Genomic imprinting and neurobehavioral programming by adverse early life environments: evidence from studying *Cdkn1c*

Anthony R. Isles[1]* and Rosalind M. John[2]

[1]Behavioural Genetics Group, MRC Centre for Neuropsychiatric Genetics and Genomics, School of Medicine, Cardiff University, Cardiff, CF24 4HQ, UK
[2]Biomedicine Division, School of Biosciences, Cardiff University, Cardiff, UK, CF10 3AX, UK
*Correspondence to: IslesAR1@cardiff.ac.uk

Abstract

Imprinted genes are subject to epigenetic regulation that leads to monoallelic expression from one parental allele only. Brain expression of the imprinted gene *Cdkn1c* is sensitive to early life adversity, including exposure to maternal low protein diet (LPD) where increased expression of *Cdkn1c* is due to de-repression of the normally silent paternal allele. Maternal LPD also leads to changes in the dopamine system and reward related behaviours in offspring. We have recently demonstrated that these brain and behavioural phenotypes are recapitulated in a transgenic model in which *Cdkn1c* expression alone is increased. Here we summarise these findings and suggest that the loss of imprinting of *Cdkn1c* in the offspring following maternal low protein diet is a key contributor to the associated changes in the dopamine system and behavior reported after early life adversity.

Introduction

An adverse early life environment (*in utero* and/or early post-natal) can impact on the developing offspring, often resulting in a poor health lasting well into later life. Physiologies affected by exposure to such a sub-optimal environment include the cardiovascular, metabolic and immune systems. An optimal early life environment is also critical for neurodevelopmental outcomes [1] and later-life behavioral phenotypes [2]. Many deleterious effects of adverse environment exposure whilst *in utero*, such as malnutrition and exposure to stress, have been demonstrated in large epidemiological studies (e.g. "Dutch Hunger Winter" and "1959-61 Chinese famines"). These include reduced cognitive abilities [3], as well as increased incidence of schizophrenia [4,5], personality disorder [5] and addiction and gambling problems [6]. These human studies are supported, and indeed have been extended, by a large number of animal studies that show clear associations between *in utero* and/or early post-natal exposure to stress, toxins, low protein and high fat diets, and abnormal brain and behavior in the offspring [1].

There is now a growing need to understand the mechanisms that link exposure to an adverse early life environment and the observed changes in brain and behavior. One suggestion is that an adverse early life environment leads to changes in the epigenetic status, and therefore expression potential, of genes that are important during

neurodevelopment. In this respect, imprinted genes have been posited as possible mediators of early life environmental effects. This group of genes are subject to developmentally determined epigenetic regulation which renders one parental allele transcriptionally silent [7]. Although relatively small in number, imprinted genes are developmentally important [8,9] and even subtle increases or decreases in expression can have significant phenotypic consequences for neurodevelopment [10,11]. Furthermore, a change in the imprinting status of imprinted genes is a recognized functional mechanism within the brain, with de-repression of the normally silent alleles of *Dlk1* and *Igf2* playing a role in neurogenesis [12,13]. As a consequence, imprinted genes may be well placed for mediating environmental effects on neurodevelopment via changes in their epigenetic regulation. However, although limited, current data suggests that generally imprinted genes are relatively insensitive to environmental manipulations, at least in the context of *in utero* nutritional programming and gene expression in metabolic tissues [14,15]. Nevertheless, although studies thus far indicate there is not an enhanced effect of early life programming on changes in expression of this class of genes as a whole, some key imprinted genes do appear to be more sensitive to the effects of a suboptimal maternal diet [14,15].

***Cdkn1c* expression is altered by an adverse early life environment**

One such exception is *cyclin dependent kinase inhibitor 1c* (*Cdkn1c* aka *p57Kip2),* an imprinted gene that is normally silenced on the paternal allele [16]. Expression of *Cdkn1c* in the offspring brain is elevated in response to a number of adverse early life events. Specifically, both maternal low protein diet (LPD) [17], and the degree of early post-natal maternal care [18] lead to increased (2-3 fold) *Cdkn1c* expression. Such alterations in gene expression may reflect changes in expression of the normally active allele, or even changes in cellular composition. However, using a novel *Cdkn1c-luciferase* animal model, we demonstrated that increased expressed of *Cdkn1c* in response to maternal LPD was definitively due to de-repression of the normally silent paternal allele, and accompanied by reduced DNA methylation at the *Cdkn1c* promoter (loss-of-imprinting; LOI) indicating an epigenetic response to prenatal adversity [19]. Importantly, aberrant *Cdkn1c* expression was initiated *in utero* and persisted into adulthood.

