Variable allelic expression of imprinted genes at the *13*, , *2* cluster in single neural cells

fi Trappc9 fi

KEYWORDS

genomic imprinting, allelic expression, single-cell analysis, neurosphere, neural stem cell, 13, 9, A 2

Introduction

Genomic imprinting has long been recognized as a paradigm of epigenetic regulation of a specific subset of ~200 genes that have important roles in mammalian embryogenesis, regulation of nutrient supply and demand between mother and offspring, as well as brain development and neural functions (Ferguson-Smith, 2011; Peters, 2014; Tucci et al., 2019). Imprinted genes are defined by their parent-of-origin dependent mono-allelic or strongly biased allelic (>70%) expression. This expression bias towards a specific parental allele is a consequence of DNA methylation marks that are differentially established in either the male or female germ cells, respectively, at a defined set of CpG-rich islands (CGIs). Such germline differentially methylated regions (gDMRs) are maintained after fertilization in the somatic cells of the developing and adult offspring; they are only erased in its developing germline cells for re-setting according to its sex and transmission to the next generation (Plasschaert and Bartolomei, 2014). The germline DMRs often regulate the allelic expression of a cluster of neighboring genes and are, therefore, also called imprinting control regions (ICRs) (Ferguson-Smith, 2011; Peters, 2014; Tucci et al., 2019). In addition to DNA methylation, the mechanisms involved in the regulation of imprinted gene expression comprise histone modifications, non-coding RNAs and boundary or insulator elements that are recognized by CTCF, a methylation-sensitive DNA binding factor. CTCF binding at unmethylated sites within DMRs of imprinted genes has been shown to regulate access to tissue-specific enhancers and the formation of allelic topologically associated domains (TADs), thereby controlling the expression of neighboring genes in an allele-specific way (Bell and Felsenfeld, 2000; Lleres et al., 2019).

The imprinting status of most genes is conserved between human and mouse, although some genes do not show an allelic expression bias in one or the other species (Court et al., 2014; Tucci et al., 2019). Furthermore, while many imprinted genes have the same strong parental allele-specific expression bias in all tissues analyzed, others show tissue-specific imprinting effects, examples of which are Gnas in defined brain regions, endocrine glands and proximal renal tubules,

mono-allelic and bi-allelic expression of specific imprinted genes in brain sections. Novel single-cell RNA-seq methods have also been applied to analyze imprinted gene expression in single cortical neurons after labeling them with fluorescent proteins and FACS sorting (Laukoter et al., 2020). Their findings indicate

modifications. Briefly, hippocampi were dissected from newborn

Single-cell isolation and allelic expression analysis

Single C57BL/6J × Cast/EiJ neurosphere cells were obtained via dissociation with Accutase, dilution in growth medium and either FACS sorting or manual isolation via capillary action under a

