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Developmental Brain Research 92 (1996) 91–100

DEVELOPMENTAL BRAIN RESEARCH

Research report

Genomic imprinting and the differential roles of parental genomes in brain development

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Accepted 5 December 1995

Abstract

Certain genes are expressed either from the maternal or the paternal genome as a result of genomic imprinting, a process that confers functional differences on parental genomes during mammalian development. In this study we focus on the cumulative effects of imprinted genes on brain development by examining the fate of androgenetic (Ag: duplicated paternal genome) and parthenogenetic/gynogenetic (Pg/Gg: duplicated maternal genome) cells in chimeric embryos. Striking cell autonomous differences in the phenotypic properties of the uniparental cells were observed. Ag cells contributed substantially to the hypothalamic structures and not the cortex. By contrast, Pg/Gg cells contributed substantially to the cortex, striatum and hippocampus but not to the hypothalamic structures. Furthermore growth of the brain was enhanced by Pg/Gg and retarded by Ag cells. We propose that genomic imprinting may be responsible for a change in strategy controlling brain development in mammals. In particular, genomic imprinting may have facilitated a rapid non-linear expansion of the brain, especially the cortex, during development over evolutionary time.

Keywords: Genomic imprinting; Chimera; Parthenogenetic cell; Androgenetic cell; Hypothalamus; Telencephalon; Development

1. Introduction

An intriguing aspect of mammalian development is the functional non-equivalence of the parental genomes [14]. Such effects of parental genomes were demonstrated by the striking phenotypic differences in embryonic and extraembryonic development of parthenogenetic/gynogenetic (Pg/Gg: embryos with duplicated maternal genome) and androgenetic embryos (Ag: embryos with duplicated paternal genome) [28–30]. These functional differences arise as a result of imprinted genes whose expression is dependent on their parental origin. Genetic studies have identified specific chromosomal regions that are likely to contain these imprinted genes, including Ch2, 6, 7, 11, 12 and 17 [5]. Further confirmation for the functional differences between parental genomes has been achieved by the identification of imprinted genes. *Igf2* and *Snrpn* are expressed from the paternal genome while *H19, Igf2r* and *Mash2* are expressed from the maternal genome [9,16]. Further imprinted genes are currently being identified.

Insights into the cumulative effects of imprinted genes on development has come from studies on chimeric embryos that contain Pg/Gg and Ag cells. Chimeric embryos containing androgenetic and wild type cells (Ag → Wt) show enhanced growth with the Ag cells making a disproportionate contribution to many mesodermal tissue but contributing little to the brain [4,20]. By contrast, chimeras with parthenogenetic/gynogenetic cells (Pg/Gg → Wt) are growth retarded and the Pg/Gg cells contribute substantially to the brain but not to the mesodermal tissues where Ag cells were found [13,4,22]. The influence of imprinted genes on development is most striking in Ag chimeras which develop severe skeletal abnormalities. It is likely that proliferation and differentiation of androgenetic cells is affected by the deficiency of maternally expressed imprinted genes and by the excess gene dosage of paternally

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0165-3806/96/$15.00 © 1996 Elsevier Science B.V.
SSDI 0165-3806(95)00209-X

expressed genes. The phenotypic effects observed in chimeras suggest both the cell autonomous effects of imprinted genes as well as the presence of imprinted genes encoding for the cell signalling factors and their receptors such as *Igf2* and *Igf2r*.

The previous studies on chimeras strongly indicated a possible influence of imprinted genes on brain development. In these studies, GPI isozyme was used as a marker which showed that the contribution of Pg/Gg cells to the brain was approximately 30–40% while that of the Ag cells was less then 5% [4]. However the precise location of the experimental cells could not be determined as no in situ markers were used. These studies are also of considerable interest in the context of certain human behavioural disorders that are apparently associated with imprinted genes that occur due to the duplication of specific maternal or paternal chromosomal regions that contain imprinted genes. For instance, children who have Prader–Willi syndrome inherit both copies of imprinted chromosomes 15q from their mothers and suffer sexual dysfunction, hyperphagia and obesity [24]. Children who either lack portions of maternal chromosome 15q or possess two copies of paternal chromosome 15q exhibit mental retardation and puppet-like movements, and are prone to seizures as part of Angelman's syndrome [23]. Genetic evidence also suggests that the mouse chromosome 2 may contain imprinted genes that are likely to affect behaviour [6]. Therefore a number of imprinted genes throughout the genome may have an important role in regulating behaviour and development of the brain.

In this study, we carried out a systematic investigation on the role of imprinted genes in development of the brain by a detailed analysis of the fate of parthenogenetic/gynogenetic and androgenetic cells in the developing brain. The precise fate of the experimental cells was assessed by using two different in situ markers to determine the distribution and location of the experimental cells. The distribution Pg/Gg and Ag cells was found to be non random; both the Pg/Gg and Ag cells were detected in highly specific and reciprocal locations in the developing brain. These results have provocative and wider implications for the control of development of the mammalian brain as a result of the differential roles of parental genomes and expression of the imprinted genes.

2. Experimental procedures

2.1. Animals

Mice used in these experiments were (C57BL/6J × CBA/Ca) F_1 (*Gpi-1b/Gpi-1b*) (AFRC colony bred from Bantin and Kingman stock), hereafter referred to as F_1, 129HD (*Gpi-1a/Gpi-1a*, dilute pink eyed from OLAC Ltd) and CFLP outbred albino (*Gpi-1a/Gpi-1a*), bred from OLAC stock. Animals were housed in standard conditions with a 14 h light and 10 h dark lighting schedule.

To obtain fertilised 1-cell eggs and blastocysts 3- to 4-week-old females were superovulated by injection of 7.5 i.u. pregnant mare's serum (PMSG, Intervet Ltd., Cambridge, UK) followed 48 h later by an injection of 7.5 i.u. human chorionic gonadotrophin (hCG, Intervet), caged singly with stud males and checked for vaginal plugs the following morning; the day of finding the plug was designated day 1.

To obtain pseudopregnant recipients, naturally cycling mature F_1 females were mated with vasectomised males of proven sterility.

