REVIEW

P i, ia t ti t im it *Gnas*, t a it t i a ia t Gα a XLα i t ma a m t

A t i P a , Ga i K, [1] and E i L G ai -L [2]

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being mostly confined to neural and endocrine tissues
(Pasolli ˏ *a*. 2000
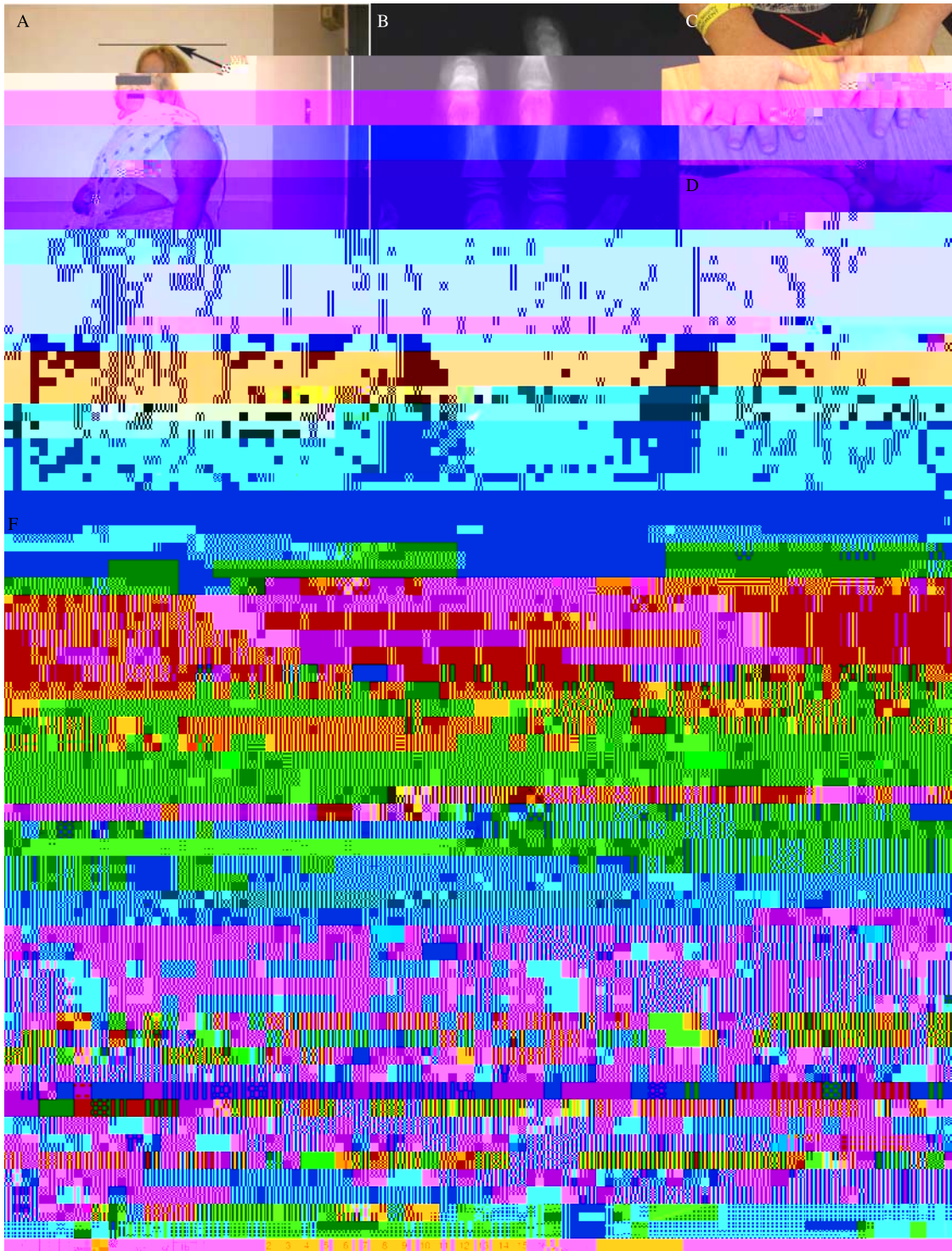
Gα_s (Kehlenbach ˌa. 1994). In contrast to G a

is ablated on the paternal allele, N and $G\alpha$ become derepressed, while $G\alpha$ and the exon 1A transcript are downregulated. Furthermore, the N DMR loses and the exon 1A DMR gains methylation on the paternal allele (Williamson a. 2006). The molecular mechanisms through which this ICR controls the imprinted expression of all transcripts of the $G\alpha$ locus remain to be elucidated.

