Loss of XLαs (extra-large αs) imprinting results in early postnatal hypoglycemia and lethality in a mouse model of pseudohypoparathyroidism Ib

E , D , P
P E , s
A s ; C P s , s s
P , B s , C CB22 3A ,
1 ,

E * J .P s, ss s s , C

GNAS c
GNAS ΔN , 55
G a,
N
α α
α
ΔN , 55
N
ΔN , 55
α
G a,
ΔN , 55 G a}
ΔN , 55
G a}
α
ΔN , 55
ΔN , 55
fi,
fi *GNAS* d *Gnas*) a e ca le , i i ed laci laca ed i hi ch a asa al egia s af ca se ed s e (dis al ch a asa e 2 i ice, 20 13.32 i h a s) a d ha e si ila a e all a ga i a ia s (3–5). *GNAS*/*Gnas* ge e a es l i le ge e ad c s h a gh he se af

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diffe e ial a i e ? a i ad fi e a s he lice (2–15 h i a s a d 2–12 h ice) (Fig. S1). The as da as ea al e a i e fi s e a is Gαs e a 1, hich e ea s a asc Ste d ig le bi h s e esse Gαs (6). The Gαs a a e esides hi a a e h la ed C G islands. Besides he absence of diffe e ial e h la i a is Gαs has s ed i a l a e ale essia i s e iss es, i cl di g i i a , h aid, e al a i al b les, a d ga ads (7–10); Gαs e essia is biallelic i as a he iss es (11–13). The f nes s ea al e a i e a e ge e a es asc i s ha e cade he e ae dac i e-s ecific a ei af 55 kDa (NESP55; a se Nes 55), a ch a ag a i-like a ei , he cod i g se e ce af hich is laca ed i hi a s ecific s ea , 8036 , e a ; Gαs e a s 2–13, eside i hi he 3′ a Ga ed egi a af NESP55 asc ic s (14). This RNA sh se cl si es a e Gαs al , C CB2 e essia , beca se i s a a e is e h la ed a he a e al allele (12, 15, 16). A hi d al e a i e a e ge e a es a- sc i s ca di g he e al ge Gαs (XLαs) (17). XLαs has a la g a i a- e i al e essia e caded b i s s ecific fi s e a , he eas he e ai de af he a ei is ide ical a Gαs. XLαs is i i ed a si el a NESP55; i.e., i s a e is e h la ed a he a e al allele a d asc i ia all ac i e al a he a e al allele (12, 15, 16). Wi hi he sa e diffe - e ial e h la ed egia (DMR) a d j s s ea af he XLαs a e is a a e d i g e essia af a Ae al ca ib ia s E.F.-R., H.J., a d M. e fa ed esea ch; L.F.F., A.P., a d G.. ca ib ed e eage s/a al ic als; E.F.-R., A.M., H.J., a d M.B. a al ed da a; a d E.F.-R., H.J., a d M.B. a e he a e .

The a ha s decla e a ca fl s .

* s D ss .

1P s ss: D s s , E , s ' s s B s A s P , s C B , 08028 B , .
2 s s ss . E- : s @ . . . .

s ss . s. / x /s / :10. 1073/ s.1117608109/-/DC .

(23, 24). This finding s gges s ha e on A/B con ols iss e-s ecific Gαs i in ing ia he esence of one o o e eg -la o cis-ac ing ele en s ha a e bo h iss e-s ecific and e h la ion-sensi i e. Gene ic ic odele ions iden ified in AD-PHP-Ib s gges ha cis-ac ing ele en s i hin he nea b STX16 loc s and he s ea NESP55 DMR o an sc i ion f o he NESP55 o o e a be c i ical fo he es ablish en and/o ain enance of e on A/B a e nal-s ecific e h la ion (25–29).

We ecen l de elo ed a o se odel of AD-PHP-Ib b dele ing he a e nal Nes 55 DMR (30) in a anne si ila o he dele ions desc ibed in so e a ien s i h his disease (26). This o se s ain, ΔNesp55 , he noco ies AD-PHP-Ib i h es ec o he GNAS i in ing defec s—i.e., loss of all of he a e nal Gnas e h la ion i in s co bined i h inc eased (biallelic) 1A an sc i ion—and i h es ec o he abno al eg la ion of ine al ion ho eos asis—i.e., h ocalce ia, h e -hos ha e ia, and seconda h e a a h oidis (30). Ho -e e , nlike he findings in a ien s i h dele ions in ol ing NESP55 and an isense e ons 3 and 4 (AD-PHP-Ib[delNAS]), he e is 100% ea l os na al le hali in ΔNesp55 ice, he eas ice in hich he a e nal Nes 55 DMR is dele ed (ΔNesp55 ice) sho no e igene ic and bioche ical abno ali ies and ha e an a a en l no al heno e and life s an. The le hali in ΔNesp55 ice, hich as ass ed o eflec o se ning h o-calce ia d ing he fi s 5 d of life, e en ed addi ional in-es iga ion of his o se odel of AD-PHP-Ib ega ding he

