Schizophrenia: a neurodevelopmental disorder - integrative genomic hypothesis and therapeutic implications from a transgenic mouse model

E.K. Stachowiak[1], I. Klejbor[3], A. Kucinski[1], S. Narla[1], C. Terranova[1], D. R. Curl[1], C. Syposs[1], Y. Yang[2], M. Bencherif[4], B. Birkaya[1], E. S. Tzanakakis[6], S. Wersinger[1,2], J. Morys[3], M.K. Stachowiak[1]

SUNY, Buffalo, NY, US, Departments: [1] Pathology and Anatomical Sciences, [2]Psychology, [6]Chemical and Biological Engineering, [1,6] Western New York Stem Cell Culture and Analysis Center, [3] Medical University, Gdansk, Poland; [4]Targacept Inc. Winston-Salem, NC 27101;

Abstract: Schizophrenia is a neurodevelopmental disorder featuring complex aberrations in the structure, wiring, and chemistry of multiple neuronal systems. The abnormal developmental trajectory of the brain appears to be established during gestation, long before clinical symptoms of the disease appear in early adult life. Many genes are associated with schizophrenia, however, altered expression of no one gene has been shown to be present in a majority of schizophrenia patients. How does altered expression of such a variety of genes lead to the complex set of abnormalities observed in the schizophrenic brain? We hypothesize that the protein products of these genes converge on common neurodevelopmental pathways that affect the development of multiple neural circuits and neurotransmitter systems. One such neurodevelopmental pathway is Integrative Nuclear FGFR1 Signaling (INFS). INFS integrates diverse neurogenic signals that direct the postmitotic development of embryonic stem cells, neural progenitors and immature neurons, by direct gene reprogramming. Additionally, FGFR1 and its partner proteins link multiple upstream pathways in which schizophrenia-linked genes are known to function and interact directly with those genes. A *th-fgfr1(tk-)* transgenic mouse with impaired FGF receptor signaling establishes a number of important characteristics that mimic human schizophrenia - a neurodevelopmental origin, anatomical abnormalities at birth, a delayed onset of behavioral symptoms, deficits across multiple domains of the disorder and symptom improvement with typical and atypical antipsychotics, 5-HT antagonists, and nicotinic receptor agonists. Our research suggests that altered FGF receptor signaling plays a central role in the developmental abnormalities underlying schizophrenia and that nicotinic agonists are an effective class of compounds for the treatment of schizophrenia.

Integrative Nuclear Fibroblast Growth Factor Receptor 1 Signaling" (INFS) – a point of convergence in neurodevelopmental pathways affected in Schizophrenia.

