Acta Genet Med Gemellol 45: 111-125 (1996)

Imprinting and Transgenerational Modulation of Gene Expression; Human Growth as a Model

M. Pembrey

Institute of Child Health, University of London, London, United Kingdom

Abstract. It is proposed that transgenerational modulation of gene expression might be possible, if the metabolic response of the parent to some physiological or social stress modified imprint setting. Transcription regulators could theoretically mediate this process. The nature of imprinted genes poised, as it were, between a transcriptionally active and silent state, makes them good candidates for incorporation into the evolution of transgenerational adaption systems where coordinated changes in gene expression over the generations is a selective advantage. The coordination of human fetal (head) growth with the existing size of the mother's pelvis is suggested as just such a circumstance. The reduced birth weight of Dutch babies where their grandmothers suffered acute starvation in mid pregnancy, supports the notion of transgenerational adaption to nutrition, as does the secular change (increase) in child growth over the last century. The recent indication that there may be functional polymorphism in the imprinting of the human IGF2 and IGF2R genes suggests these ideas could be explored using association studies at the population and individual level.

Key words: Human growth, Imprinting, Trasgenerational effects.

INTRODUCTION

Ten years of research into the genetics of Angelman syndrome (AS) and personal acquaintance with many people affected by the remarkable AS phenotype have proved highly provocative to an inveterate speculator as I am. Clinical genetics is, of course, primarily concerned with helping individual families cope with their genetic disadvantage, but many of us feel keenly the dual responsibility to "care for" and "learn from" the patients we see. Recent advances in human genetics encourage both a developmental and an evolutionary perspective to one's deliberations, not least when a mutation causes a striking abnormality of behaviour. What is the role of the AS gene in normal human development and how critical was this function to recent human evolution? Individuals

with AS have an absence of speech that seems out of proportion to their mental retardation and have a remarkably low threshold for excited, handflapping laughter. Speech and laughter, of course, are at the very heart of what is distinctive about human behaviour. People with the Prader-Willi syndrome have a striking lack of satiation which leads to marked overeating if they are not controlled.

It could be argued that a highly developed and refined sense of satiation was essential for the move from hunter gatherer behaviour to an early human agricultural society where the harvest would have to be stored for future consumption. When it became clear that both these disorders involved imprinted genes it was natural to start wondering if imprinting *per se* had played a part in the apparent rapidity of recent human evolution with respect to social behaviour.

Evolution of behaviour is, in essence, an evolution of *responsiveness*. There are costs in not being able to respond appropriately; the more suited the average response is to survival, reproduction and parenting, the greater the selective advantage. The recurring problem, of course, is that the various environmental and social pressures keep changing. These challenges are not only changes during an individual's lifetime, but on a longer timescale – "sea changes" in the climate, food supply and social organisation over a few, or moderate number, of generations. If some biological mechanism (other than truly learned behaviour) existed that could result in a person's physiological response to these environmental pressures influencing gene expression in their *offspring*, a form of transgenerational adaption to slowly changing circumstances could evolve. This general idea is not new, being addressed by Jablonka and Lamb (1989), for example, in their model of inheritance of acquired epigenetic variations, but there are now opportunities to more directly test some of the ideas. It is likely that this form of transgenerational adaption would need to be reversible (changing back if the inducing circumstances reverted) if it is to be advantageous and therefore maintained. Is imprinting such a biological mechanism, and has it indeed become involved in transgenerational adaption?

In this paper, I wish to briefly:

a) distinguish between transgenerational modulation of gene expression itself (the term I will use for the reversible adaptive process indicated above) and the process by which such an adaptive system might have evolved, and

b) explore the idea using human growth as an example.

Human behaviour is, of course, not the only responsive system to have evolved, and human growth represents a simpler example to explore. Not only is the genetics of behaviour too complicated and poorly understood, but growth can be relatively easily measured and some transgenerational data exist.

Transgenerational modulation of gene expression

The modulation proposed concerns the (level of) transcription of imprinted genes during development and thereafter. In the simplest scenario, the developmental and physiological impact of the gene product on the phenotype would be expected to be dose dependent, with the dose ranging from biallelic (100%) down to at least monoallelic (50%).