Physical constructs in the *GNAS* products as revealed by mutations in cell and human studies

It has been known for some time that inactivating mutations in the human *GNAS* gene are associated with the inherited disorder 'Albright's hereditary osteodystrophy' (AHO)/'pseudo-hypoparathyroidism' (PHP; Levine a. 1980, 1983a, Patten a. 1990, Weinstein a. 1990, Davies & Hughes 1993). Fuller). lls 6.9(e) -1.1ght-32.t i-14.hi.8(m)2s.8(i-16.5(i)26.ol)2463.162emgluei6 6.9(

profound obesity in adulthood (discussed in detail below). The increase in adiposity arises already during the post-natal stage, as has been documented in mice with maternally inherited mutations of exons 2 and 6 (Cattanach *et al*. 2000, Yu *et al*. 2000, Plagge & Kelsey 2006). Despite their increased lipid accumulation and adipose tissue mass, these mice remain underweight until after weaning.

Comparatively little information on post-natal symptoms is available from case studies of AHO/PHP-Ia patients who carry mutations in *GNAS* exons on the maternal chromosome. An s.c. oedema has not been documented. However, a

Tab. 1 Continued

T tati	Di t i , i a ti		R
	Human	Mouse	
	•Severe obesity	•Severe obesity, increased body weight, hyperlipidae-mia, hyperglycaemia, glu-cose intolerance, hyperinsulinaemia, insulin resistance, reduced energy expenditure (hypometa-bolic)	
	•Variable mental retardation and neurological symptoms	•Reduced SNS activity, reduced mothering behaviour towards offspring •Reduced locomotor activity	
Human: imprinting defects affecting GNAS expression; e.g. loss of methyl-ation at exon A/B; STX16 deletions; Nesp deletions	PHP-Ib •Resistance to PTH, elevated PTH levels, hypocalcaemia, hyperphosphataemia •Mild TSH resistance •Brachydactyly, short stature, round face •Obesity •Abnormal ossifications		Human: Liu et al. (2003), Bastepe & Jüppner (2005), Linglart et al. (2007), Mantovani et al. (2007) and de Nanclares et al. (2007) see also text)

(a) Post-natal stage

T tati	Human	Mouse	R
Gα$_s$ (paternal allele-specific expression)	AHO/PPHP •S.c. ossifications •Brachydactyly	•Normal development (but 31–40% lethality on 129/Sv strain background)	Human: Eddy et al. (2000), Shore et al. (2002), Faust et al. (2003), Chan et al. (2004), Riepe et al. (2005) and Gelfand et al. (2006, 2007) Mouse: Chen et al. (2005) and Germain-Lee et al. (2005)

Human: missense or nonsense mutations in GNAS exons 1–13; (point mutations, small deletions, splice site mutations)
Mouse: deletion of Gnas exon 1

(b) Adult stage

Human	Mouse	R
AHO/PPHP •Short stature, brachydactyly •S.c. ossification, progressive osseous heteroplasia (POH)	•Reduced body length •S.c. ossification	Human: see text; also reviewed in: Aldred & Trembath (2000), Weinstein et al. (2001, 2006), Bastepe & Jüppner (2005), Germain-Lee (2006) and Mantovani & Spada (2006) Mouse: Chen et al. (2005) and Germain-Lee et al. (2005)

