been incriminated both during both the peri-natal (first wave) period and during adolescence. Genetic and environmental impacts at least partially act *via* epigenetic misprogramming of neurodevelopment. Throughout the disorder, adverse environmental events can trigger crises.

**Figure 1B.** The course to and progression of schizophrenia can be related to three fundamental phases in the “life” of the brain - though depicted sequentially they are *interlinked* and there is no absolute demarcation. Each phase is anomalous in schizophrenia, with disruption of brain formation and (re)organisation implicated in causal pathophysiology. Both of these phases, as well as brain “upkeep”, embrace a spectrum of processes *potentially* available for therapeutic intervention. Pre and post-diagnostic course-altering interventions are conveniently labelled “prevention” and “reversal”, respectively, but only relative to the *clinical* picture: underlying pathophysiological changes will have set in much *earlier*. Currently, the most compelling “Window of Opportunity” is around the first episode to impede onset, or block early progression.

**Figure 2 Course-alteration and disease-modification: core facets.**

The notion of disease-modification, more familiar for neurodegenerative disorders, refers to a *direct* and enduring impact on the disease process: that is, on causal pathophysiological mechanisms. This leads to a delay or halting of the onset or progression of the disorder. Neurobiological substrates of schizophrenia are incompletely defined, multiple and, at least to some extent, symptom-specific.

Further, causal processes are subject to modulatory neural influences so a more liberal interpretation of disease-modification incorporates agents that respectively block or promote processes favouring or hindering disease evolution. This notion can be integrated into the more general concept of course-alteration. In addition, precocious relief of certain classes of symptom (like impaired social cognition) and countering risk factors (like drug-seeking behaviour and “stress”) may slow the path to schizophrenia (Suppl Figures 2 and 3). While “prevention” and “rescue” are relative to *diagnosis*, the pathophysiological road to schizophrenia is enacted far earlier (Figure 1).

**Figure 3 Clinical trajectories of help-seeking young subjects at high risk for developing schizophrenia: diverse strategies for their detection.**

**Figure 3A.** Amongst CHR people seeking help, many will need treatment for psychiatric problems like depression, anxiety and even sub-diagnostic psychotic episodes. Based on observations in specialized centres (Box 2), about a third convert to schizophrenia or another psychosis within 2-5 years. A minority will remit, some will remain in a comparable state of impairment, while others will transit to another psychiatric disorder - not necessarily associated with psychosis. This emphasizes the importance of trans-nosological thinking for strategies designed to reduce conversion. The values shown are based on a study performed (1993-2006) in a specialised Australian clinic of a CHR sample<sup>45</sup>. **Figure 3B.** Comprehensive clinical assessment is crucial for identifying CHR subjects, especially when coupled to information on life-style like recreational drug abuse and social isolation. A broad palette of measures are also instructive for predicting conversion, though no single measure *alone* has adequate fidelity for reliably predicting the fate of individuals. Multi-modal strategies help enhance sensitivity and specificity of predictions, even at the individual level, and are useful for stratification of subgroups. Abbreviations not in text: EEG; Electroencephalography; MRS, Magnetic Resonance Spectroscopy and PET, Positron Emission Tomography.

**Figure 4 Overview of core pathophysiological mechanisms implicated in the genesis of schizophrenia: potential targets for course-altering intervention.**

**Figure 4A.** Several, potentially-targetable mechanisms are incriminated in the genesis of schizophrenia. The antipsychotic-sensitive hyperactivity of subcortical dopaminergic projections is a comparatively late repercussion of other (upstream) pathophysiological events, and the high-risk phase is characterised by several anomalies like disruption of cortisol secretion, perturbed activity of several classes of neurotransmitter and, probably, anomalous pruning of synapses. Anticipating such changes is a disruption of GABAergic-glutamatergic networks in corticolimbic regions, including a hypoactivity of NMDA receptors on GABAergic interneurons. Even earlier, cellular modulators of neural differentiation, migration and plasticity are deregulated. Finally, three other global mechanisms playing a role in the genesis of schizophrenia are indicated: neuroinflammation, epigenetic misprogramming and aberrant patterns of cortical myelination. **Figure 4B.** Anomalies are apparent at

several interacting levels of regulation, from molecules to cells to cerebral circuits to social networks. All present opportunities for intervention and, though drugs engage with molecular targets, they influence higher echelons of this hierarchy. G-protein coupled receptors and ion channels (not shown) are relevant at all levels. Epigenetic mechanisms exert a broad-based influence upon networks of proteins. Mitigation of “stress” would have multifarious, beneficial effects in protecting circuits from disruption. Agents that normalise GABAergic interneuron activity should re-coordinate perturbed cortico-subcortical networks controlling mood and cognition. Protecting oligodendrocytes may reconfigure neural networks where myelin has been damaged by inflammation or other insults. Axons themselves are disrupted in schizophrenia, so promoting their structural and functional integrity would be of interest. Agents acting in the PFC to favour top-down control of neurocognition and social function could be effective, while CBT and stimulation techniques engage widespread cortical circuits. Drug associations and multi-target drugs with complementary mechanisms of action may also be especially effective for network reconstitution and course-alteration. ErbB is a tyrosine receptor kinase receptor. Abbreviations not in text: CRT; cognitive-remediation therapy; Glu, glutamate; mTOR; mammalian target of rapamycin; NCAM, Neural cell adhesion molecule; rTMS, rapid transcranial magnetic stimulation and DCS, direct current stimulation.

**Figure 5 Schematic representation of a “hybrid” strategy as compared to other therapeutic approaches for treating schizophrenia and its genesis.**

Currently, we are limited to symptomatic control of schizophrenia. However, treatment needs to be improved, in particular as regards resistant patients and symptoms other than positive (Suppl Figure 1). Early-life, “disease-modifying” medication to interrupt the path to schizophrenia presents formidable problems of validation, clinical development and safety. Accordingly, therapeutic exploitation is not yet a viable proposition. Nonetheless, one pragmatic strategy would be to target the pathophysiology and symptoms presented by CHR help-seeking, young people. For example, palliating stress-induced HPA axis overdrive and promoting social cognition both alleviate symptoms and, at least partly as a consequence, may reduce the risk of conversion (Suppl Figure 3). Inasmuch as certain pre-diagnostic symptoms and neurobiological substrates are not unique to schizophrenia, the transition to *other* psychiatric disorders may likewise be impeded. A dual, “hybrid” strategy would have advantages in terms of developability and therapeutic utility over pure course-alteration (see text).