Suboptimal diet in pregnancy and poor maternal care are both linked to the abnormal development and function of the offspring dopamine system [20-23]. Increased levels of both Tyrosine hydroxylase (Th, the rate-limiting enzyme in dopamine synthesis) and dopamine have been reported, as well as abnormal reward responses, a behavior known to require dopamine [17,18]. *Cdkn1c* is important in neurogenesis, migration and morphology of the developing nervous system [24-26]. Of particular relevance here is the fact that *Cdkn1c* cooperates with *Nurr1* to promote the proliferation and differentiation of midbrain dopaminergic neurons [27]. Maternally inherited loss of function of *Cdkn1c* results in reduced numbers of Nurr1-positive and Th-positive cells in the ventral midbrain at embryonic day (E)18.5 [27]. Taken together with the recently reported epigenetic sensitivity of *Cdkn1c* to the early life environment, this suggests the possibility that *Cdkn1c* may

contribute to the programming of the offspring dopamine system by abnormal early life environments. However, although its neurodevelopmental function is consistent with a causal role, it is highly likely that *Cdkn1c* is one of many genes whose expression changes following early life adversity. Consequently, to what extent does this subtle elevation in *Cdkn1c* expression contribute to altered behavior later in life?

We recently addressed this question directly using an established *Cdkn1c* transgenic model (*Cdkn1c*^{BACx1}) based on a single copy insertion of bacterial artificial chromosome (BAC) spanning the *Cdkn1c* locus into the mouse genome [28]. In this model, *Cdkn1c* is expressed from the transgene in the developing nervous system with temporal and spatial accuracy [28] at approximately 2-fold the normal level modeling LOI [29]. Neural analysis of these animals revealed increased Th staining intensity in the striatum and ventral tegmental area (VTA), increased whole tissue dopamine levels in the striatum, and an enhanced immediate early gene (IEG) response to the stimulant, amphetamine [30], changes reminiscent of those reported after early life adversity [17,22]. In addition to these neuronal changes, the *Cdkn1c*^{BACx1} animals showed changes in a number of behaviors linked to the dopamine system.

Dissociation of liking and wanting

The hyperdopaminergic phenotype predicted a change in reward-related behaviors [30,31]. We assessed the hedonic reaction of *Cdkn1c*^{BACx1} mice via examination of the microstructure of their consummatory behavior [32]. Rodents typically produce repeated clusters of licks separated by pauses when consuming liquids. The mean number of licks in a cluster (cluster size) is directly related to the perceived palatability of solution being consumed, independent of the overall amount consumed, and is therefore a measure of "liking". *Cdkn1c*^{BACx1} animals displayed a lower lick cluster size relative to their wild-type (WT) littermate controls suggesting a reduced hedonic response [12].

Interestingly, when assessed on a progressive ratio schedule (a test of "wanting"), *Cdkn1c*^{BACx1} mice were far more motivated by the food reward (8% sucrose), reaching a higher breakpoint (the maximum ratio reached by an animal within a session indicating the point at which they will no longer work for the reward) than their WT littermate controls [30]. Although a dissociation of this kind has been suggested from separate studies [32], the *Cdkn1c* LOI model is one of only two manipulations to show a double-dissociation between wanting and liking within the same animals. Strikingly, the other example is also a hyperdopaminergic animal, produced by knockdown of the dopamine transporter gene, *Dat* [33].

Behavior in the social group

In addition to showing alteration in hedonism, we found that *Cdkn1c*^{BACx1} mice were more likely to win a tube-test encounter with unfamiliar animals than their WT littermates, indicative of altered social dominance behaviors [30]. Social dominance has not been shown to be influenced by an adverse early life environment directly, although rat offspring raised

by high licking and grooming mothers do show reduced social interaction and concomitant increased *Cdkn1c* expression [18]. However, previous work indicates that social dominance is governed, in part, by the dopamine system in rodents [34] and that more dominant animals show increased motivation for reward [35].

The enhanced social dominance shown by *Cdkn1c*^{BACx1} mice is also interesting in the context of imprinted function generally. Previous work with another imprinted gene, *Grb10*, demonstrated that mice carrying a paternal knockout (*Grb10*^{patKO}) were also more likely to win a tube-test encounter with unfamiliar animals [36], paralleling the findings seen in *Cdkn1c*^{BACx1} mice. This is the first explicit demonstration of a convergent role for imprinted genes on a behavioral function, paralleling other functional studies indicating a convergent role for imprinted genes in placental function, energy homeostasis and thermogenesis [37]. Moreover, the direction of effects is apparently opposite for maternal *Cdkn1c* and paternal *Grb10*, as twice as much expression of maternal *Cdkn1c* and loss of paternal *Grb10* both increase wins in this test. This pattern fits with the prevailing theory for the evolution of imprinting, namely intragenomic conflict [38], and suggests that the maternal interest is to increase, whereas the paternal interest is to decrease, social dominance.