There could be circumstances where even the remaining active allele becomes silenced altogether, or all transcription stops earlier during development in all or selected tissues. The situation in which such an adaptive system is likely to have evolved is where there is a survival cost if a balance or some accord between successive generations is not maintained. There are various possible scenarios involving neuroendocrine controlled behaviour, but the most extreme example is likely to be where a member of one generation resides for a period within a member of the previous generation!

There has been much discussion on the possible role of imprinting in maintaining a balance between the demands of the growing fetus and the interests of the mother (and father). One hypothesis that has received a lot of attention is the parental tug-of-war hypothesis of Haig and colleagues (Moore and Haig 1991). It predicts that genes where the maternal allele is silent and the paternal allele active *promote* fetal growth (putting the mother's nutritional stores at risk), and genes imprinted the other way around, where only the maternal allele is expressed, *restrict* fetal growth (putting the survival of the father's offspring at risk). Their hypothesis was built on the early observation that the insulin-like growth factor 2 (Igf2) and the Igf2 receptor (Igf2r) are both imprinted in the mouse, but in opposite directions. Only the paternal Igf2 allele is active during prenatal life and it increases fetal growth (DeChiara et al 1991).

In contrast, Igf2r is only expressed from the maternal allele (Barlow et al 1991). This hypothesis has not, however, been developed much as an *existing* system of transgenerational adaption (to maternal starvation over several generations for example) as opposed to an evolutionary mechanism by which these oppositely imprinted genes might be maintained in the murine population. As discussed below the Haig model is not particularly appropriate for the human situation where a key intergenerational balance must be struck between the growth of the fetal head and the existing size of the mother's pelvis.

The mechanism of the transgenerational adaption I have in mind is a specific metabolic or hormonal response of the parent (to their nutritional state for example) directly modifying the setting of the gametic imprint of one or more genes determining fetal growth. Whether "marking" of the parent's imprinted genes involves DNA methylation or a change in chromatin configuration, it remains theoretically possible that changes in the level of transcriptional regulators in the germline cells, gametes or zygote could modify the setting of the imprint. In somatic cells, at least, transcriptional regulators can induce sequence specific demethylation (Lichtenstein et al 1994) or modify chromatin structure (Lewin 1994).

Numerous examples of transgenerational effects of drug or hormonal treatments in mammals have been observed, and have been critically reviewed by Campbell and Perkins (1988). What is remarkable is that in many experiments the physiological and metabolic disturbances caused by these treatments persisted for three and sometimes more generations. The crossing of treated males to normal females can give offspring with the altered phenotype. No really satisfactory mechanism is proposed, but direct modification of imprints is clearly a possibility. Marked effects were observed in chemically or surgically induced diabetes in rats. It is noteworthy that the insulin loci 1 and 2 show evidence of imprinting with only the paternal allele expressed in the mouse yolk sac (Giddings et al 1994) and paternal uniparental disomy of chromosome 6 causes transient neonatal diabetes in humans (Temple et al 1995). Other experimental transgenera-

tional effects reviewed by Campbell and Perkins (1988) concerned the parathyroid and thyroid hormones, and here it is noteworthy that the hormone resistance of Albright's hereditary osteodystrophy shows parent-of-origin effects highly suggestive of tissue-specific imprinting (Wilson et al 1995).

Thus in summary, circumstantial evidence suggests the existence of some systems of transgenerational modulation of gene expression, in which the question of the involvement of imprinted genes should soon become directly testable. Repeated appropriate physiological or metabolic stress over several generations is predicted to result in shifts towards, or away, from monoallelic expression. This type of shifting if indeed it is demonstrable would be expected to be reversible. It would represent the functioning of a transgenerational adaptive system that had already evolved. Is it, however, likely that such a system could have evolved?

Evolution of transgenerational adaptive systems dependent on imprinting

The key considerations with regard to our deliberations are:

a) whether the characteristics of the imprinted loci that have evolved might permit modification of the imprint-setting process by the molecular changes that accompany a parental physiological response to environmental or social stress, and

b) what situations, if any, have such large survival costs associated with intergenerational discordance of response that selection for any potential coordinating system that exists would be inevitable.