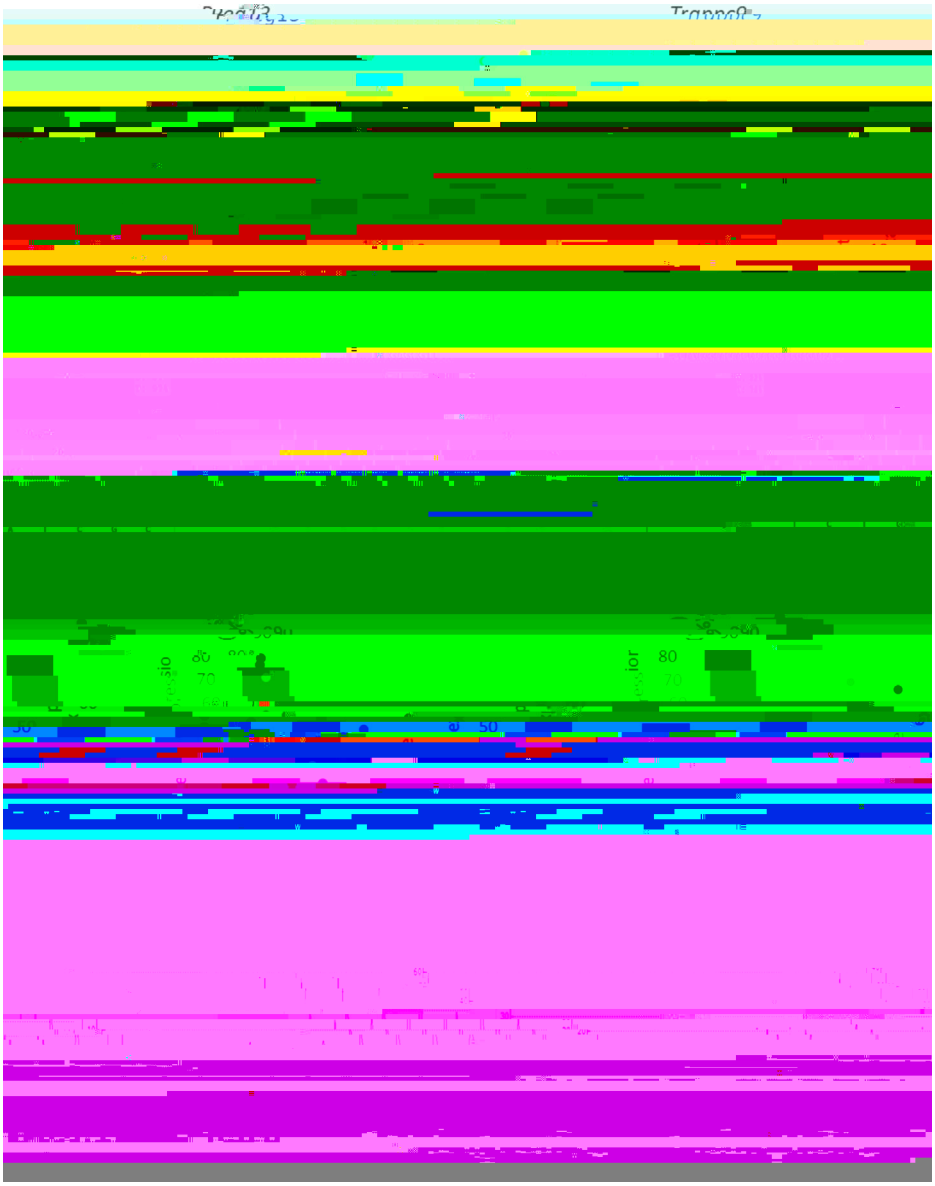

FIGURE 2

via

which is in line with human brain data (Court et al., 2014) and their status as actively transcribed genes. This excludes any secondary DMRs at this imprinted gene cluster.

Overall, our data confirm brain-specific imprinting, i.e., preferential expression from the maternal allele, of Trappc9 and Ago2 in mouse, while maternal allelic expression of Kcnk9 occurs in brain and some peripheral tissues, e.g., kidney. Unexpectedly, Trappc9 and Ago2 have no allelic expression bias in hippocampal neurosphere cultures.

Varying allelic expression biases of *13*, and *2* in single neural stem cells and differentiated neurons

Analysis of imprinted gene expression on a bulk tissue level raises the question of whether the observed allelic bias is reflected in every cell of the lysate in the same way, or whether individual cells differ in their mono-/bi-allelic transcriptional status of the gene and, thus, deviate from the tissue average. Large-scale single-cell imprinted gene expression analysis is still in its infancy, but novel

half of the NSCs showed biased bi-allelic expression: 21% predominantly maternal, 31% predominantly paternal (Figure 4B). Considering all single-NSC categories in proportion, the data are in line with the bulk neurosphere analysis (Figure 2

expression (Figures 5A–C). Most NSCs had a biased bi-allelic expression of Ago2 (29% predominantly maternal, 33% predominantly paternal). Overall, the proportions of NSCs falling into the various expression categories did not indicate a clear allele bias and are in line with the equal bi-allelic Ago2 expression found in bulk neurosphere samples (Figure 2). Compared to the NSCs, among the differentiated neurons more cells showed equal bi-allelic (21%), mono-allelic maternal (16%) and biased maternal (32%) expression (Figure 5B), while the proportions of neurons with mono-allelic paternal (9%) and biased paternal (23%) expression were reduced (Figure 5B). However, as with Trappc9, the in vitro differentiated neuron categories did not overall reflect the same strong bias of 75% maternal allele-specific Ago2 transcripts that were detected in whole-brain tissue (Figure 2).

Since the non-coding RNA Peg13 is transcribed from the core imprinting regulatory region (DMR) of the locus, findings of Peg13 expression other than mono-allelic paternal or paternally biased bi-allelic (Figures 3B,C) were unexpected. This raises the question of whether there is a specific pattern of allelic expression of the other imprinted genes of the locus associated with Peg13 transcription from the maternal allele. When analyzing the expression status of Trappc9 and Ago2 in those cells that showed equal bi-allelic, maternally biased or mono-allelic maternal expression of Peg13, we did not find any specific patterns or correlations (Supplementary Table S2). Some of these cells displayed the expected maternal bias of Trappc9 and/or Ago2, but other allelic biases, including mono-allelic expression states, were also observed and in varying combinations within individual cells.

In summary, our single-cell analysis of the three imprinted genes indicates a surprising variability of allelic expression states in individual cells, ranging from mono-allelic maternal to mono-allelic paternal transcription, even for the core imprinted gene of the locus, Peexp.96-dTcga5.28x9t1216at6-dTcga5c19)26v)rufu

silencer elements might contribute to the regulation of tissue-specific expression of Trappc9, Chrac1 and/or Ago2 in vivo. Reg-D might also contribute specifically to the reduced transcription of their paternal alleles in brain, thereby generating an imprinted expression bias in this tissue, although any allele-specific

expression and becomes bi-allelically expressed in postnatal NSCs of the ventricular zone and hippocampal subgranular zone (Ferron et al., 2011; Montalban-Loro et al., 2021). The change to bi-allelic expression of Dlk1 is associated with gain of methylation at its germline DMR and is a requirement for

variable allelic expression states of imprinted genes in individual cells are largely unclear. The cases of Dlk1 gain of methylation and loss of imprinting in NSCs (Ferron et al., 2011; Montalban-Loro et al., 2021

statistical analysis. MC and AP wrote the manuscript. DM and AP conceived, designed and supervise