However, whilst highlighting a possible conflict of interest over a behavioral phenotype, the use of this one tube test outside of the normal social group has been criticized as being unlikely to reflect actual social dominance differences within the normal social group [39]. To address this, we explored the social dominance behavior of *Cdkn1c*^{BACx1} mice more deeply and in the more relevant context of the cage-group. We found that levels of *Cdkn1c* expression had no influence on the social dominance rank within the normal home-cage [40]. Nevertheless, the pattern of data did indicate that over-expression of *Cdkn1c* leads individuals to disrupt the normally stable social hierarchy. We interpret this to be because the *Cdkn1c*^{BACx1} mice are more territorial than their wild-type cage-mates, shown in other tests, and may contest the social dominance hierarchy more frequently, which in turn leads to a greater incidence of fighting within the social group [40].

Conclusions

These neural and behavioral data suggest that doubling the expression of *Cdkn1c*, mimicking loss-of-imprinting, leads to a hyper-dopaminergic animal [33,41]. In turn, this leads to whole raft of behavioral abnormalities, impacting on reward and social function. Moreover, the phenotype of this model of *Cdkn1c* LOI recapitulates many of the effects seen in animals where manipulation of the early life environment leads to changes in behavior, the midbrain dopamine circuitry and *Cdkn1c* expression [17,18] (Figure 1). This suggests that, although it is likely to result in a number of gene expression changes, the loss of imprinting of *Cdkn1c* in the offspring following maternal low protein diet is a key contributor to the associated changes in the dopamine system and behavior reported after early life adversity. However, whether this particular gene expression increase is *the* critical change, still remains to be fully established.

Acknowledgements
Thanks to Prof Dominic Dwyer for helpful comments on the manuscript.

References

1. Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nemeroff CB, Reyes TM, Simerly RB, Susser ES, et al.: **Early life programming and neurodevelopmental disorders**. *Biol Psychiatry* 2010, **68**:314-319.
2. McGowan PO, Meaney MJ, Szyf M: **Diet and the epigenetic (re)programming of phenotypic differences in behavior**. *Brain Res* 2008, **1237**:12-24.
3. Li J, Na L, Ma H, Zhang Z, Li T, Lin L, Li Q, Sun C, Li Y: **Multigenerational effects of parental prenatal exposure to famine on adult offspring cognitive function**. *Sci Rep* 2015, **5**:13792.
4. St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, Zheng X, Gu N, Feng G, Sham P, et al.: **Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961**. *JAMA* 2005, **294**:557-562.
5. Neugebauer R, Hoek HW, Susser E: **Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood**. *JAMA* 1999, **282**:455-462.
6. Franzek EJ, Sprangers N, Janssens AC, Van Duijn CM, Van De Wetering BJ: **Prenatal exposure to the 1944-45 Dutch 'hunger winter' and addiction later in life**. *Addiction* 2008, **103**:433-438.
7. Ferguson-Smith AC: **Genomic imprinting: the emergence of an epigenetic paradigm**. *Nature reviews. Genetics* 2011, **12**:565-575.
8. McGrath J, Solter D: **Completion of mouse embryogenesis requires both the maternal and paternal genomes**. *Cell* 1984, **37**:179-183.
9. Surani MA, Barton SC, Norris ML: **Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis**. *Nature* 1984, **308**:548-550.
10. Jiang YH, Tsai TF, Bressler J, Beaudet AL: **Imprinting in Angelman and Prader-Willi syndromes**. *Current Opinion in Genetics & Development* 1998, **8**:334-342.
11. Isles AR, Ingason A, Lowther C, Walters J, Gawlick M, Stober G, Rees E, Martin J, Little RB, Potter H, et al.: **Parental Origin of Interstitial Duplications at 15q11.2-q13.3 in Schizophrenia and Neurodevelopmental Disorders**. *PLoS Genet* 2016, **12**:e1005993.
12. Ferron SR, Radford EJ, Domingo-Muelas A, Kleine I, Ramme A, Gray D, Sandovici I, Constancia M, Ward A, Menheniott TR, et al.: **Differential genomic imprinting regulates paracrine and autocrine roles of IGF2 in mouse adult neurogenesis**. *Nat Commun* 2015, **6**:8265.
13. Ferron SR, Charalambous M, Radford E, McEwen K, Wildner H, Hind E, Morante-Redolat JM, Laborda J, Guillemot F, Bauer SR, et al.: **Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis**. *Nature* 2011, **475**:381-385.
14. Ivanova E, Chen JH, Segonds-Pichon A, Ozanne SE, Kelsey G: **DNA methylation at differentially methylated regions of imprinted genes is resistant to developmental programming by maternal nutrition**. *Epigenetics* 2012, **7**:1200-1210.