Neumann et al (1995) have recently discussed the molecular characteristics of imprinted genes drawing attention to the presence of direct DNA repeats that are of distinct type but unique to each imprinted gene. They suggest that transcriptionally induced supercoiling stress, in repeat containing regions, might induce formation of non-B DNA and thereby influence transcriptional progression. These configurational aspects together with the observed mono-parental methylation that probably makes the difference between transcription or not, provide two routes by which modification of the imprint setting in response to the parental metabolic state could occur – methylation changes or chromatin structure. As we saw above, both can be modified by transcriptional regulators in somatic cells, so it is theoretically possible that a similar mechanism could operate in the germ cells. Work to date in characterising imprinted genes suggests there are at least two ways in which monoallelic expression can be achieved. One is methylation of specific promotor sequences that then prevent transcription – the imprinting of the mouse Xist gene provides a good example (Ariel et al 1995, Zuccotti and Monk 1995). Another mechanism is methylation of the binding site of a repressor molecule that by preventing the repressor from binding maintains the imprinted allele in a transcriptionally active state, whilst the other allele is silenced. Here the imprinting of a sequence in intron 2 of the mouse Igf2r gene provides an example (Stoger et al 1993, Denise Barlow personal communication). The "availability" of different mechanisms for achieving monoallelic expression provides evolutionary flexibility. In one sense imprinted genes are, by defini-

tion, so poised between being transcriptionally active and silent that just the sex difference in gametogenesis can (incidentally) make all the difference; an ideal "substrate" on which transgenerational adaption might evolve. Once established and whilst such an adaptive process remains a selective advantage, the imprinting, the "poise", of the locus will remain.

The basic evolutionary "drive" in all this is the high rate of change of non-coding/repetitive DNA, including the movement of transposable elements, all of which are likely to lead to the transcriptional silencing of nearby genes. This tendency is offset by any natural selection against the altered phenotype so produced. Genomic imprinting as we observe it now could in fact just be a phase in a slow drift into transcriptional silence. In the absence of exhaustive, transgenerational studies of allelic expression, parent-of-origin effects would be the only "window" we have on this process (Fig. 1). Figure 1 deliberately omits an indication of the parental origin of the active allele in the "window", because this is not the point of interest, only that the transcriptional potential of the gene is so susceptible to influences that just the differences between spermatogenesis and oogenesis can switch it on or off. If genes can "drift" to become silent no matter which parent transmits them, there will be a silent state before they accumulate disabling mutations. I have called these "Sleeping Beauty" genes for it is possible that changing circumstances over several generations might recruit them back into the active genome. One way in which this drift into transcriptional silence might be circumvented (under the influence of selective pressure of course) is by adoption of a passing promotor sequence. Sleeping Beauty's prince may arrive in the form of a transposon. During fetal development only the paternal allele of Igf2 is expressed in both mouse and human, but after birth there is a species difference. The expression of Igf2 declines altogether in the mouse, whilst in the human adult liver and chondrocytes expression is maintained and is biallelic (Vu and Hoffman 1994). The explanation is in different promotor usage. In the adult human, control of IGF2 expression switches from "fetal" promotors P2-P4 shared with the mouse to a Pl promotor some 20kb upstream of the gene, that the mouse does not have.

What circumstances might be compelling enough to lead to evolutionary selection for a system of transgenerational adaption? This brings us back to the regulation of fetal growth. There is a major difficulty with the Moore and Haig parental tug-of-war hypothesis where humans are concerned, because the hypothesis works best when each litter is sired by a different male. It is also assumed a high energy/nutritional cost to the mother during pregnancy, the cost being proportional to the growth and number of fetuses. Fetal growth in mice and humans is very different, with different constraints.

Constraints and influences on human fetal growth

You do not have to be an obstetrician to appreciate that the size of the mother's pelvis is the main constraint on human fetal growth. The human birth process courts natural selection. Human evolution, so dominated by successive increases in brain size and the associated increases in cognitive function, had also to take account of delivery through a birth canal the size of which was largely determined a generation before.