Tab. **1** Continued

T tati		Di t i , i a t ti		R
		Human	Mouse	
		• Variable mental retardation and neurological symptoms		
		(a) Post-natal stage		
$XL\alpha_s$	Human: chromosomal abnormalities of the 20q13.2–13.3 region, which affect $XL\alpha_s$ among other genes (maternal uniparental disomies, paternally inherited deletions); Repeat length polymorphism in Gnasxl exon Mouse: Gnasxl exon mutation; paternally inherited Gnas exon 2 and exon 6 mutations; maternal duplication of distal chromosome 2 (matDp.dist2)	• Growth retardation • Hypotonia • Feeding difficulties • Adipose tissue abnormalities • Mental retardation (but no GNASXL specific null mutations available for confirmation)	• Growth retardation • Hypotonia, hypoactivity • Poor suckling • Lack of lipid reserves in adipose tissue • Hypoglycaemia • Hypoinsulinaemia • ∼80% mortality	Human: Chudoba et al. (1999), Eggermann et al. (2001), Salafsky et al. (2001), Aldred et al. (2002), Velissariou et al. (2002) and Genevieve et al. (2005) Mouse: Cattanach & Kirk (1985), Williamson et al. (1998), Yu et al. (1998, 2000), Cattanach et al. (2000), Skinner et al. (2002) and Plagge et al. (2004)
		(b) Adult stage	• Reduced body weight and length • Reduced BAT and WAT mass and lipid content, stimulated lipolysis • Increased food intake • Increased metabolic rate • Hypoglycaemia • Hypoinsulinaemia • Hypolipidaemia • Increased glucose tolerance and glucose uptake in	Mouse: Cattanach et al. (2000), Yu et al. (2000, 2001), Skinner et al. (2002), Chen et al. (2004) and Xie et al. (2006).

cases of PHP-Ia hypercalcitoninaemia has been reported, which seems to be contradictory at first sight to the indication of hypocalcaemia in these patients (Wagar *et al*. 1980, Fujii *et al*. 1984, Kageyama *et al*. 1988, Vlaeminck-Guillem *et al*. 2001, Zwermann *et al*. 2002). However, resistance to calcitonin signalling (via its $G\alpha_s$-coupled receptor) and reduced levels of 1,25-dihydroxycholecalciferol, which normally downregulate calcitonin production, have been implicated in causing this symptom (Vlaeminck-Guillem *et al*. 2001).

The PTH resistance was also apparent in $G\alpha_s$ knockout mouse models after maternal inheritance of the mutations. On a normal diet, PTH levels were significantly higher (two- to threefold) in $m-/p+$ mice when compared with wild-type littermates (Yu *et al*. 1998, Germain-Lee *et al*. 2005). On a high phosphate diet, the PTH levels were increased by approximately sixfold over levels in mice fed a standard diet, and the $m-/p+$ mice showed again significantly elevated levels (2·9-fold) of PTH compared with wild types. The $m+/p-$ mice had PTH levels that were intermediate, trending approximately twofold higher than in wild types, but lower than in $m-/p+$ mice (Germain-Lee *et al*. 2005), indicating that a low level of $G\alpha_s$ expression might normally occur from the paternal allele in renal proximal tubules.

Growth hormone–releasing hormone (GHRH) resistance GHRH is a hypothalamic hormone, whose receptor on pituitary somatotroph cells is G_s-coupled, leading to stimulation of GH release. It was demonstrated that $G\alpha_s$ is expressed predominantly from the maternal allele in normal pituitary tissue (Hayward *et al*. 2001), thereby strengthening the hypothesis that subjects with a defective maternal *GNAS* allele could have $G\alpha_s$ deficiency in somatotrophs and a reduced GH response to GHRH. Previous scattered case reports of patients with PHP-Ia indicated a broad range of GH status from deficiency to sufficiency (Urdanivia *et al*. 1975, Wagar *et al*. 1980, Faull *et al*. 1991, Scott & Hung 1995, Marguet *et al*. 1997). A recent systematic analysis confirmed a markedly increased prevalence of GH deficiency in patients with PHP-Ia due to resistance to GHRH, thus expanding the range of multi-hormone resistances in PHP-Ia (Germain-Lee *et al*. 2003, Mantovani *et al*. 2003). The penetrance of GH deficiency is not 100% though, e.g. ~68% of PHP-Ia patients (Germain-Lee *et al*. 2003, Mantovani *et al*. 2003), which is in agreement with partial imprinting and incomplete silencing of the paternal allele of *GNAS*