15. Radford EJ, Isganaitis E, Jimenez-Chillaron J, Schroeder J, Molla M, Andrews S, Didier N, Charalambous M, McEwen K, Marazzi G, et al.: **An unbiased assessment of the role of imprinted genes in an intergenerational model of developmental programming**. *PLoS Genetics* 2012, **8**:e1002605.
16. Hatada I, Mukai T: **Genomic imprinting of p57KIP2, a cyclin-dependent kinase inhibitor, in mouse**. *Nat Genet* 1995, **11**:204-206.
17. *Vucetic Z, Totoki K, Schoch H, Whitaker KW, Hill-Smith T, Lucki I, Reyes TM: **Early life protein restriction alters dopamine circuitry**. *Neuroscience* 2010, **168**:359-370.

Here the authors replicate previous findings showing that maternal low protein diet leads to changes in the dopamine circuitry and related behaviour. They also demonstrate a number of concomittant molecular changes, including a 2-3 fold increase in expression of imprinted *Cdkn1c.

18. Pena CJ, Neugut YD, Calarco CA, Champagne FA: **Effects of maternal care on the development of midbrain dopamine pathways and reward-directed behavior in female offspring**. *Eur J Neurosci* 2014, **39**:946-956.
19. *Van de Pette M, Abbas A, Feytout A, McNamara G, Bruno L, To WK, Dimond A, Sardini A, Webster Z, McGinty J, et al.: **Visualizing Changes in Cdkn1c Expression Links Early-Life Adversity to Imprint Mis-regulation in Adults**. *Cell Rep* 2017, **18**:1090-1099.

***Here the authors demonstrate that increased expression of *Cdkn1c* following exposure to a maternal low protein diet *in utero* is due to a de-repression of the paternal allele of *Cdkn1c* and is accompanied by reduced DNA methylation at the *Cdkn1c* promoter indicating an epigenetic response to prenatal adversity**

20. Ong ZY, Muhlhausler BS: **Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring**. *FASEB J* 2011, **25**:2167-2179.
21. Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM: **Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes**. *Endocrinology* 2010, **151**:4756-4764.
22. Palmer AA, Brown AS, Keegan D, Siska LD, Susser E, Rotrosen J, Butler PD: **Prenatal protein deprivation alters dopamine-mediated behaviors and dopaminergic and glutamatergic receptor binding**. *Brain Res* 2008, **1237**:62-74.
23. Hall FS, Wilkinson LS, Humby T, Robbins TW: **Maternal deprivation of neonatal rats produces enduring changes in dopamine function**. *Synapse* 1999, **32**:37-43.
24. Tury A, Mairet-Coello G, DiCicco-Bloom E: **The cyclin-dependent kinase inhibitor p57Kip2 regulates cell cycle exit, differentiation, and migration of embryonic cerebral cortical precursors**. *Cereb Cortex* 2011, **21**:1840-1856.
25. Ye W, Mairet-Coello G, Pasoreck E, Dicicco-Bloom E: **Patterns of p57Kip2 expression in embryonic rat brain suggest roles in progenitor cell cycle exit and neuronal differentiation**. *Dev Neurobiol* 2009, **69**:1-21.
26. Itoh Y, Masuyama N, Nakayama K, Nakayama KI, Gotoh Y: **The cyclin-dependent kinase inhibitors p57 and p27 regulate neuronal migration in the developing mouse neocortex**. *J Biol Chem* 2007, **282**:390-396.
27. Joseph B, Wallen-Mackenzie A, Benoit G, Murata T, Joodmardi E, Okret S, Perlmann T: **p57(Kip2) cooperates with Nurr1 in developing dopamine cells**. *Proc Natl Acad Sci U S A* 2003, **100**:15619-15624.