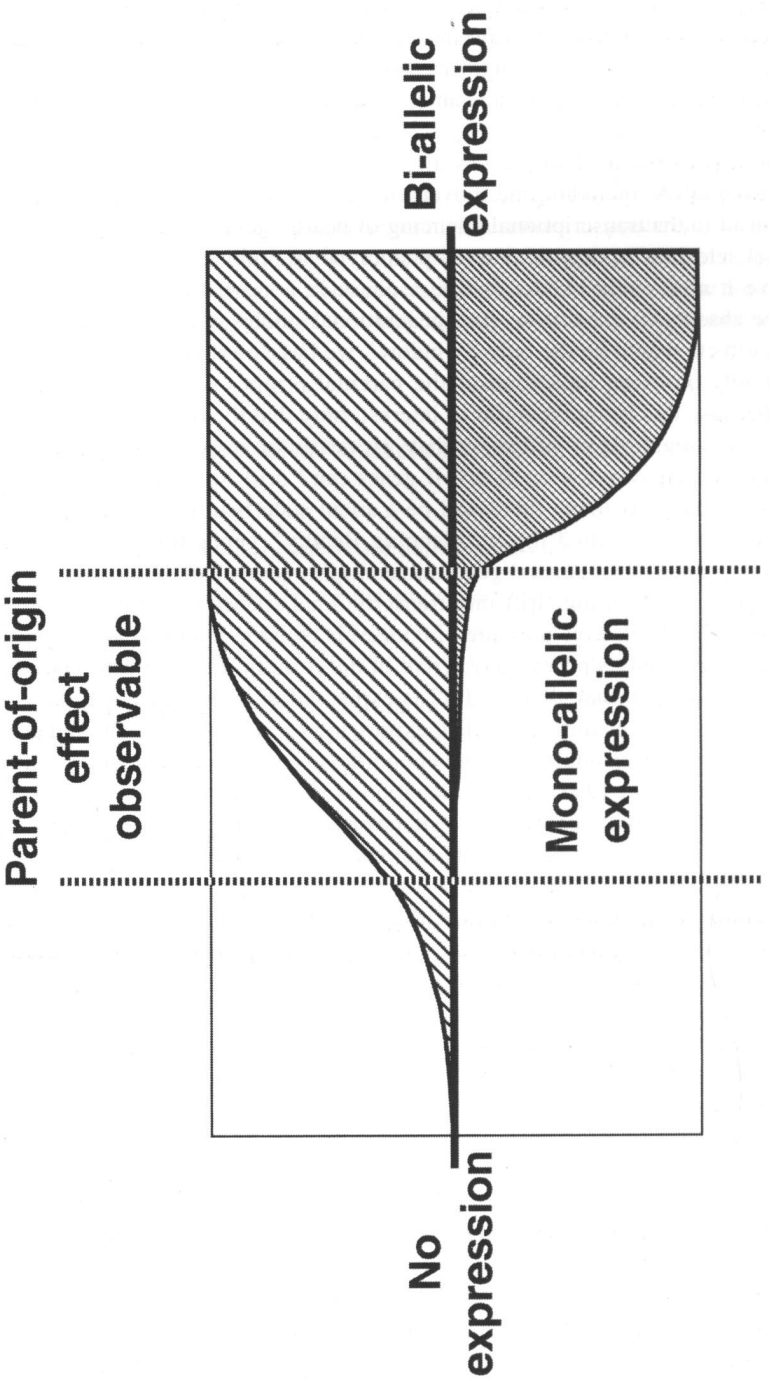

Fig. 1 - Genomic imprinting portrayed as a phase or "window" on an evolutionary drift towards complete transcriptional silencing. This is likely to be driven by changes in non-coding DNA, but could be due to transgenerational adaption and the shift could be in either direction (see text).

https://doi.org/10.1017/S0001566000001197 Published online by Cambridge University Press

The evolution of human reproduction would appear to have provided the circumstances in which some form of intergenerational co-ordination in growth would be a selective advantage.

In their review of reproduction in Gambian women "Reproduction against the odds", Prentice and Prentice (1988) address some of the ways in which human reproduction is different from the general mammalian scenario upon which the Moore and Haig hypothesis depends. In contrast to experimental laboratory animals or farm animals, the growth of the human fetus is relatively unaffected by marked variation in the mother's food intake during pregnancy. Pregnant women receiving 700 kilocalories a day, equivalent to only a third of the recommended daily intake, have babies with birthweights down by just 10% or so. This protection from maternal semi-starvation is due in part to large maternal fat stores (in contrast to most other primates living in the wild) and the long gestation. The very slow growth of the fetus spreads the metabolic cost to the mother over a long period. The reverse protection of the fetus is also evident; maternal over-eating does not cause large babies that will jam in the birth canal. These buffering effects on fetal growth with respect to maternal nutrition should not be taken to mean that there are no metabolic changes in the fetus, only that regulatory systems exist that maintain fetal growth within tight limits. It is possible the biallelic expression of the IGF2R gene in humans, in contrast to the mouse, (Kalscheuer et al 1993) is part of this tight control on human fetal growth.