2.2. Ag/Pg chimeras using the β-globin marker for experimental cells

Superovulation of females, the collection, activation and handling of eggs and embryos, and embryo aggregation was carried out according to standard procedures described in detail elsewhere [3]. We used a mouse line designated Tg-MβG-1 [19] (henceforth called line 83), to prepare Ag and Pg embryos. This is homozygous for a transgenic locus of 1000 tandemly repeated copies of β-globin gene which is detectable by in situ hybridisation [17]. Ag zygotes were constructed from eggs taken from a (C57BL/6 × CBA/Ca)F_1 × 83/83 crossing and Pg zygotes were from activated 83/83 eggs. These experimental zygotes were cultured in vitro for 5–6 days to the blastocyst stage. Ag → Wt chimeras were constructed after isolating Ag inner cell mass and introducing them into the CFLP host blastocyst. Pg ↔ Wt chimeras were constructed either by aggregating Pg morulae with CFLP morulae or by injecting Pg inner cell masses into CFLP host blastocyst. The reconstituted blastocysts were transferred to recipient mice and allowed to develop to different stages. The transgenic marker has been used in the past for cell lineage studies in chimeric mice [7]. The experimental cells were detected by in situ hybridisation for the transgene insert exactly as described previously [1]. 7Ag, 9 Pg and 7 control embryos were prepared using this method.

2.3. Ag/Gg chimeras using Lac Z marker

Chimeric mice were prepared with Ag and Gg inner cell mass or embryonic stem cells that contained the ubiquitously expressed *Lac Z* transgene from the ROSA26 mice [11]. Expression of this transgene is not affected by its parental origin. The ROSA transgenic line was prepared in a 129/Sv embryonic stem cell line and we have subsequently maintained these mice on a C57B16/CBA background. The androgenetic zygotes were prepared from F_1 × ROSA26/ROSA26 and F_1 × 129/Sv (giving ROSA26/129 Ag). The gynogenetic zygotes were constructed from ROSA26/ROSA26 × F_1 and 129/Sv × F_1 eggs (giving ROSA 26/129 Gg). Both the R26Ag and

R26Gg zygotes were cultured to the blastocyst stage (5–6 days). The ICMs were released by immunosurgery, and injected into F_2 (F_1 mice were the same as the ones described above) blastocysts obtained on day 4 of gestation to produce R26Ag → F_2 and R26Gg → F_2 chimeras. Chimeric embyros were fixed and stained for *Lac Z* as described previously [1]. 42 Ag, 31 Gg and 14 control embryos from different developmental stages were prepared using this method.

2.4. Cell counts in chimeric brains

For the quantification of Ag and Pg contribution three brain sections were evaluated from each brain area for 12 chimeric mice. Counting of cells was made in area of 0.13 sq mm of each section using computerised image analysis for cell counts. Coat and eye colour were only used as a guide to which animals were chimeric and did not bear any obvious relationship to brain chimerism. Since the contribution of Ag/Pg cells to the medulla was quantitatively similar and showed no obvious patterning or differences from control chimeric embryos, this region of the brain was used as a control area against which Pg/Ag cell distribution was compared.

2.5. Brain size

Brains from six Rosa 27 Gg chimeras, six littermate controls matched for age, sex and strain, and six control chimeras were used. Thirty sections were taken from each brain at intervals through the forebrain (anterior striatum to posterior hippocampus) and measured using computerised image capture with image analysis software (NIH). The mean brain size was calculated for each animal and expressed relative to body weight for statistical analysis.

Rosa 27 Ag chimeras rarely survive birth and were therefore removed on day 17 of development, weighed and left overnight in PBS 2% paraformaldehyde fixative. The following day brains were removed from 35 embryos and weighed. Bodies were sectioned and left in Xgal overnight to determine level of chimerism. Eight embryos, although treated in an identical manner were found to have no Ag cell contribution and were used as matched control littermates.