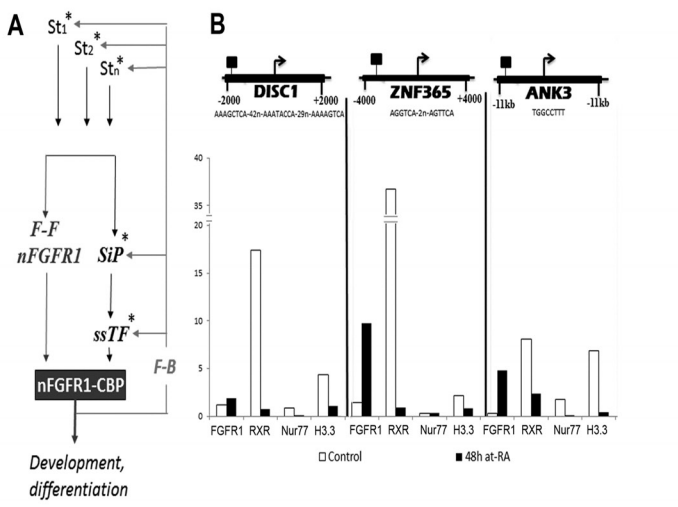


Figure 1-*INFS - Neurogenic signals generated by diverse extracellular stimuli (St; neurotransmitters, hormones, growth factors, cell contact receptors) in embryonic and brain stem cells are propagated through signaling pathways (SiP; cAMP, Ca++/PKC, MAPK) to sequence specific transcription factors [(ssTF; CREB, AP1, NfkB, Smads, nuclear retinoid receptors (RXR/RAR) and orphan Nur receptors)]. In parallel, a newly synthesized FGFR1 translocates into the nucleus and "feeds forward" (F-F) neurogenic signals directly to CREB binding protein (CBP), an essential transcriptional co-activator and gene-gating factor. The coupled activation of CBP by nuclear (n) FGFR1 and cascade signal transduction to ssTF are responsible for cell differentiation. * marks signaling pathways in which schizophrenic-related genes have been found, including cAMP, G-protein signaling, PKC, MAPK, NfkB, CREB, RXR, and Nurr1 which have also been shown to engage INFS.*

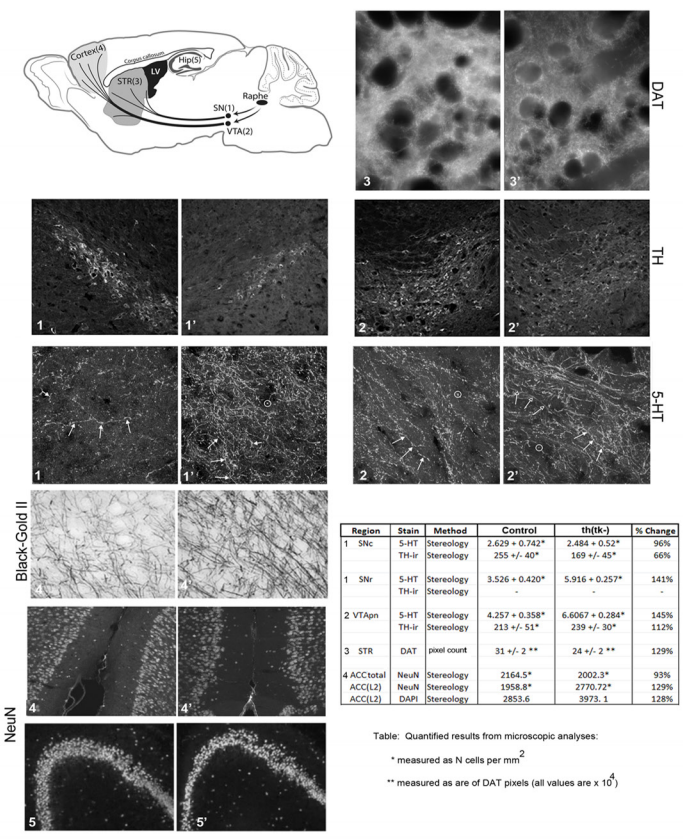
(B) Chromatin Immuno-Precipitation-sequencing (ChIP-seq) analysis shows binding of FGFR1, RXR, Nur77 and histone 3 variant H3.3 to potential Nur/RXR target regions in selected schizophrenia-linked genes: DISC1, ZEP365, ANK3 was verified by independent ChIP experiments in human (h)ESC as shown. Bars illustrate RA-induced increase of FGFR1 biding to the indicated regions of the genes.

In pluripotent hESC (control) FGFR1 binds along with its RXR and Nur77 and with H3.3, a marker of transcribed chromatin, to DSIC1 and ZFP365, with little or no binding to ANK3. The FGFR1/RXR and FGFR1/Nur77 complexes are known to activate transcription and thus may support the DISC1, and ZEP365 gene activities. All-trans retinoic acid (RA) increases binding of FGFR1 and reduces RXR, Nur77 and H3.3. Increased binding of FGFR1 alone, may act to repress gene activities.

Conclusion: Our observations suggest a transcriptional circuit in which INFS integrates the incoming developmental signals (St) through the Feed-Forward (FF) module and reinforces/turns off those input signals via a feedback (FB)module. SZ mutations, including "weak" copy variations may deregulate this self-controlled genomic circuit and thus lead to broad molecular and developmental dysfunctions.