This relative independence of fetal growth from the mother's nutrition is in marked contrast to postnatal growth and maturation, where adult height is considerably influenced by the food intake (see secular trends in height discussed later). These two features of human growth raise the question of the risks posed by the poorly fed and stunted mother having a pelvis that is too small to deliver her normal-sized baby. If transgenerational adaption had indeed evolved along the lines indicated earlier, then one might expect semi-starvation over several generations to gradually result in smaller babies, thereby restoring the balance between fetal and maternal pelvic size. However, if this was observed it would be difficult to disentangle ongoing direct metabolic effects from true transgenerational modulation of gene expression by metabolic modification of the imprint-setting process or some other mechanism. What is needed is a transgenerational study of a single episode of starvation, such as the, now classic, studies of the Dutch famine at the end of the Second World War.

A blockade by the occupying forces caused precisely documented mass malnutrition between October 1944 and May 1945 in cities of the western Netherlands. Impaired nutrition of pregnant women caused a sharp drop in their own pregnancy weights and was associated with a modest drop in birth weight of their full term babies of 6-10% of pre-famine levels (Smith 1947, Sindram 1953). Lumley (1992) has now reported a follow up of the babies born to women who were themselves exposed *in utero* to the famine, in order to assess the impact on the grandchildren of the women who suffered the wartime starvation. Figure 2 summarises the key findings. There is sufficiently precise data on the timing of the pregnancy in relation to the famine period to present the results by stage of pregnancy. The children born to mothers who as fetuses were exposed to the famine in the mid 6 months of gestation showed a 6.2% drop in birthweight compared to pre-famine controls despite a generation of good nutrition. This could be a direct effect on the developing uterus that somehow compromises its later response to