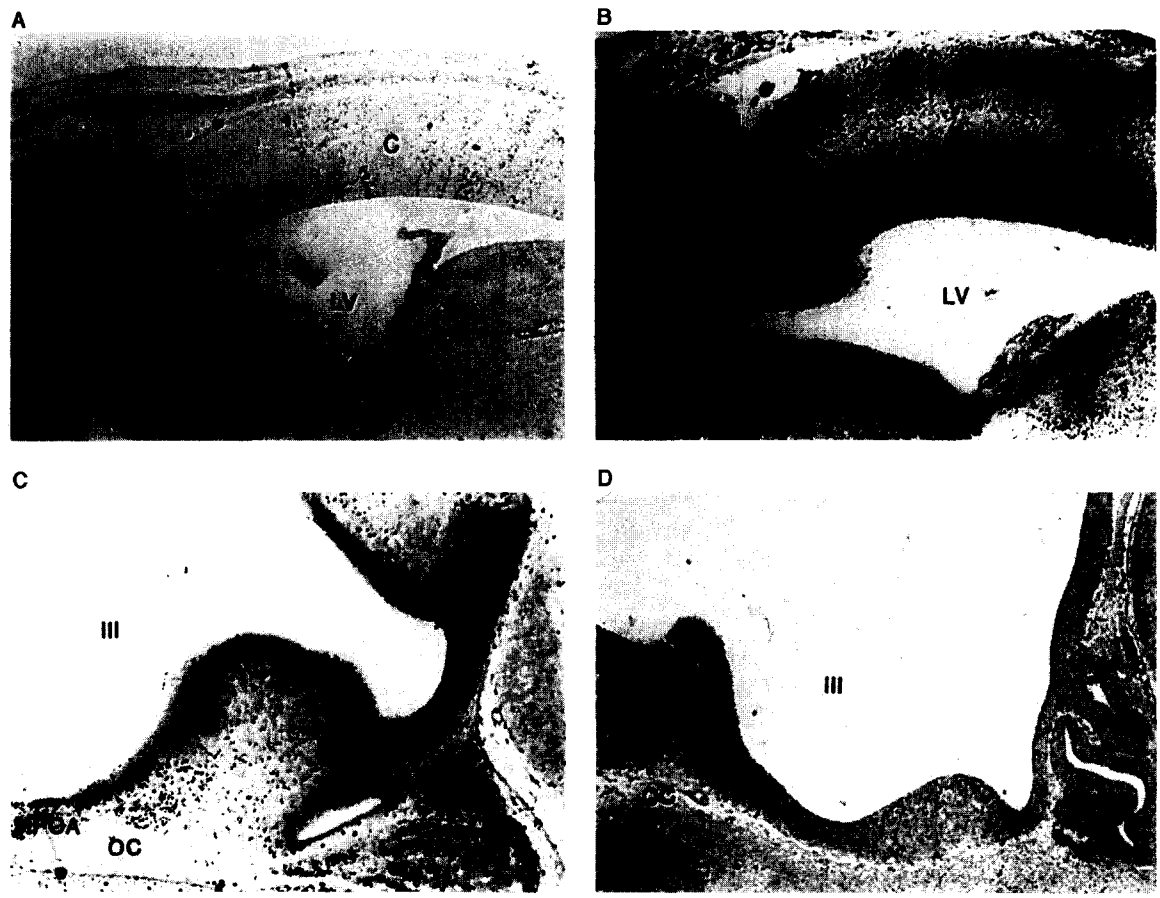


Fig. 1. Parasagittal sections of the mouse brain (embryonic Day 17) illustrating differential contribution of Ag cells (A and C) and Pg cells (B and D). While relatively few Ag cells can be seen in the cortex (A) they make a substantial contribution to the hypothalamus (C). The converse is seen for Pg cells which contribute substantially to cortex and striatum (B) but are virtually absent from the hypothalamus (D). OB, olfactory bulb; III, third ventricle; C, cortex; OC, optic chiasma; LV, lateral ventricle; Pit, pituitary; St, striatum; POA, preoptic area.

3. Results

3.1. Distribution of Ag and Pg cells using β-globin marker

Ag cells accumulated in a very specific region of the diencephalon (Fig. 1A and C) but only a few Ag cells are seen in the forebrain. The pre-optic area and hypothalamus, particularly medio-basal and posterior hypothalamus, contained a relatively large number of Ag cells (Fig. 1C). By contrast, Pg cells were found predominantly in the cortex and striatum (Fig. 1B) where Ag cells are virtually undetectable (Fig. 1A). It is important to note that the Pg cells were virtually undetectable in the hypothalamus (Fig. 1D). The degree of chimerism and hence absolute numbers of experimental cells in different embryos is, of course variable but the reciprocal patterning of Ag and Pg cell distribution was consistently observed in all the embryos examined. In chimeras with control cells carrying the β-globin transgene insert, a uniform distribution of cells was observed throughout the brain without any differences in distribution to specific regions of the brain.

The relative distribution of experimental cells was determined by controlling for the variable levels of chimerism (Fig. 2). In embryos on day 13 of gestation (E13), the relative number of Pg cells in the cortex is approximately three fold higher than that of control cells while the number of Ag cells in the cortex are about half that of control cells. The same pattern of differential distribution was seen for the striatum. In contrast, the hypothalamus showed a reciprocal distribution, with Pg cells being half that of controls, while Ag cells were almost threefold higher than the number of control cells in this area. By E17, Pg cells were still present at a higher level in cortex compared to the control cells, while the number of Ag cells remained relatively low. The distribution of Ag cells in the hypothalamus by a factor of 6 over normal cells, and 20 over Pg cells is particularly striking. Therefore, the distribution of Pg and Ag cells is apparently distinct and reciprocal when comparing the hypothalamus with the cortex/striatum (Figs. 1 and 2). After taking into account the different degrees of chimerism, Ag and Pg cells in their respective areas of the brain increased in absolute terms with developmental time, between E13 and E17, suggesting that these cells are able to proliferate. However, the same was not true for Pg cells in the hypothalamus or Ag cells in the cortex. In these areas Pg and Ag cells decreased in number relative to control cells over developmental time.

