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Professor Robert Freedman and his contributions to psychiatric research

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FIGURE 1 Photograph of Prof. Robert Freedman

Prof. Freedman successfully finished his outstanding 12-year service at the *American Journal of Psychiatry* as its editor-in-chief in 2018. He was educated and trained at Harvard College and Harvard Medical School, National Institute of Mental Health (NIMH) Intramural Research Program, and University of Chicago since late 1960s till late 1970s. He has since then worked at Department of Psychiatry and Pharmacology, University of Colorado Denver as Professor. He was the Chair of the Experimental Therapeutics Clinical Trials Initial Review Group NIMH 2012-15, member of NIMH National Advisory Council 2002-8, and importantly, he served as a member of the editorial boards of *New England Journal of Medicine, Biological Psychiatry, Neuropsychopharmacology and Schizophrenia Research*.

He was elected membership of the Institute of Medicine, National Academy of Sciences in 2006. He was awarded the NIMH Merit Award in 1990, William K. Warren Research Award, International Congress for Schizophrenia Research in 1999, and Connie and Steve Lieber Award for Schizophrenia Research, Brain and Behavior Research Foundation in 2015.

Prof. Freedman is highly interested in research on the effects of prenatal maternal immune activation by infection on human fetal brain development. He has developed auditory P50-potential cerebral inhibition as a physiologic endophenotype associated with schizophrenia genetic risk, specifically at *CHRNA7*, the gene for the α7-nicotinic cholinergic receptor. He then realized that their earlier in vitro observation that choline is an agonist at this receptor meant that it could be used prenatally in pregnant women to increase activation of the receptor. He then designed a clinical trial that used newborn P50 inhibition as an outcome of the effect of perinatal choline supplementation in 2013 and 3 years later found that enhanced newborn P50 inhibition predicted improved problems in child behavior at 40 months of age. The choline supplement enhanced the development of newborn P50 inhibition even in fetuses with mothers who had schizophrenia. With the late Paul Patterson's group, one of the pioneers of the maternal immune activation model, they investigated the effects of immune activation in mice made genetically vulnerable by *CHRNA7* null mutation, the translational basis for the proposed study. Prof. Freedman most recently designed and directed the human study that found initial evidence

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that maternal gestational infection decreases newborn P50 inhibition and has subsequent effects on early behavior. His current work builds on that initial study, to identify the mechanisms that are responsible for the effects of maternal infection and other early life complications on fetal brain development.[1-4]

His contributions to research on schizophrenia can be further divided into the following 4 particular fields.

Characterization of a heritable endophenotype in schizophrenia

A heritable endophenotype had been proposed by Holzman, Gottesman, and others as key to understanding the genetics of schizophrenia. Prof. Freedman led the first group to develop such an endophenotype, deficits in the normal inhibition of the P50 auditory evoked response to repeated stimuli, and to find its genetic basis. He realized that earlier evoked potential researchers in schizophrenia had failed to recognize that the long "recovery time" of cerebral potentials was not synaptic fatigue, but rather an active inhibitory process that could be assessed used a classical paired-pulse conditioning-testing paradigm. His team demonstrated loss of inhibition in patients with schizophrenia and subsequently in some of their first-degree relatives. Using genetic linkage, they found that the P50 inhibition endophenoptype was linked to the chromosome 15 locus of *CHRNA7* and eventually to polymorphisms in its promoter, a gene that they had earlier shown was responsible for this physiological trait in a mouse model. Recently, in the NIMH COGS Collaboration, the team showed that P50 inhibition is heritable in schizophrenia in families who also have evidence for the heritability of schizophrenia itself. In individuals with schizophrenia but no family history of schizophrenia, who are frequently studied in GWAS cohorts, there was no heritability. Thus, the heritability of the endophenotype is closely related to the familial transmission of the illness itself. P50 inhibition has been used as a tool in 340 studies by other investigators worldwide for their research. He also felt that it was important to continue reverse translation of this endophenotype to animal models. At the Karolinska Institute, he found that the α7-nicotinic receptor is primarily expressed on interneurons, consistent with *CHRNA7*'s linkage to P50 inhibition, and later he showed that its expression is reduced in schizophrenia postmortem hippocampal tissue. More recently, they have studied the expression in living patients with a newly synthesized positron emission tomography (PET) ligand.[5-8]