adults with AHO is ~80% (Nagant de Deuxchaisnes & Krane 1978). An extensive search of the literature and of historical controls from patients (Germain-Lee *et al*. 2003 and unpublished) has revealed that the mean height is ~5 ft 0·5 in ±0·7 in (153·4 cm ±1·8 cm) in adult males and 4 ft 8·7 in ±0·7 in (144 cm ±1·8 cm) in females.

During childhood PHP-Ia patients with GH deficiency follow the same pattern as other patients with AHO/PPHP, i.e. they are usually not short at this stage (Fig. 3F). In most GH-deficient PHP-Ia children IGF-I levels were slightly below the normal range, but seemed adequate enough to maintain normal growth velocities. The growth curves of GH-deficient PHP-Ia patients revealed normal stature until approximately early adolescence, at which time there is a cessation in growth and an apparent lack of pubertal growth spurt (Fig. 3F; Germain-Lee *et al*. 2003). This is consistent with a premature epiphyseal closure in bones as an important factor causing short stature and brachydactyly in PHP-Ia and PPHP. Both are also characterised by markedly advanced hand-wrist bone ages, thought to be secondary to premature epiphyseal fusion (Albright *et al*. 1952, Steinbach & Young 1966, Germain-Lee *et al*. 2003, Germain-Lee 2006). Several studies have implicated haploinsufficiency of $G\alpha_s$ as being responsible for the premature epiphyseal fusion (Kobayashi *et al*. 2002, Bastepe *et al*. 2004, Tavella *et al*. 2004, Sakamoto *et al*. 2005*a*,). Biallelic expression of *GNAS* has been demonstrated in human bone (Mantovani *et al*. 2004) and in mouse chondrocytes (Bastepe *et al*. 2004). A 50% reduction of $G\alpha_s$ levels in PHP-Ia and PPHP could impair signalling via the PTH/PTH-related peptide receptor, which mediates chondrocyte proliferation and inhibits differentiation. Bone mineral density does not seem to be affected (Long *et al*. 2006).

Although GH deficiency cannot fully explain short stature, as both PHP-Ia and PPHP patients have reduced heights, it seems to be playing a supplementary role to that of premature epiphyseal fusion. In support of this notion, adults with PHP-Ia and GH deficiency have a lower height SDS than GH-sufficient PHP-Ia patients (Germain-Lee *et al*. 2003). Studies are currently underway to evaluate whether recombinant GH treatment in GH-deficient PHP-Ia children can increase growth velocity and final adult height (Germain-Lee 2006 and unpublished results). GH treatment could potentially augment linear growth and permit an increased growth velocity prior to the premature fusion of the epiphyses not only in GH-deficient PHP-Ia children, but also in GH-suffi-

s.c. ossifications in ageing AHO patients (Germain-Lee unpublished), 12-month-old heterozygous mutants were analysed and revealed extensive heterotopic s.c. bone formation in the dermis (Huso *a*. 2007). Mineral deposits in the areas surrounding hair follicles were detected, and many of these areas contained bone marrow elements, consistent with true s.c. bone formation, which was confirmed by X-ray and computed tomography imaging. There were no differences in the frequency or histology of the s.c. ossifications in mice with either a maternally or paternally inherited mutation, which is analogous to its occurrence in AHO (PHP-Ia and PPHP) patients and consistent with haploinsufficiency/ lack of imprinting of $G\alpha_s$ in the relevant cell types (Levine *a*. 1983, Mantovani *a*. 2004).

