Behavioural and Molecular Changes Associated with Advanced Paternal Age: A Mouse Model

Rebecca G Smith[1], Rachel L Kember[1], Leonard C Schalkwyk[1], Joseph D Buxbaum[2], Abraham Reichenberg[1,2], Cathy Fernandes[1], Jonathan Mill[1]

1. King's College London, Institute of Psychiatry, London, UK
2. Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

E-mail : Rebecca.Smith@kcl.ac.uk Group Webpage: http://iop.kcl.ac.uk/departments/?locator=1063

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Introduction

Accumulating evidence from epidemiological research has demonstrated an association between advanced paternal age and risk for several psychiatric disorders including autism and schizophrenia. Despite the methodological advantages of epidemiological research, a major limitation is that techniques are limited to observation. By breeding mice from fathers of three different ages, two months (young), 10 months (old) and 12 months (very old) we examined the effect of paternal age on social and non-social behaviour and whether any behavioural effects seen could be explained by *de-novo* copy number variations (CNVs) or epigenetic changes.

Summary of Behavioural Results

Animal behaviour was analysed in the open field, holeboard and social behaviour tasks. The offspring of old fathers engaged in significantly less social activity with the conspecific mouse than offspring of young fathers (p=0.02, **Figure 1**). This difference was mainly seen in sniffing behaviour.

The offspring of old fathers demonstrated reduced exploration in the holeboard task, making fewer nose pokes and spending less time nose poking than offspring of young fathers (p=0.02, **Figure 2**). No differences were observed in overall locomotor activity in any task.