Genetics and biology of α7-nicotinic receptors in schizophrenia

Based on the encouraging 1997 linkage findings, his team decided to look for functional polymorphisms in the *CHRNA7* gene that would account for linkage and indicate how the gene was failing in schizophrenia. He first verified the linkage finding using classical linkage disequilibrium in the NIMH genetics initiative families not involved in the 1997 linkage study. Then he worked with his colleague Dr. Sherry Leonard to identify polymorphisms. SNPs associated with schizophrenia and P50 inhibition were primarily in the promoter and decreased expression in vitro, which suggested that the receptor could respond to agonists, but that it would be expressed at lower levels. The Icelandic group found using GWAS that deletions encompassing chromosome 15q13-14 including *CHRNA7* were a highly specific genome-wide significant factor in patients with schizophrenia, who did not have pre-existing family history. The team was able to replicate that finding in the NIMH Genetics Initiative that he was PI for at the Colorado site. The deletion excludes a nearby partial duplication of *CHRNA7*, *CHRFAM7A*, that Dr. Leonard and he were the first to report. They hypothesized that the duplication would act as a dominant negative for *CHRNA7*. They found that the duplication is more highly expressed if the patient has an infection. Working with Dr. Henry Lester they found that the duplication product, a truncated peptide, can assemble with the full-length *CHRNA7* product and decrease channel activity.[9-12]

Establishment of P50 gating as an early biomarker of fetal brain development of inhibitory neurons

While they were investigating the genetic basis of the P50 sensory gating deficit, Dr. Freedman hypothesized that *CHRNA7* SNPs associated with schizophrenia might be pathogenic in development, perhaps even more so than in the adult brain. They first confirmed in animal models that α7-nicotinic receptors are indeed expressed before birth and that their absence affects interneuron migration into the hippocampus. They thus needed to be able to measure possible effects on inhibition as soon as possible after birth in humans. They therefore extended the P50 inhibition phenotype to newborns. In newborns, as in adults, deficits in P50 inhibition are associated with familial risk for schizophrenia, i.e. babies who have schizophrenia in one of the parents, or a *CHRNA7* SNP that is associated with schizophrenia have decreased P50 inhibition. The P50 inhibition deficit is the first developmentally characterized endophenotype to become part of the Research Domain Criteria (RDoC) and has now been replicated by another group in England. Using P50 inhibition as a biomarker, they showed positive effects of phosphatidylcholine supplementation, including in babies with *CHRNA7* SNPs associated with schizophrenia.[13-16]

Perinatal prevention of mental illness

The American Medical Association in 2017 recommended that all pregnant women receive choline supplements and cited their work in their discussion. There has now been an initial discussion among public health and manufacturing

stakeholders to assess strategies for a population-wide intervention. He wishes to clarify that he has no financial interest and has refused manufacturer funding. The hypothesis that choline might be a suitable prenatal agonist at fetal α7-nicotinic receptors emerged from their early basic science studies of the properties of the receptor in rat hippocampal slices, which confirmed his earlier localization of the receptor on interneurons. He further developed the hypothesis in discussion with other Conte Center investigators, including Dr. Lester. They did not recommend the intervention beyond their research participants until the AMA's resolution, because he felt that further evidence for its safety and effectiveness were needed. Three other groups subsequently also reported positive results with no emergent safety issues through pregnancy and the first 4 years of life. He was invited to discuss the ethical issues of prenatal prevention, some of which were noted by the reviewers, at the 2019 meeting of the Schizophrenia International Research Society. He is now conducting an ongoing second double-blind, placebo-controlled trial are part of his commitment to more research. Mothers in that trial are fully informed of the potential benefit and permitted to take choline supplements or increase their dietary intake, regardless of their random assignment, as they choose. Of note, the Editor of the *Journal of Pediatrics* asked him to revise his paper in press to be more specific about supplementation, and he changed its title himself to speak more directly about choline's potential value. His publications provide the current evidence for all preventive strategies, not just choline, to physicians who care for pregnant women.[17-21]

CONFLICT OF INTEREST

None.

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