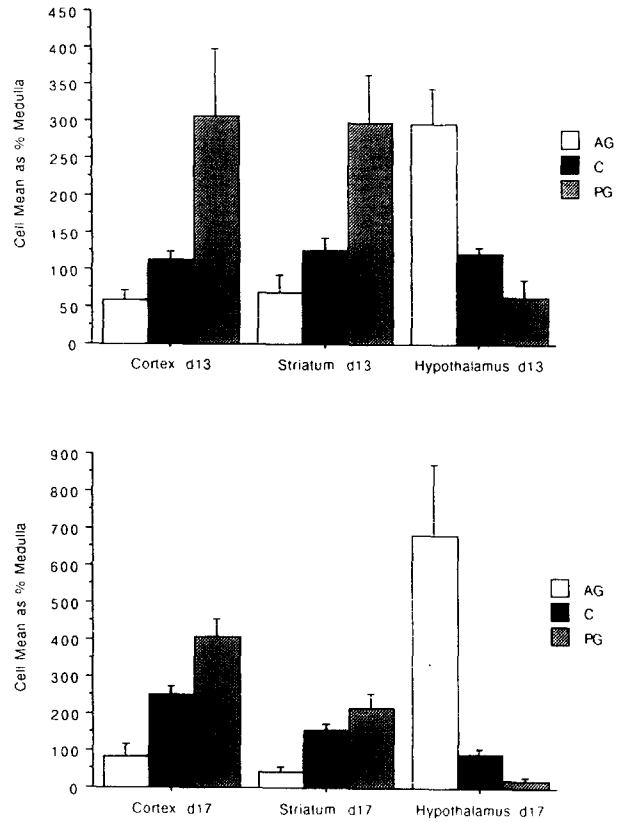
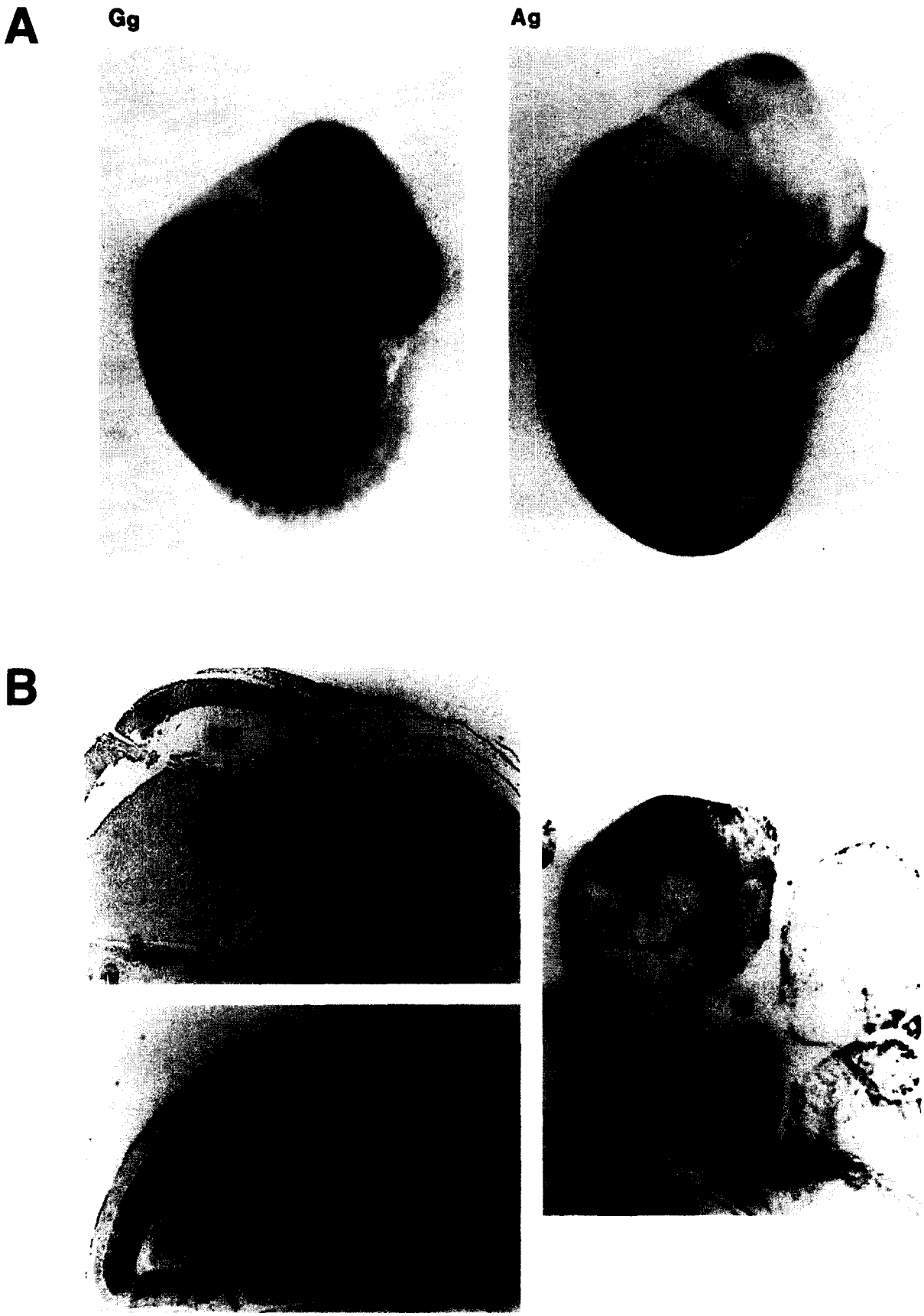
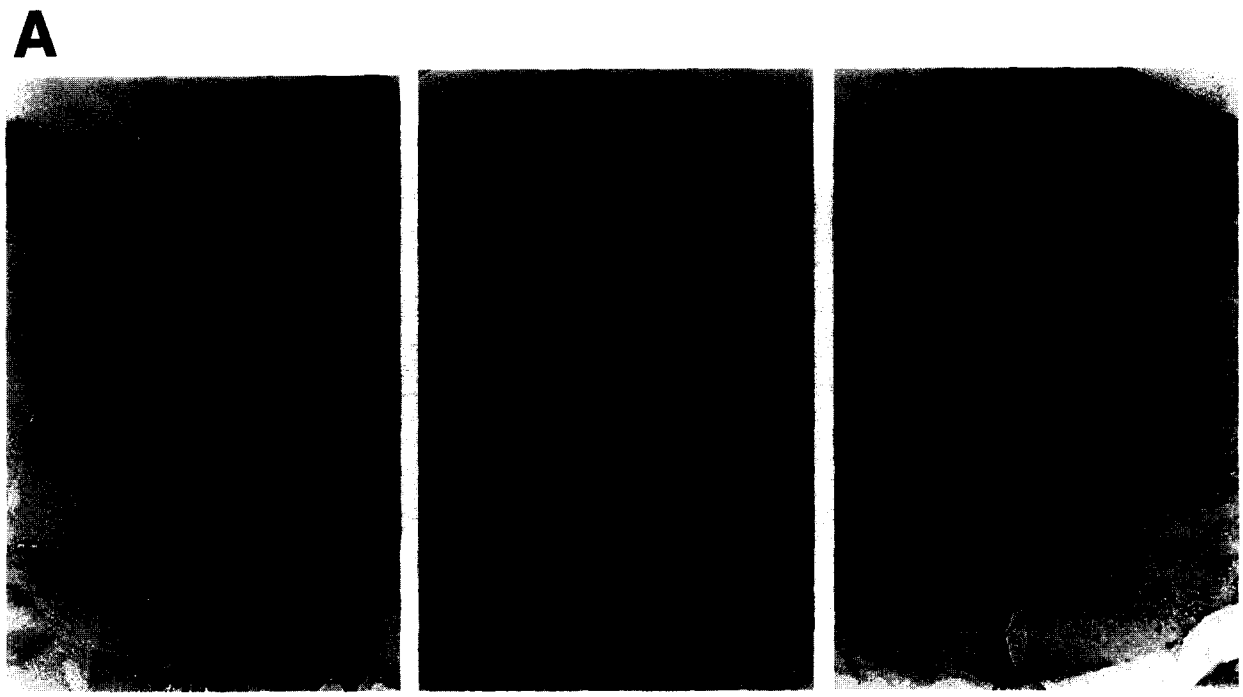


Fig. 2. Relative distribution of chimeric control, chimeric Ag and chimeric Pg cells in different brain regions on embryonic days 13 and 17. Because levels of chimerism are variable, the data has been normalised for each embryo by comparison with its own medulla where the contribution of Ag and Pg cells show no overall differential contribution. Data expressed as % medulla contribution ±S.E.