Inhibition of INFS in dopamine neurons create broad anatomical schizophrenia-like impairments

Hypothesis - blockade of INFS in developing midbrain DA neurons would alter the DA system and co-developing neuronal systems. Dominant negative FGFR1(TK-) targeted to DA-producing cells by 4.8 kb tyrosine hydroxylase (TH) promoter



Region	Stain	Method	Control	th(tk-)	% Change
1 SNc	5-HT	Stereology	2.629 + 0.742*	2.484 + 0.52*	96%
	TH-ir	Stereology	255 +/- 40*	169 +/- 45*	66%
1 SNr	5-HT	Stereology	3.526 + 0.420*	5.916 + 0.257*	141%
	TH-ir	Stereology	-	-	-
2 VTApn	5-HT	Stereology	4.257 + 0.358*	6.6067 + 0.284*	145%
	TH-ir	Stereology	213 +/- 51*	239 +/- 30*	112%
3 STR	DAT	pixel count	31 +/- 2 **	24 +/- 2 **	129%
4 ACCtotal	NeuN	Stereology	2164.5*	2002.3*	93%
ACC(L2)	NeuN	Stereology	1958.8*	2770.72*	129%
ACC(L2)	DAPI	Stereology	2853.6	3973. 1	128%

Table: Quantified results from microscopic analyses:

* measured as N cells per mm^2

** measured as are of DAT pixels (all values are x 10^4)

Figure 2-*Brain malformations in th-fgfr1(tk-) mice. Brain structures: 1–SN, 2–VTA, 3–striatum, 4–Anterior Cingulate Cortex, 5–hippocampus*

DA systems*: tyrosine hydroxylase (TH) immunoreactive DA neurons in midbrain at PD 360 of control and th-fgfr1(tk-) mice. Stereology revealed reduced size of DA neurons at PD 0 (SNc -27%, VTA -21%) and at PD 360 (SNc -15% , VTA -10%). Density of DA neurons was reduced at PD 0 (SNc -26%, VTA -34%) and at PD 360 (SNc -34%). Density of terminals in striatum (3) expressing DAT was reduced by 23% in th-fgfr1(tk-) mice.*

5-HT systems*: 5-HT-IR t neurons in midbrain at PD 180. th-fgfr1(tk-) mice have increased number of incoming **5-HT fibers** in the paranigral nuc. (1) and the parabrachial pigmentosus nuc.of the VTA, as well as in the SNr (2). These axons formed dense networks with numerous varicosities. HPLC analysis showed increased levels of 5-HT in the SN and increased 5-HT metabolite 5-Hydroxyindoleacetic acid (5-HIAA) in the VTA of adult th-fgfr1(tk-) mic.*

Cortwx (PD180)*: neurons stained with anti-NeuN; stereology -7.5% reduction of neuronal density in the Anterior Cingulate . Subregional analysis - 35% increase of NeuN+ cells and a 22% increase in DAPI+ cells in cortical layers 1 and 2. Black-Gold II -more myelinated, disorganized fibers in th-fgfr1(tk-).*

Hippocampal pyramidal layer*: Neurons stained with anti-NeuN. Preliminary analyses suggest reduced densities of pyramidal neurons in th-fgfr1(tk-) mice.*

Conclusion: impaired development of DA neurons affects neuronal systems innervated by DA neurons or receive DA input indicating that developmental hypoplasia of DA neurons can lead to wide spread structural brain disorganization as observed in schizophrenia

th-fgfr1(tk-) mice display positive, negative and cognitive SZ-like symptoms differentially corrected by anti-psychotics and novel α7 Nicotinic Receptors Agonists.