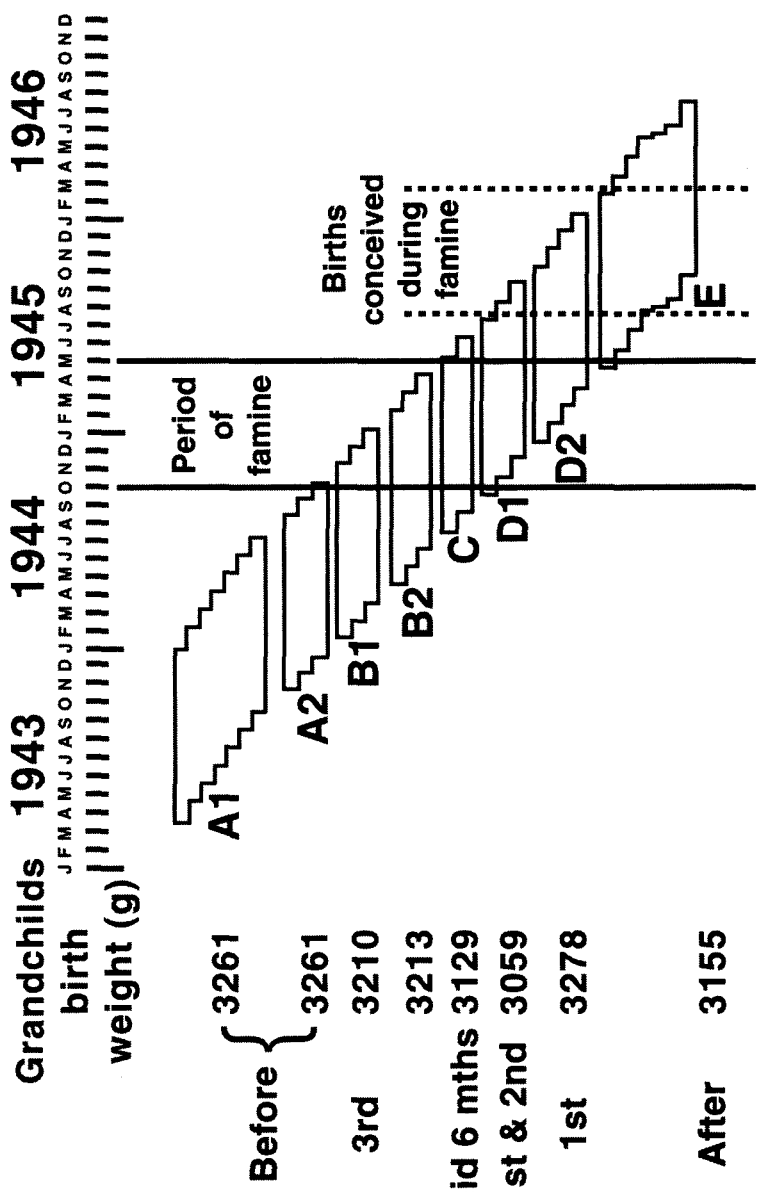

Fig. 2 - Classes (A1-E) of pregnancies based on their timing in relation to the period of famine in the West Netherlands at the end of the Second World War. The column on the left gives the average (n = 69 to 347) birthweight of children born to women who themselves were fetuses between 1943 and 1946. There is a reduction in birthweight in the grandchildren of women who suffered starvation in mid pregnancy (Class D1). Adapted from Lumey (1992).

pregnancy with a knock on effects on the growth of the baby, or it could reflect transgenerational modulation of the imprinted genes that influence fetal growth.

If indeed the Dutch starvation data are a reflection of transgenerational adaption, then what about changes in the other direction? As emphasised above the adaption would have to work to protect the fetus from any small response it might make to increasing maternal nutrition, by ensuring that the mother and her pelvis are larger. The first effect should be on adult female height (which correlates with pelvic size). Does improving nutrition over several generations lead to taller people? In many populations it seems to.

Secular changes in height

Over the last 100 years in industrialised countries, and recently in some developing ones, children have been getting larger and growing to maturity more rapidly. This is known as the secular trend or, more correctly, the secular change. A recent authoritative review of worldwide variation in human growth (Eveleth and Tanner 1990) summarises the factors that are believed to be responsible for the secular changes as – "improved nutrition, control of infectious disease through immunizations and sanitation, reduced family size, more widespread health and medical care, and population mobility. It is clearly complex and not fully understood".

If one wished to find a way into a study of the underlying mechanisms of this secular change, including the role of imprinting, exceptions to the general tendency could be useful. If shifts towards biallelic expression or complete silencing of imprinted genes were to underlie these secular changes, then comparison of populations with and without secular changes (despite increased nutrition) might be revealing. The Mexican-Americans in Texas are an example where no increase in height between 1930 and 1978 has been observed (Malina and Zavaleta 1980), although there are increases in weight.

In most developed countries the trend of increasing height seems to have slowed down or stopped during the last 10 years, but there are exceptions. A system of transgenerational adaption involving modification of imprint setting is more likely to "overshoot" by a generation or so than a direct effect of diet on the growth of children, but even so the continuing secular change in Holland is remarkable (van Wieringen 1986). Figure 3 shows the height of Dutch conscripts from 1865 to 1980. Bock and Sykes (1989) have found no levelling off in the Fels Longitudinal Research Study in Yellow Springs, Ohio, and Eveleth and Tanner (1990) conclude "The Dutch and American data make simple explanations in terms of overall calories in childhood suspect; something a bit more specific seems required".

Controlling transgenerational adaption

What I have been proposing is an intergenerational "feedback" or rather a "feedforward" control loop that links grandparental nutrition with fetal growth. Such wild speculation is dangerous, and so are feedback loops! They can spiral out of control. If indeed the epigenetic phenomenon of imprinting is involved then there is also the heretical possibility that adaption over many generations could lead to the epigenetically