The overall contribution of Ag and Pg cells to the brainstem showed no marked difference, although the distribution within the brainstem does show certain regional differences. Hence Pg cells accumulated in the trigeminal and other cranial nerve ganglia, the mid-line raphe (future serotoninergic neurons) and made a substantial contribution to the mid-brain tectum compared with the pons. Ag cells were also present in the trigeminal ganglia and tectum, and in the reticular formation of the mid-brain, pons and medulla. Ag cells were also found in the thalamus and habenula and both Ag and Pg cells contributed to the choroid plexus. However, there was no obvious or consistent pattern of distribution of the experimental cells in the brainstem.

Fig. 3. A: representative E12 chimeras showing differential distribution of Ag (left) and Gg (right) experimental cells to brain and somatic tissues. B: brain sections of Ag and Gg chimeric mice prepared from experimental cells containing *Lac Z* transgene marker. Ag cells are virtually absent from cortex and striatum of E13 brain, but present in choroid plexus (top left) while Gg cells are high in cortex, striatum (bottom left). Note the radial distribution of cortical cells. A high power parasagital section (×200) through the hypothalamus reveals a high concentration of Ag cells in this region. C, cortex; St, striatum; LV, lateral ventricle; Ch P, Choroid plexus; Di, diencephalon; Hyp, hypothalamus; Pit, pituitary; III, third ventricle; MA, mammillary area; MB, mid-brain.


A
Gg
Ag
B


A

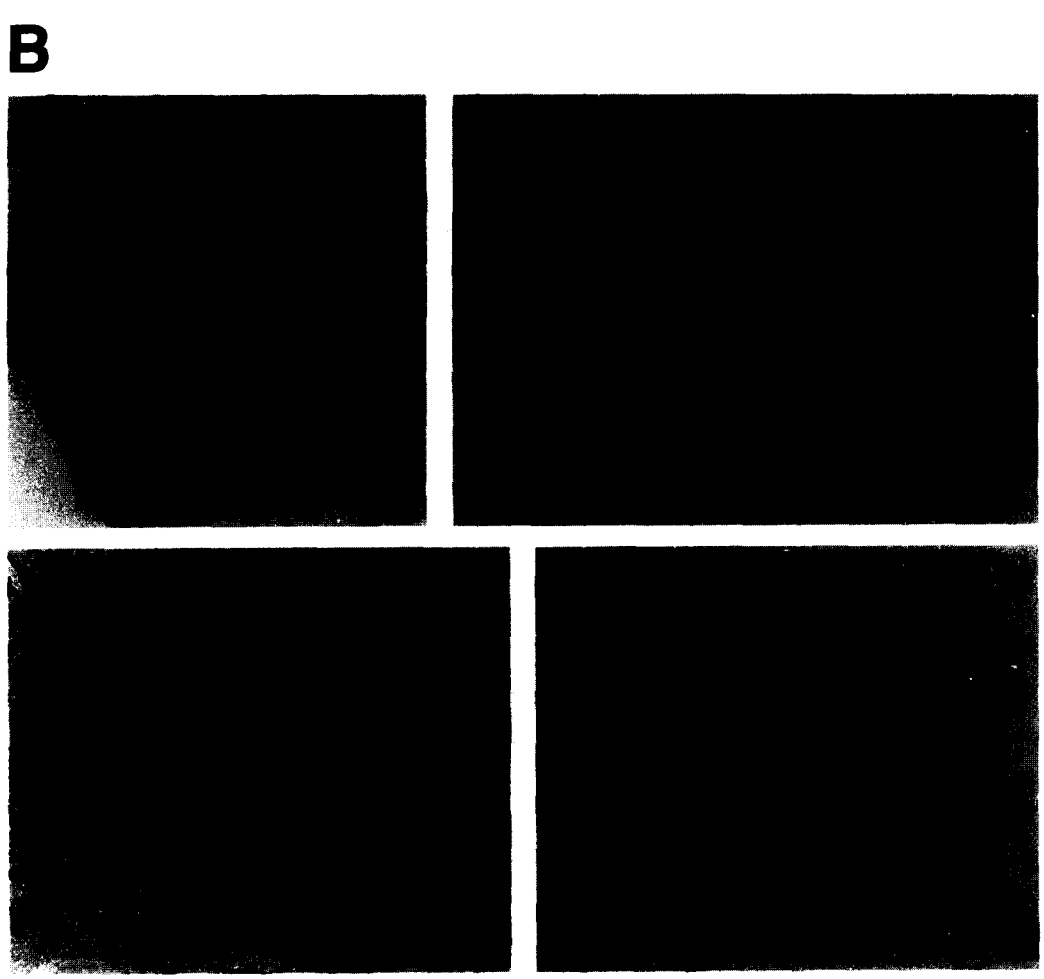

B

Table 1
(a) Body and brain weights for Ag chimeras and control

	Ag chimeras					
	Control	Low	Intermediate 1	Intermediate 2	High	
Body weight (mg)	680 ± 10	800 ± 8	850 ± 6 **	1130 ± 30 **	15207 ± 75 **	df 4, F = 92.57, P < 0.0001
Brain weight (mg)	62 ± 5	87 ± 3	69 ± 1 **	78 ± 1 **	51 ± 2 *	df 4, F = 19.57, P < 0.0001
Ratio-Brain/Body weight	9.1%	10.8%	8.1%	5.6%	3.3%	

(b) Forebrain size and body weight for Gg chimeras and control

	Control	Gg chimera	
Body weight (gm)	36.5 ± 1.8	34.0 ± 0.4	df 12 t = 1.1 P NS
Brain size (mm^2)	43.5 ± 1.9	48.2 ± 1.4	df 12 t = 1.7 P NS
Ratio-Brain/Body	1.19 ± 0.02	1.47 ± 0.06	df 12 t = 4.71 P < 0.005