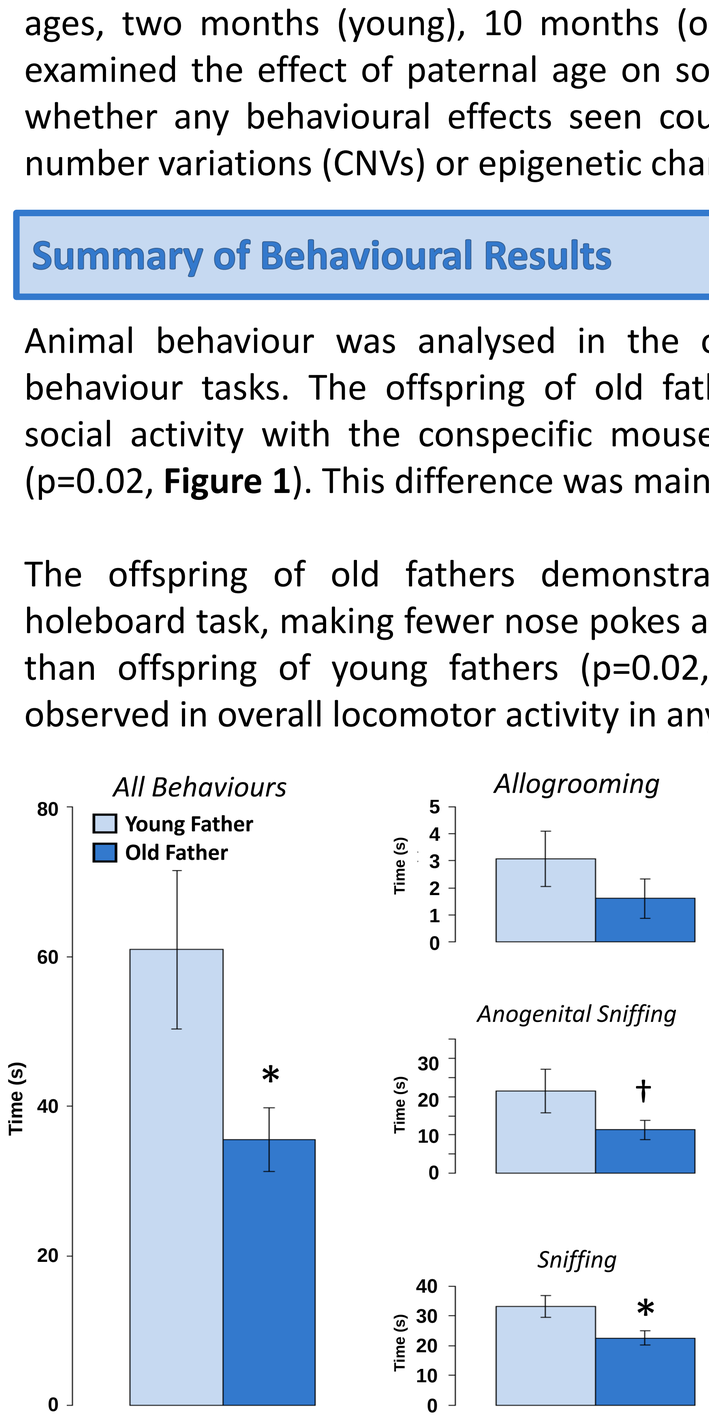

Figure 1 *Results of social behaviour task. * p-value < 0.05, † p-value = 0.06*

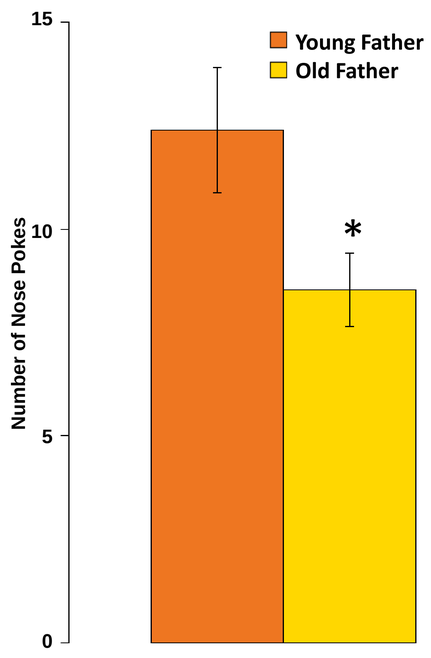

Figure 2 *Number of nose pokes into holes in the holeboard task. * p-value < 0.05*

Copy Number Variation Results

DNA from the spleen of parents and offspring of young and old fathers were hybridized against a common female reference sample and analysed for CNVs using Nimblegen 720K CGH arrays. Our screen of CNVs showed that they are widespread in the mouse genome (**Figures 3 & 4**) but no significant association was seen with the number, size or location of *de-novo* CNVs and paternal age.

Figure 3 *Mouse Karyotype showing all CNVs within a Single Family*

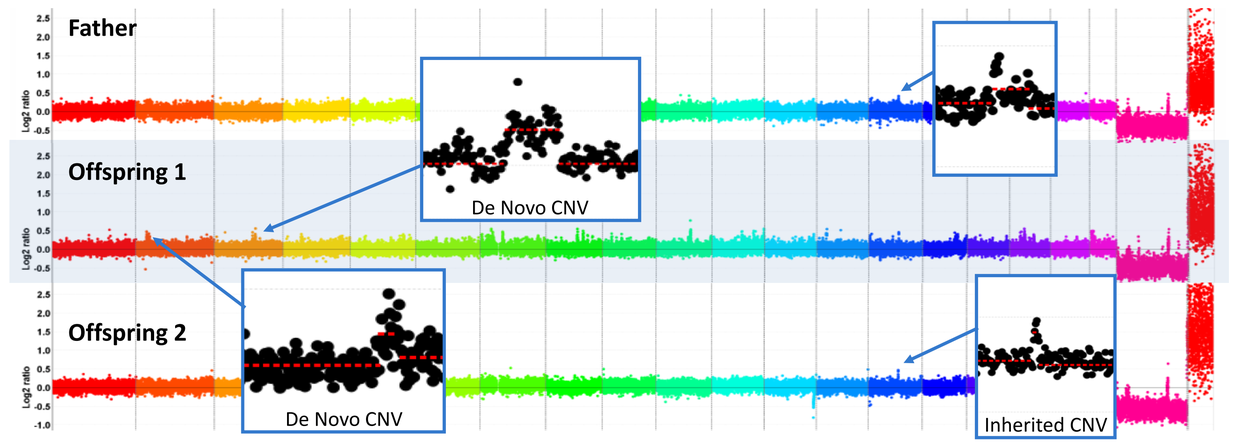

Figure 4 *Examples of inherited and de-novo CNVs in a mouse family*

Epigenetic Results

Global methylation was assessed using the Luminometric Methylation Assay (LUMA) combined with Pyrosequencing. DNA from spleen showed that the offspring of very old fathers have a significantly increased level of global methylation in a somatic tissue (p=0.005, **Figure 5**) but this higher level of methylation was not detected in DNA from the fathers themselves (**Figure 6**).

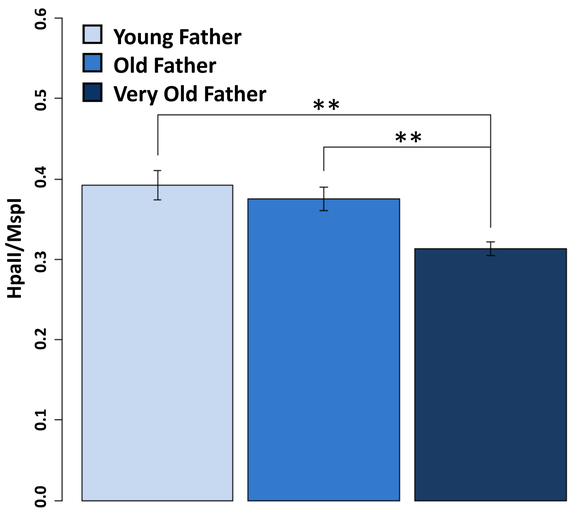

Figure 5 *Results of LUMA assay on spleen samples of offspring ** p-value < 0.01*