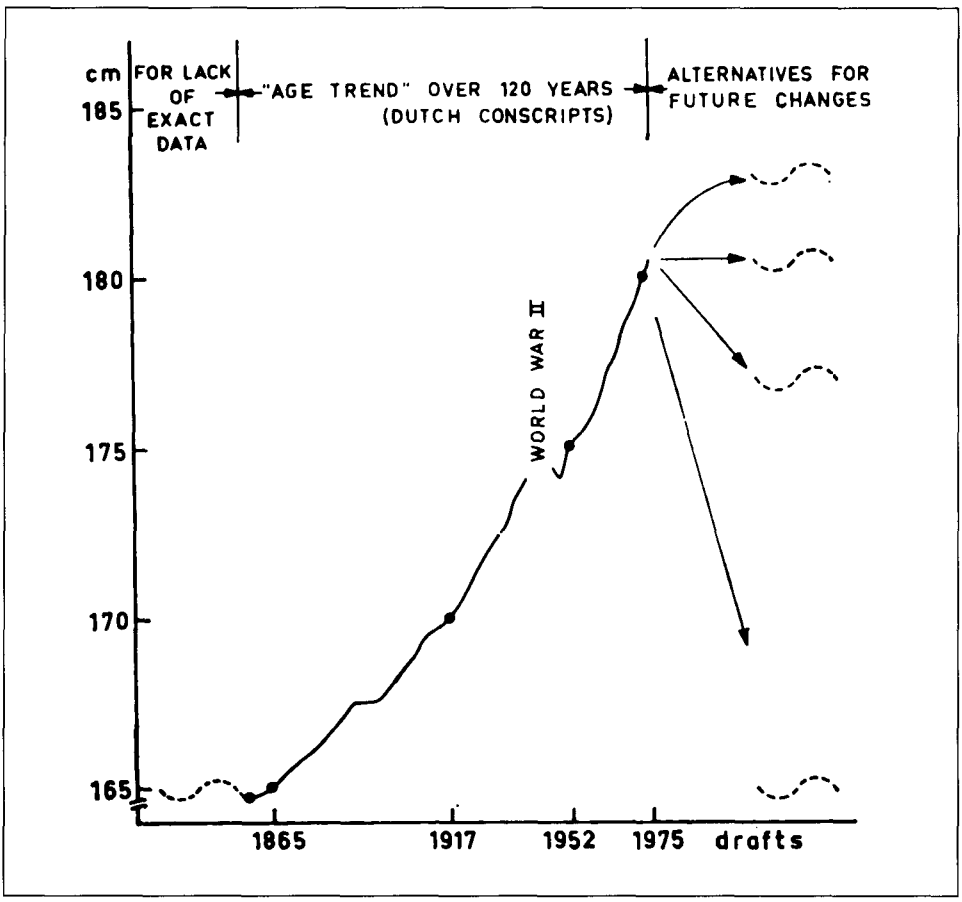

Fig. 3 - The height of Dutch conscripts from 1865 to 1980 from van Wieringen (1986).

altered gene expression being fixed in the genome by mutation, especially if it involves methylated DNA which is mutagenic. Such speculation is not new (see discussion in Jablonka and Lamb 1989, for example). Could the Patagonian giants (Fig. 4) be an example! There are many myths about races of giants, but the Patagonians are better documented than most (which isn't saying much). Gould and Pyle (1896) are dismissive (uncritically perhaps) as the following quote shows; "In the olden times there were extraordinary stories of the giants who lived in Patagonia. Some say that Magellan gave the name to this country because its inhabitants measured 5 cubits. The naturalist Turner says that on the river Plata near the Brazilian coast he saw naked savages 12 feet high; and in his description of America, Thévenot confirms this by saying that on the coast of Africa he saw on a boat the skeleton of an American giant who had died in 1559, and who was 11 feet 5 inches in height. He claims to have measured the bones himself. He says that the bones of the leg measured 3 feet 4 inches, and the skull was 3 feet and 1 inch, just about the size of the skull of Borghini, who, however, was only of ordinary

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height. In his account of a voyage to the Straits of Magellan, Jacob Lemaire says that on December 17, 1615, he found at Port Desire several graves covered with stones, and beneath the stones were skeletons of men which measured between 10 and 11 feet. The ancient idea of the Spaniards was that the men of Patagonia were so tall that the Spanish soldiers could pass under their arms held out straight; yet we know that the Patagonians exhibit no exaggeration of height – in fact, some of the inhabitants about Terra del Fuego are rather diminutive. This superstition of the voyagers was not limited to America; there were accounts of men in the neighborhood of the Peak of Teneriffe who had 80 teeth in their head and bodies 15 feet in height".

CONCLUSION

Imprinted genes are clearly involved in growth, and there are numerous aspects of human growth, some of them outlined in this paper, that need explaining. It is important to emphasise that I have not been concerned with the parental-sex-of-origin aspect of imprinting, but the fact that imprinted genes are structurally "poised" between the transcriptionally active and silent state. As far as the proposed transgenerational adaption is concerned it may not matter too much whether it is the paternal or maternal gene that is inactive in the mid, monoallelic, expression state. It is sufficient that the sex differences in gametogenesis can modify the gene's transcriptional potential so as to keep expression in the offspring poised around the 50%, monoallelic mark. This is not to deny that there could be evolutionary circumstances, such as placentation, where the sex of the parent transmitting the active allele is important, but this is not a feature of the model I have presented.