** Significantly higher than control or low chimera.
* Significantly lower than control or low chimera.

3.2. Distribution of Ag and Gg cells using Lac Z marker

In order to confirm and extend the observations described above, additional experiments were performed in which the experimental cells contained a different in situ marker. The cells were also of different genetic background. This was to ensure that the observations reported above were indeed due to the intrinsic differences in the properties of Ag and Pg/Gg cells and not because of other factors. In these studies *Lac Z* was the in situ marker. This was achieved by preparing the experimental Ag and Gg embryos from the transgenic mouse line, ROSA26, that expresses *Lac Z* ubiquitously. The results were again highly consistent with those found above using the β-globin 83 transgene as a marker. A total of 7 R26Ag → Wt and 12, R26Gg → Wt chimeras were prepared and examined on E12 and E13 by whole mount staining for *Lac Z*, a representative example of which is shown (Fig. 3A). The R26Ag cells were largely absent in the cortex and striatum, but clearly visible in choroid plexus (Fig. 3B–upper left) and were present in large numbers in the hypothalamus (Fig. 3B–right). The R26Gg made a substantial contribution to the cortex and striatum with clear evidence of cortical columns and what appeared to be a patch/matrix organisation of the striatum (Fig. 3B–lower left). The patterning in distribution of R26Ag and R26Gg cells were similar in later embryos (E17) to that observed in the previous experiments using the β-globin transgene marker. Sections through the forebrain revealed a high contribution of Gg cells in the cortex (Fig. 4) while Ag cells were virtually excluded from cortical regions (Fig. 4). Few Gg cells were found in the hypothalamus (Fig. 4) while large numbers of Ag cells were seen to accumulate here (Fig. 4). In comparison with the earlier Ag chimeras (E13), Ag cells become particularly concentrated in certain hypothalamic areas (mammillary bodies, arcuate nucleus, ventro-medial nucleus and proptic area). Control Rosa 26 cells were distributed uniformly throughout these regions (Fig. 4). Chimeras with R26Ag and R26Gg from earlier stages of development showed that the distinct pattern of Ag and Gg cell distribution was obvious in E12 embryos, but it was not pronounced in E10 embryos. Extensive studies with R26Ag embryonic stem cells show that the distribution of these cells in chimeras is very similar when comparing R26AgES → Wt and R26AgICM → Wt chimeras even at the eighth passage of ES in vitro, indicating the stability of the intrinsic properties of these cells.

The distribution of Ag and Pg/Gg cells is apparently not affected by the sex of host or donor cells since the distribution of Ag and Pg/Gg cells was highly consistent in all chimeras. The donor Pg/Gg is always XX (excepting XO) and since the host can be either male or female in the ratio of 1:1, differences in the distribution of these cells would be expected if there was any influence of the host cells based on their sex. No such sex related differences were found. The situation with the Ag cells is more complex since these cells can be XX or XY. We have sexed three Ag → Wt chimeras which showed characteristic distribution of the Ag cells [12]. One phenotypically male chimera was found to be Ag XX → Wt XY. Two other phenotypically female chimeras were Ag XX → Wt XX and Ag XY → Wt.XX, respectively. Finally, we have preliminary evidence from two chimeric embryos produced from embryonic stem cells one of which is Ag.XO

Fig. 4. A: horizontal section of E17 Rosa 26 chimeras through diencephalon and cortex. Left Gg, centre Ag and right control chimeras. C, cortex; T, thalamus; Hyp, hypothalamus. B: cortex and hypothalamus shown at higher magnification for Gg and Ag chimeras. C, cortex; Hip, hippocampus; Ch P, choroid plexus; III, third ventricle; Hyp, hypothalamus; Tg, trigeminal ganglion.

and the second is Gg.XO; both of these produced the characteristic Ag and Pg distribution patterns described here [M.N., S.C.B and M.A.S., unpublished].

3.3. Brain size

Gg chimeric embryos tend to be smaller than controls matched for age and strain, and it is well established that brain size is strongly correlated with body weight. Measurement of brain size from adult Gg chimeras revealed the forebrain to be significantly larger than controls even though body weight was lower (Table 1b). The ratio of brain size to body weight was significantly higher in Gg chimeras. Ag chimeras vary in body weight according to the degree of chimerism. However, when the body weight was highest, brain weight was paradoxically lowest (Table 1a). Indeed the brain weight was reduced below normal levels for age matched controls despite their body weight being more than double that of normal embryos. The ratio of brain weight to body weight was in the opposite direction to Gg chimeras with the largest Ag chimeras having the smallest brains (Table 1).

4. Discussion

We have demonstrated a novel aspect of development in the mouse that suggests differential roles of parental genomes during brain development. The reciprocal distribution of parthenogenetic/gynogenetic (Pg/Gg) and androgenetic (Ag) cells in telencephalic and diencephalic structures respectively, suggests functional differences of parental genomes in the brain. The differences in Ag and Gg/Pg cells in chimeras may arise as a result of the cumulative effects of imprinted genes whose expression is dependent on their parental origin. The cell autonomous phenotypic properties of Ag and Pg/Gg cells could be due to loss and/or gain of function for imprinted genes in the uniparental cells. For instance, the Pg/Gg cells will be affected by loss of function of imprinted genes expressed from the paternal genome, and an excess gene dosage for imprinted genes from the maternal genome. The uniparental cells also produced effects on the overall growth of the brain; Pg cells enhanced growth of the forebrain while the presence of Ag cells resulted in growth retardation. Therefore imprinted genes may also influence cell–cell interactions and include short range paracrine factors. These functional differences between parental genomes could provide a mechanism for the exquisite control of the proliferation and differentiation of neuronal patterning that is perhaps unique to the development of the mammalian forebrain. Moreover, the radial deployment of *lac Z* staining neurones in Pg/Gg chimeras may illustrate their clonal origin and the pattern of their migration to the cortex. These data lend support to the radial unit hypothesis of Rakic [27] by showing that cells in a given radial column are clonally related.