28. John RM, Ainscough JF, Barton SC, Surani MA: **Distant cis-elements regulate imprinted expression of the mouse p57(Kip2) (Cdkn1c) gene: implications for the human disorder, Beckwith--Wiedemann syndrome**. *Hum Mol Genet* 2001, **10**:1601-1609.
29. Andrews SC, Wood MD, Tunster SJ, Barton SC, Surani MA, John RM: **Cdkn1c (p57Kip2) is the major regulator of embryonic growth within its imprinted domain on mouse distal chromosome 7**. *BMC Dev Biol* 2007, **7**:53.
30. **McNamara GI, Davis BA, Browne M, Humby T, Dalley JW, Xia J, John RM, Isles AR: **Dopaminergic and behavioural changes in a loss-of-imprinting model of Cdkn1c**. *Genes Brain Behav* 2018, **17**:149-157.
31. **McNamara GI, Davis BA, Dwyer DM, John RM, Isles AR: **Behavioural abnormalities in a novel mouse model for Silver Russell Syndrome**. *Hum Mol Genet* 2016, **25**:5407-5417.

****In these two studies we demonstrate that two-fold overexpression of *Cdkn1c* specifically, modeling loss-of-imprinting, leads to changes in the dopamine system. In turn these animals show reduced hedonic reponse to, but increased motivation for a food reward.**

32. Dwyer DM: **EPS Prize Lecture. Licking and liking: the assessment of hedonic responses in rodents**. *Q J Exp Psychol (Hove)* 2012, **65**:371-394.
33. Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X: **Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards**. *J Neurosci* 2003, **23**:9395-9402.
34. Jupp B, Murray JE, Jordan ER, Xia J, Fluharty M, Shrestha S, Robbins TW, Dalley JW: **Social dominance in rats: effects on cocaine self-administration, novelty reactivity and dopamine receptor binding and content in the striatum**. *Psychopharmacology (Berl)* 2016, **233**:579-589.
35. Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC: **Dominant rats are natural risk takers and display increased motivation for food reward**. *Neuroscience* 2009, **162**:23-30.
36. Garfield AS, Cowley M, Smith FM, Moorwood K, Stewart-Cox JE, Gilroy K, Baker S, Xia J, Dalley JW, Hurst LD, et al.: **Distinct physiological and behavioural functions for parental alleles of imprinted Grb10**. *Nature* 2011, **469**:534-538.
37. Cleaton MA, Edwards CA, Ferguson-Smith AC: **Phenotypic outcomes of imprinted gene models in mice: elucidation of pre- and postnatal functions of imprinted genes**. *Annu Rev Genomics Hum Genet* 2014, **15**:93-126.
38. Moore T, Haig D: **Genomic imprinting in mammalian development - a parental tug-of-war**. *Trends in Genetics* 1991, **7**:45-49.
39. Curley JP: **Is there a genomically imprinted social brain?** *BioEssays : news and reviews in molecular, cellular and developmental biology* 2011, **33**:662-668.
40. *McNamara GI, John RM, Isles AR: **Territorial Behavior and Social Stability in the Mouse Require Correct Expression of Imprinted Cdkn1c**. *Frontiers in Behavioral Neuroscience* 2018, **12**.

***This paper examines the social-group behaviour in mice over-expressing *Ckdn1c*, showing social stability is altered in these mice rather than social dominance *per se*.**

41. Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA: **Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: differential effects of D1 and D2 receptor antagonists**. *J Neurosci* 2001, **21**:305-313.

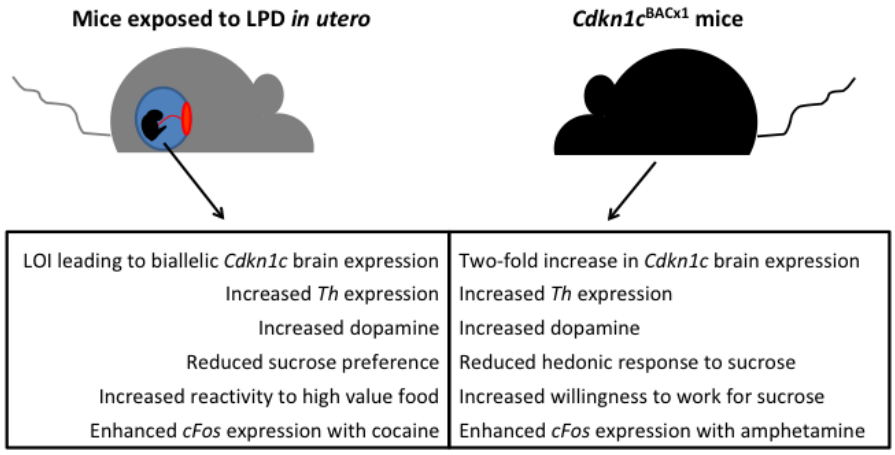

Figure 1 Schematic summarising similar brain and behavioural phenotypes seen mice exposed to maternal LPD and transgenic *Cdkn1c*^{BACx1} mice (LOI = loss of imprinting).

Formatted: Font: Bold