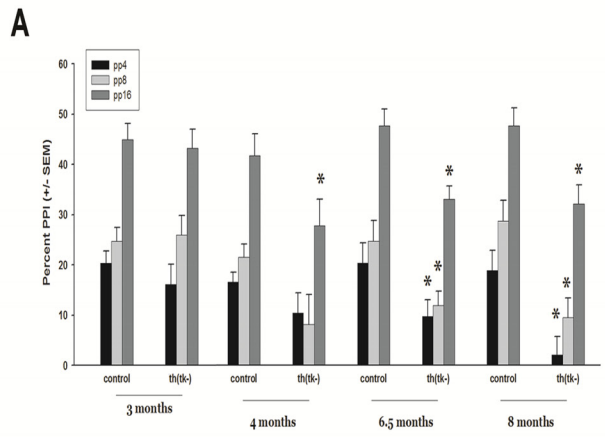


Figure 3-*Impaired sensory-gating and social behavior in th-fgfr1(tk-) mice and their normalization by drugs. (A) Deficits in sensory gating appear in adulthood and are reversed by clozapine. Time dependent changes in prepulse inhibition (PPI) in th-fgfr1(tk-) mice. At 6 and 8.5 month groups there were significant reductions in PPI in th-fgfr1(tk-) mice at each prepulse intensity. (B) There was* a treatment x genotype interaction with clozapine treatment at 3.0 mg/kg ($p<0.05$).

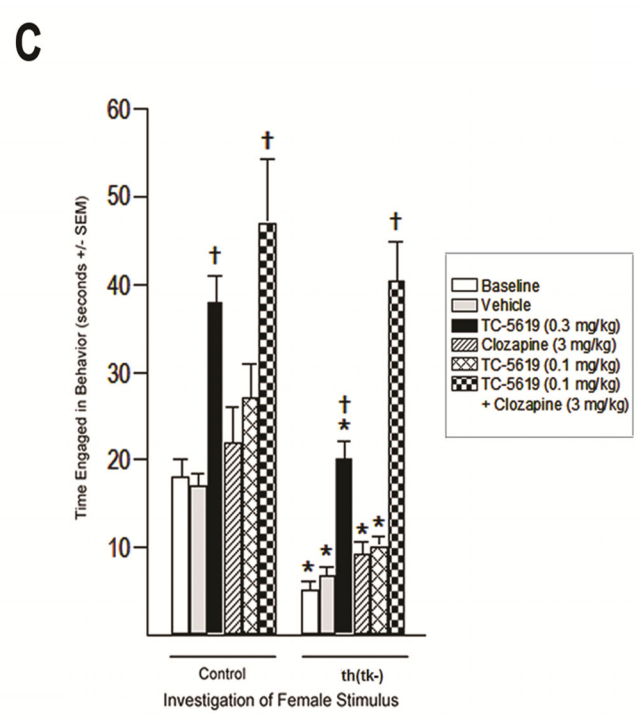


Figure 3*(C) Effects of treatment with TC-5619 and Clozapine on Social Investigation (acute drug injections). In the absence of drug (baseline and vehicle groups), control mice spend more time investigating stimulus animals (nontransgenic mice) than th-fgfr1(tk-) mice. TC-5619 (0.3 mg/kg) increased the time both genotypes spent investigating the stimulus animal. There was no difference in investigation time between drug-treated control mice and drug-treated TK- mice. Low doses of clozapine (3.0 mg/kg) or TC-5619 (0.1 mg/kg) alone have no effect on investigation time of either a female or male stimulus animal. These behaviorally inactive doses of TC-5619 and clozapine administered together increase social investigation of female stimulus animals in both genotypes.*

Another key feature of schizophrenia is **cognitive impairment (i.e.,** deficits in working memory). *th-fgfr1(tk-)* mice were unable to distinguish between old and recently investigated objects, a distinction that was successfully made by control mice