Previous studies have shown that androgenetic cells enhance growth of the body, which is opposite to the effects of these cells on the brain. In the case of chimeras with Ag cells, it might have been anticipated that the greater the contribution of Ag cells, the larger the embryo and therefore greater the number of Ag cells accumulating in the brain. However, even in chimeras with the highest number of Ag cells, there were very few cells in the forebrain and most of these cells always accumulated in the same area, namely the basal forebrain from the hypothalamus to the pre-optic area. In embryos at an earlier stage (day 10–12), Ag cells are found throughout the brain [4]. This suggests that Ag cells in the presumptive forebrain tissue (cortex, striatum, hippocampus) fail to produce a lineage and are subsequently eliminated. The precise mechanism for the elimination of these cells remains to be determined. The Pg/Gg cells made the highest contribution to the cortex with some growth enhancement. Despite this, Pg/Gg cells were barely detectable in the hypothalamus where Ag cells were present. The distribution of Ag and Pg/Gg cells is not, therefore, merely a consequence of the degree of chimerism. This has also been shown by estimating their contribution to specific parts of the brain with respect to the brain stem, an area where no differences in the contribution of the donor cells were found. These observations argue strongly for a precise role of the parental genomes in brain development as a result of the differential expression of the imprinted genes.

From genetic evidence it is clear that there are important imprinted genes that remain to be identified especially on Ch2, 6 and 12 [5]. Based on the evidence from this study, some of these genes are likely to have a significant role in brain development. As we show here, both the spatial distribution and the overall growth of the brain are linked as far as imprinted genes in the brain are concerned. An obvious way that such a process may be regulated is through the control of proliferation of neuronal progenitors, and/or the terminal differentiation and/or apoptosis of neuronal cells. This type of control fits with the reciprocal effects observed with the parental genomes in non-neuronal tissues. Subtle alterations in the temporal regulation of expression of maternally and paternally imprinted genes could in this way have a major effect on brain development. It is possible that imprinted genes on the maternal genome are concerned with regulating the proliferation of the neuronal stem cells. The genes on the paternal genome would then act to counterbalance this growth through accelerating differentiation of neuronal progenitors, and by apoptosis. The rostro-caudal gradient seen in the distribution of Pg/Gg cells is puzzling but it may indicate spatial regulation of cortical growth and or differentiation.

A paradigm for how imprinted genes can regulate development comes from studies on *Igf2* and *Igf2r* [9]. *Igf2* is expressed from the paternal genome while the *Igf2r* is expressed from the maternal genome only [2]. These two genes are apparently involved in the control of embryonic

growth. *Igf2* is an embryonal mitogen and its gain and loss of function can cause excess and deficiency of growth, respectively [8,10]. The role of *Igf2r* is more complex since this gene is not involved in signal transduction for *Igf2*. This role is fulfilled by *Igf1r*, which does not behave as an imprinted gene since both parental copies of this gene are expressed. *Igf2r* may in fact restrict embryonic growth by removing excess IGF2 [21]. However, *Igf2* and *Igf2r* are not the imprinted genes controlling brain development, as they are not widely expressed here. What is more important, brain growth is positively affected by the maternal genome and not the paternal genome as described above. Hence, although maternally and paternally imprinted genes appear to influence both brain and body growth by a reciprocal action, this reciprocity is different in these somatic tissues. Brain growth is enhanced by maternally expressed genes while body growth is enhanced by paternally expressed genes.

A number of studies and certain disorders in the human show why it is essential to maintain complementary roles of parental genomes during development. Chimeras with Pg/Gg cells are growth retarded with a loss of Pg/Gg cells from many of the mesodermal tissues. In chimeras with Ag cells, there is abnormal proliferation of the mesodermal tissues resulting in severe skeletal and other abnormalities as well as limb deformities [4,20]. These effects are the result of excess or deficiency of gene dosage for imprinted genes. Such abnormal gene dosage also results in human disease, such as growth abnormalities in Beckwith Wiedemann [32] syndrome and Wilm's tumour [25], as well as behavioural disorders seen in the Prader Willi and Angelmanns syndromes. The gene *SNRPN* on the syntenic human Ch 15q11-q13 contribute to the behavioural disorder, Prader-Willi, that is associated with maternal 15q11–13 deletions [26,31] while 2NF 127, a gene incoding a potential transcription factor is linked to *SNRPN* and is only paternally expressed [15]. It is noteworthy that Ag cells are seen predominantly in the medial pre-optic area and hypothalamus, regions of the brain concerned with neuroendocrine function and primary motivated behaviour, including feeding and sexual behaviour that is disturbed in Prader–Willi syndrome. A magnetic resonance imaging study of the cortical morphology showed that the Sylvian fissures were significantly anomalous in Angelman patients (due to maternal 15q11–13 deletion). This may be significant since the Angelman children are severely mentally retarded, with speech and movement disorders [18] a finding not inconsistent with the distribution of Pg cells.

References

[1] Allen N.D., Keverne, E.B. and Surani M.A., A position-dependent transgene reveals patterns of gene expression in the developing brain, *Dev. Brain Res.*, 55 (1990) 181–190.

[2] Barlow, D.P., Stoger, R., Herrman, B.G., Saito, K. and Schweifer, N., The mouse insulin-like growth factory type-2 receptor is imprinted and closely linked to the Tme locus, *Nature*, 349 (1991) 84-87.

[3] Barton, S.C., Adams, C.A., Norris, M.L. and Surani, M.A., Nuclear transplantation in fertilized and parthenogenetically activated eggs. In M. Monk (Ed.), *Mammalian Development. A Practical Approach*, IRL Press, Oxford, 1987, pp. 235–253.

[4] Barton, S.C., Ferguson-Smith, A.C., Fundele, R. and Surani, M.A., Influence of paternally imprinted genes on development, *Development*, 113 (1991) 679–687.

[5] Cattanach, B.M. and Beechey, C.V., Autosomal and X-chromosome imprinting, *Development, Suppl.*, (1990) 63–72.

[6] Cattanach, B.M. and Kirk, M., Differential activity of maternally and paternally derived chromosome regions in mice, *Nature*, 315 (1985) 495–498.