In taking these ideas forward, it seems sensible to exploit any imprinting polymorphisms that can be discovered and look for associations at a population and individual level in the context of the various hypotheses concerning the evolutionary and physiological role of imprinting. Xu et al (1993) have claimed there is functional polymorphism in the imprinting of the human IGF2R gene, and the same group have reported that 8 of 87 individuals studied had persistent monoallelic expression of IGF2 in blood cells after birth (Giannoukakis and Deal, personal communication). The transgenerational growth trends of the populations from which these individuals come should be born in mind. Perhaps it is time for the closet neo-Lamarkians to come out!

Acknowledgements: Marilyn Monk and David Hart for supportive discussions over the years, and Peter Beighton for the Gould and Pyle reference on the Patagonian giants. Marcus Pembrey is funded by Mothercare (UK).

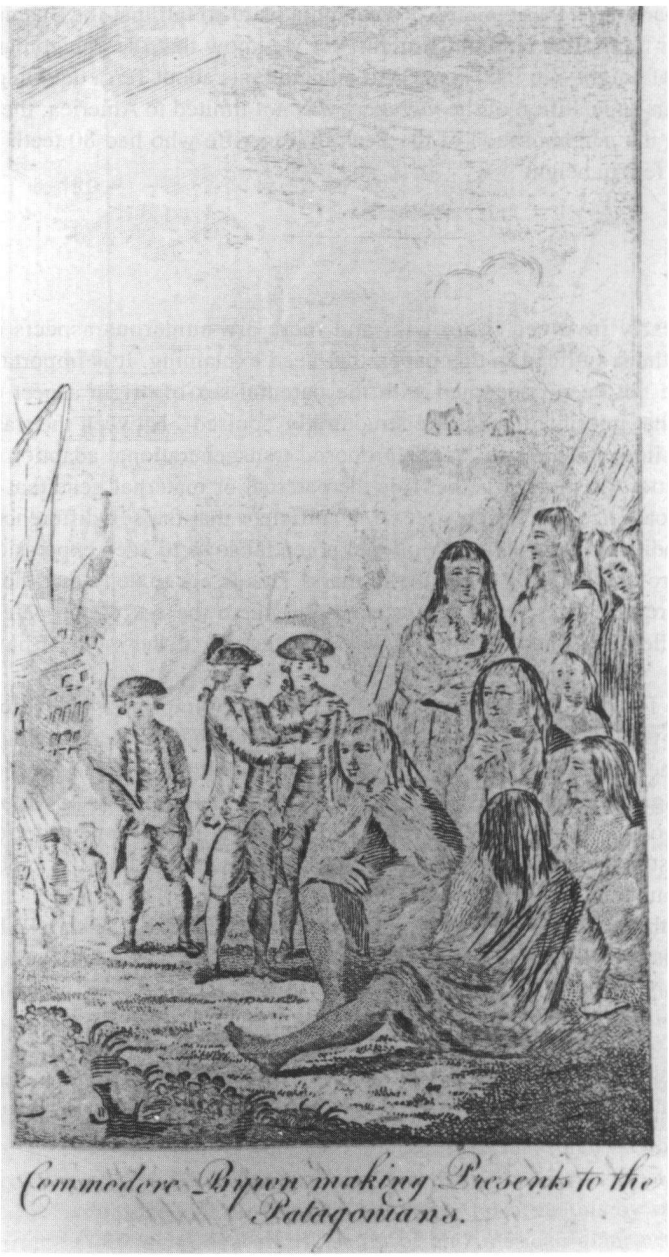

Fig. 4 - Copper plate illustrations of the Patagonians taken from "Geography for Youth or a plain and easy introduction to the Science of Geography for the use of young gentlemen and ladies", printed by W. Lowndes, 76 Fleet Street, London 1797.

https://doi.org/10.1017/S0001566000001197 Published online by Cambridge University Press

A Sailor giving a Patagonian Woman a Biscuit for her Child.

https://doi.org/10.1017/S0001566000001197 Published online by Cambridge University Press

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Correspondence: Prof. Marcus Pembrey, BSc MD FRCP, Institute of Child Health, University of London, 30 Guilford Street, London WC1N 1EH, United Kingdom.