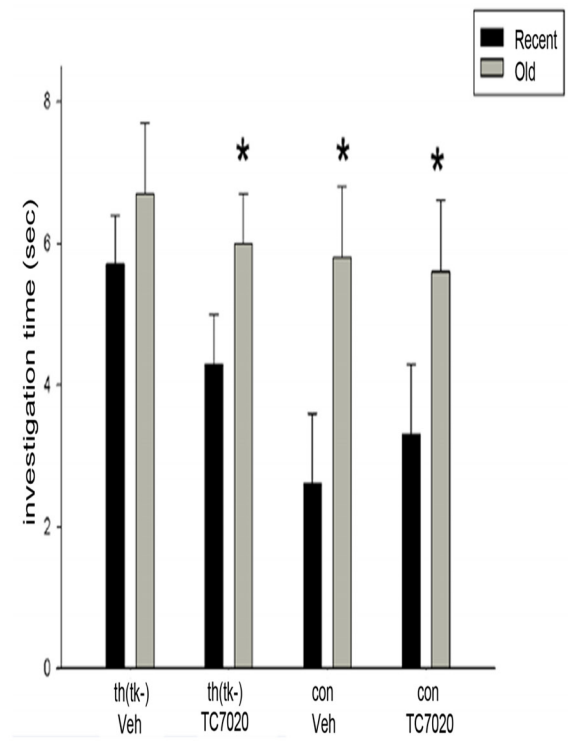


Figure 4 *TC-7020 improves a measure of cognition that is impaired in th-fgfr1(tk-) mice. During Phase I, mice were placed into the arena for 10 minutes and allowed to investigate four identical "old" objects. Subjects were then returned to their home cage for 50 minutes. During Phase II, four "new" identical objects were placed in the arena and mice were given 10 minutes to explore the objects. After returning to their home cages again, mice were then injected with either saline or TC-7020 (1.0 mg/kg, i.p.). 40 minutes after injections, mice were placed in the arena for Phase III and investigation time of each object (2 "old" and 2 "new") was measured. In subjects with no impairments, it is common to spend more time investigating the old objects presented in Phase I, which are less familiar than the more recent objects from Phase II ("new" objects). With saline, male th-fgfr1(tk-) mice cannot distinguish between objects presented in the recent (Phase II, "B" objects) and in the distant past (Phase I, "A" objects). This deficit may reveal working memory impairment. In mice administered TC7020, this deficit was ameliorated. Bars represent means +/- SEM. * significantly greater than recent, one way ANOVA ($p<0.05$). Preliminary account of these observations was given in (Yi Yang, 2010)*

8. Summary

In conclusion, inhibition of INFS that integrates several schizophrenia related pathways, recapitulates several anatomical, neurochemical and behavioral features of the human disease. Targeting of the dominant negative FGFR1(TK-) to developing postmitotic catecholamine neurons in which the INFS mechanism is activated impairs their development producing hypoplastic and hyperactive DA neurons. This initial abnormality is accompanied by secondary changes in other neuronal systems including brain stem serotonergic neurons and cortical neurons, which were not directly targeted by the FGFR1(TK-) transgene. Thus, in schizophrenia, malformation of DA neurons could be the initial defect that gradually affects other monoamine and cortical circuitries underlying the progression of the disease and gradual behavioral deterioration. Importantly, the onset of sensorimotor gating impairments in *th-fgfr1(tk-)* mice parallels the time course of positive symptom progression of the human disease. We also show the nicotinic acetylcholine system has the potential to alleviate symptoms associated with both cortical and subcortical malformations by mediating subcortical DA release and possibly the activity of other neuronal networks impaired in schizophrenia.

Stachowiak, M.K., A. Kucinski, R. Curl, C. Syposs, Y. Yang, S. Narla, C. Terranova, D. Prokop, I. Klejbor, M. Bencherif, B. Birkaya, T. Corso, A. Parikh, E.S. Tzanakakis, S. Wersinger, and E.K. Stachowiak. 2013. Schizophrenia: a neurodevelopmental disorder - integrative genomic hypothesis and therapeutic implications from a transgenic mouse model. Schizophr Res. 143:367-376.