[7] Clarke, H.J., Varmuza, S., Prideaux, V.R. and Rossant, J., The developmental potential of parthenogenetically-derived cells in chimeric mouse embryos: implications for action of imprinted genes, *Development*, 104 (1988) 175–182.

[8] DeChiara, T.M., Efstratiadis, A. and Robertson, E.J., A growth-deficient phenotype in heterozygous mice carring an insulin-like growth factor II gene disrupted by targeting, *Nature*, 344 (1990) 78–80.

[9] Efstratiadis, A., Parental imprinting of autosomal mammalian genes, *Curr. Opin. Genet. Dev.*, 4 (1994) 265–280.

[10] Ferguson-Smith, A.C., Cattancach, B.M., Barton, S.C., Beechey, C.V. and Surani, M.A., Embryological and molecular investigations of paternal imprinting on mouse chromosome 7, *Nature*, 351 (1991) 667–670.

[11] Friedrich, G. and Soriano, P., Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice, *Genes and Dev.*, 5 (1991) 1513–1523.

[12] Fundele, R.H., Barton, S.C., Christ, B., Krause, R. and Surani, M.A., Distribution of androgenetic cells in foetal mouse chimeras, *Roux's Arch Dev. Biol.*, 204 (1995) 484–493.

[13] Fundele, R.H., Norris, M.L., Barton, S.C., Reik, W. and Surani, M.A., Systematic elimination of parthenogenetic cells in mouse chimeras, *Development*, 106 (1989) 29–35.

[14] Fundele, R.H. and Surani, M.A., Experimental embryological analysis of genetic imprinting in mouse development, *Dev. Genet.*, 15 (1994) 515–522.

[15] Glenn, C.C., Nicholls, R.D., Robinson, W.P., Saitoh, S., Niikawa, N., Schinzel, A., Horsthemke, B. and Driscoll, D.J., Modification of 15q11–q13 DNA methylation imprints in unique Angelman and Prader–Willi patients, *Hum. Mol. Genet.*, 2 (1993) 1377–1382.

[16] Guillemot, F., Caspary, T., Tilghman, S., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Anderson, D.J., Loyner, A.L., Rossant, J. and Nagy A., Genomic imprinting of *Mash2*, a mouse gene required for trophoblast development, *Nature Genet.*, 9 (1995) 235–241.

[17] Jagerbauer, E., Fraser, A., Herbst, E.W., Kothary, R. and Fundele, R., Parthenogenetic stem cells in postnatal mouse chimeras, *Development*, 116 (1992) 95–102.

[18] Lenoard, C.M., Williams, C.A., Nicholls, R.D., Agee, O.F., Voeller, K.K., Honeyman, J.C. and Staab, E.V., Angelman and Prader–Willi syndrome: a magnetic resonance imaging study of differences in cerebral structure, *Am. J. Med. Genet.*, 46 (1993) 26–33.

[19] Lo, C.W., Localization of low abundance DNA sequences in tissue sections by in situ hybridization, *J. Cell Sci.*, 81 (1986) 143–162.

[20] Mann, J.R., Gadi, I., Harbison, M.L. Abbondanzo, S.J. and Stewart, C.L., Androgenetic mouse embryonic stem cells are pluripotent and cause skeletal defects in chimeras: implications for genetic imprinting, *Cell*, 62 (1990) 251–260.

[21] Moore, T. and Haig, D., Genomic imprinting in mammalian development: aparental tug-of-war, *Trend Genet.*, 7 (1991) 45–49.

[22] Nagy, A., Sass, M. and Markkula, M., Systematic non-uniform distribution of parthenogenetic cells in adult mouse chimeras *Development*, 106 (1989) 321–324.

[23] Nicholls, R.D., Genomic imprinting and candidate genes in the Prader–Willi and Angelman syndromes, *Curr. Opin. Genet. Dev.*, 3 (1993) 445–56.

[24] Nicholls, R.D., Knoll, J.H.M., Butler, M.G., Karam, S. and Lalande, M., Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader–Willi syndrome, *Nature*, 342 (1989) 281–285.

[25] Ogawa, I.O., Eccles, M.R. Szeto, J., McNoe, L.A., Yun, K., Maw, M.A., Smith, P.J. and Reeve, A.E., Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour, *Nature*, 362 (1993) 749–751.

[26] Oszcelik, T., Leff, S., Robinson, W., Donlon, T., Lalande, M., Sanjines, E., Schinzel, A. and Francke, U., Small nuclear ribonucleoprotein polypeptide N *(SNRPN)*, an expressed gene in the Prader–Willi syndrome critical region, *Nature Genet.*, 2 (1992) 265–269.

[27] Rakic, P., A small step for the cell, a giant leap for mankind: a hypothesis of neorcortical expansion during evolution, *Trends Neurosci.*, 18 (1995) 383–388.

[28] Solter, D., Differential imprinting and expression of maternal and paternal genomes, *Annu. Rev. Genet.*, 22 (1988) 127–146.

[29] Surani, M.A., Barton, S.C. and Norris M.L., Nuclear transplantation in the mouse: heritable differences between parental genomes after activation of the embryonic genome, *Cell*, 45 (1986) 127–136.

[30] Surani, M.A., Allen, N.D., Barton, S.C., Fundele, R., Howlett, S.K., Norris, M.L. and Reik, W., Developmental consequences of imprinting of parental chromosomes by DNA methylation, *Phil. Trans. Roy. Soc. (Lond.)*, 326 (1990) 313–327.

[31] Wagstaff, J., Knoll, J.H.M., Glatt, K.A., Shugart, Y.Y., Sommer, A. and Lalande, M., Maternal but not paternal transmission of 15q11–13-linked nondeletion Angelman syndrome leads to phenotypic expression, *Nature Gen.*, 1 (1992) 291–294.

[32] Weksberg, R., Shen, D.R., Fei Y.L., Song, W.L. and Squire, J., Insulin-like growth Factor 2 imprinting in Beckwith–Weidemann syndrome, *Nature Gen.*, 5 (1993) 143–150.