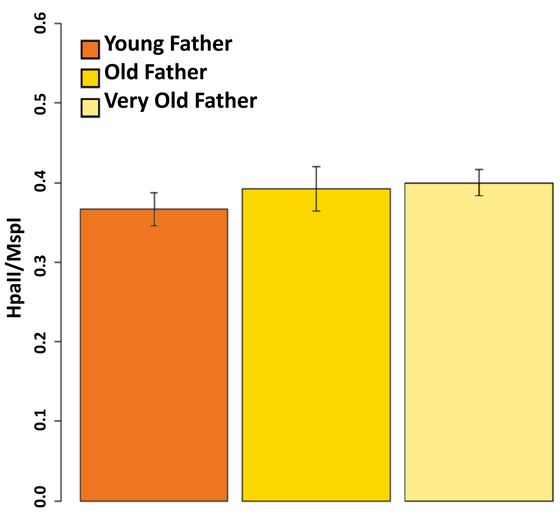

Figure 6 *Results of LUMA assay on spleen samples of fathers*

DNA methylation across differentially methylated regions (DMRs) in brain expressed imprinted genes were assayed using the Sequenom EpiTyper. Four DMRs showed consistent significant differences in methylation between the offspring of young fathers and offspring of old fathers (**Figure 7**).

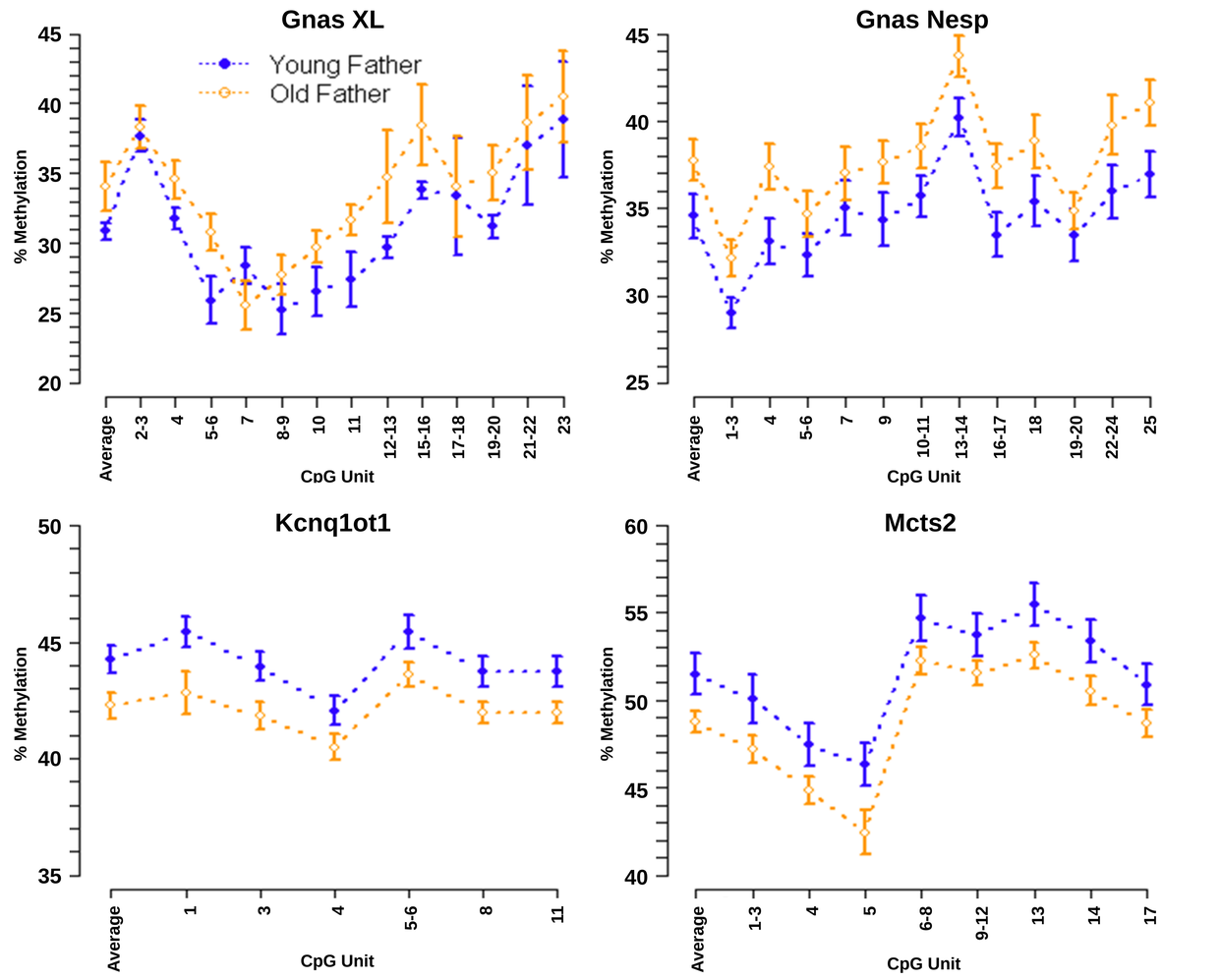

Figure 7 *Methylation Results of four imprinted genes from Sequenom EpiTyper analysis*

Conclusion

The behavioural results provide evidence for the paternal age effect observed in epidemiological studies and although there was no association between *de-novo* CNV events and paternal age there is evidence for the role of epigenetics in mediating the paternal age effect.