Submitter name: Caroline Brennan
Submitter email: c.h.brennan@qmul.ac.uk

Population based forward genetic screen of mutagenized zebrafish identifies loci associated with nicotine preference and human smoking behavior

Alistair J. Brock[1], Judit García-González[1], Matthew O. Parker[2], David Jolliffe[3], Ari Sudwarts[1], Muy-Teck Teh[4], Elisabeth M. Busch-Nentwich[5], Derek L. Stemple[5], Adrian R. Martineau[3], Jaakko Kaprio[6,7], Teemu Palviainen[6], Valerie Kuan[8], Robert T. Walton[3], Caroline H. Brennan[1]

[1]School of Biological and Chemical Sciences, Queen Mary, University of London; [2]Brain and Behaviour Laboratory, School of Pharmacy and Biomedical Science, University of Portsmouth; [3]Barts and The London School of Medicine and Dentistry, Blizard Institute; [4]Centre for Immunobiology and Regenerative Medicine, Institute of Dentistry, Barts and The London School of Medicine and Dentistry; [5]Wellcome Trust Sanger Institute; [6] Institute for Molecular Medicine FIMM, HiLIFE; [7]Department of Public Health, Faculty of Medicine, University of Helsinki; [8]Institute of Cardiovascular Science, University College London

BACKGROUND: Although there is clear evidence of genetic contributions to susceptibility to nicotine addiction, it has proved difficult to identify causal alleles and pathways from studies in humans. Mutagenesis in model species generates strong phenotypes not present in wildtype populations and can be used to identify biological mechanisms underlying quantifiable behaviours. We tested the hypothesis that a forward genetic screen for nicotine preference in zebrafish can predict loci and biological mechanisms influencing human smoking behaviour.

METHODS: A population-based forward genetic screen of ethylnitrosurea- mutagenized zebrafish was used to identify lines of fish showing altered nicotine preference. Immunohistochemical, behavioral and quantitative PCR analyses were used to characterize mutant larvae. Focussed SNP analysis of the homologous human locus in cohorts from the UK and a Finnish Twin study assessed the predictive validity of the zebrafish data for human smoking behavior.

RESULTS: We show nicotine preference is heritable in fish as in humans and identify loss-of-function mutations in the zebrafish *Slit3* gene as associated with increased nicotine preference. *Slit3* mutant larval fish showed altered sensitivity of habituation to acoustic startle to dopaminergic antagonists and increased *Drd2* and *Drd3* mRNA expression. Dopaminergic neuronal pathfinding was unaffected. Analysis of the *Slit3* locus in two independent human cohorts identified 2 genetic markers that predict level of cigarette consumption and likelihood of cessation.

CONCLUSION: These findings suggest a role for SLIT3 signaling in development of dopaminergic pathways affecting behaviours associated with nicotine dependence and confirm the translational relevance of the zebrafish model in exploring complex human behaviors.