C t e a d t e CNS ab al t es AHO is often, but not always, accompanied by cognitive deficits ranging from learning disabilities to severe retardation (Marguet *a*. 1997, Rutter & Smith 1998, Levine *a*. 2000, 2002, Weinstein *a*. 2001). Reductions in $G\alpha_s$ levels have been associated with

disagreement over whether $Gs\alpha$ is imprinted in adipose tissues. In addition, $Gs\alpha$ is abundantly expressed in adipose tissues in neonatal mice, but is strongly downregulated around weaning (Plagge *et al*. 2004, Xie *et al*. 2006), implying that the enhanced metabolic rate in adults is not caused by increased sensitivity intrinsic to the tissue. An explicit test of the sensitivity of adipose tissues in the mutants is the metabolic response to an agonist of the adipose-specific β3-adrenoreceptor: such studies have revealed essentially normal responsiveness in $Gnas2^{-/+}$ and $Gs\alpha^{+/-}$ mice (Yu *et al*. 2000, Xie *et al*. 2006). These results rather suggest a differential effect of maternal Gα$_s$ and XLα$_s$ on sympathetic activity towards adipose tissues, and support for this proposition comes from the finding of reduced urinary excretion of noradrenalin in $Gnas2^{-/+}$ and increased excretion in $Gs\alpha^{+/-}$ mice (Yu *et al*. 2000, Xie *et al*. 2006).

In keeping with their lean phenotype, $Gs\alpha^{+/-}$ and $Gnas2^{+/-}$ mice have strongly increased insulin sensitivity, as evidenced by improved glucose tolerance and an exaggerated hypoglycaemic response to injected insulin. Euglycaemic–hyperinsulinaemic clamp studies demonstrated increased glucose uptake into skeletal muscle, WAT and BAT. The mutants also respond to an oral triglyceride load with an increased clearance rate (Yu *et al*. 2001, Chen *et al*. 2004, Xie *et al*. 2006). Gene expression analysis in $Gs\alpha^{+/-}$ mice reveals a profile in adipose tissues consistent with increased sympathetic activation and induction of genes associated with triglyceride uptake and hydrolysis, lipid oxidation and the adipogenic pathway (Xie *et al*. 2006). In contrast, the paucity of expression changes in skeletal muscle of genes associated with energy metetl51(g)-11. -8.212 50.3musir0.3(genes)vnfvl51(g)713 T1_0 1 Tf 9.4646 0 0 9.(tr)1t]TJ -8.21cJ -8.37(pn)-clee51(g)7i11

abnormalities or develop a PHP-Ib-like phenotype (Fröhlich *et al.* 2007). Whilst in most PHP-Ib cases methylation loss is limited to exon A/B, in others there are additional methylation changes across the *GNAS* locus, and these do not have *STX16* deletions (Bastepe *et al.* 2001, 2003, Linglart *et al.* 2007). Instead, two families with loss of methylation of the exon A/B, *GNASXL* and *NESPAS* DMRs have been found to have deletions and/or rearrangements spanning the *NESP* exon (Bastepe *et al.* 2005). Again, the mechanism by which these deletions result in failure to establish or maintain methylation of the maternal allele is currently unclear. In contrast to these familial forms, most PHP-Ib cases with more extensive methylation defects present as sporadics with no evidence of *STX16* or *NESP* deletions. In some such cases, unaffected sibs have the same maternal 20q13 haplotype, suggesting the presence of a newly acquired mutation in cis or that the defect is not linked to the 20q13 region (Linglart *et al.* 2007). It is interesting to note that a 'maternal hypomethylation syndrome' has been described in which affected individuals have loss of methylation at more than one maternal DMR, so that some sporadic PHP-Ib cases may be a manifestation of a more global imprinting defect (Mackay *et al.* 2006). An intriguing difference between the various forms of PHP-Ib is that sporadics appear to be more severely affected, while as many as 40% of individuals identified with maternally inherited *STX16* deletions are asymptomatic (Linglart *et al.* 2007). It is not possible at present to exclude ascertainment bias as the basis for this observation, but it might relate to different molecular events in the establishment of the abnormal methylation patterns or how they impact on the regulation of

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