i e a ia s af hese si es a d, he eb , leads a feedi g defec h gh a ela ed echa is . Al e a i el , he feedi g diffic l i *ΔNesp55* ice ca es l f a a ge e ali ed e alogical a a defec ha e e s he s f a ge i g access a he a he . I fac , sa e af he *ΔNesp55* s a ea h e ac i e (30), hich a eflec a e alogical defec .

The aa feedi g a d he de le ia af li e gl cage likel ca ib e a he h agl ce ia abse ed i *ΔNesp55* ice a d hei *Gnasxl* $^{+/-}$ li e a es. I e es i gl , ha e e , a a alsis af *Gnasxl* $^{+/-}$ s a P2 e ealed a fas i g gl case afile ha is ca sis e i h a defec i gl case ca e eg la ia . S ch a defec has bee s gges ed fa *Gnasxl* $^{+/-}$ ice based a i a a ia el la gl caga a d i a a ia el a al e ie h i e, a e i e h i e, a d ca ica s e a e le els (31). Sa e *Gnasxl* $^{+/-}$ ice, des i e ha i g a a e l defec i e gl case

b les h a gh he se af lase ca e ic asca , e fa d ha he ed c ia i Gαs le els as al 50%. Th s, he sile ciig af a e al Gαs e essia a a be ca le e de a al ca di ia s, a d/a his eg la a e e a acc al i a s b- se af a i al b la cells. O *ΔNesp55 /Gnasxl* $^{+/-}$ ice de a s a ed a a al calce ic es a se a PTH ad i is a- ia , i dica iig ha he ac ia s af PTH a ba e a e a i ai ed, as is alsa e i a ie s i h PHP-Ib (48). This es l likel eflec s he abse ce af a e al Gαs sile ciig i his iss e (13).

Like *ΔNesp55 /Gnasxl* $^{+/-}$ ice, 10-d-ald *Gnasxl* $^{+/-}$ ice a e alsa h acalce ic a d h e has ha e ic, s gges iig e- ha s ha XLαs ca i ib es a he e al ac ia s af PTH. This e la a ia a ld be ca sis e i h e ia s e a s ha XLαs ca i ic Gαs ac ia s (49, 50), a d he abse ce af h acalce ia a d h e has ha e ia i ad l *Gnasxl* $^{+/-}$ ice a i dica e ha he ca ib ia af XLαs a ei a edia iig he e al effec s af PTH a decli e i h age (50). Ca e sel , he calce ic es a se a PTH is bl ed i ad l *Gnasxl* $^{+/-}$ ice (Fig. 4*D*), s gges iig ha XLαs igh s ill la a ale i edi- a iig PTH ac ia s i ba e. Ca sis e i h his i e e a ia , XLαs a ei e essia has bee de ec ed i ad l a se as eac es (51), hich a e i a a l i al ed i he PTH-de e de eg la ia af ba e e adeliig a d calci ha ea- s asis (52–54).

B ge e a iig he *ΔNesp55 /Gnasxl* $^{+/-}$ ice, e e e able a es ablish a iable a se adel af AD-PHP-Ib. Al ha gh hei s i al a e as fa d a be di i ished, a s bs a ial be af hese da ble- a ice (c e l >30) s i ed a ad l haad a d had see i gl a al life s a s. The echa is s de l iig he a hage esis af h acalce ia a d h e has ha e ia e- s l iig f a PTH esis a ce ca ld a be i es iga ed f he i he s i iig *ΔNesp55 /Gnasxl* $^{+/-}$ ice. F he a e, e- ea i g le hali af hese da ble- a ice a i dica e ha a e al Gαs sile ciig acc s i a e iss es ha e ia sl ecagi ed a ha a e e essia af he a he a e all e essed *Gnas* ad c s—e.g., 1A a d Nes as—has a ega i e effec a s i al. These es ia s e ai a be add essed.

Materials and Methods

Mouse Models. *Gnasxl* $^{+/-}$ *ΔNesp55* s (30, 31). *ΔNesp55* ss CD1 s s - s *ΔNesp55* *Gnasxl* $^{+/-}$ *Gnasxl* $^{-/+}$, s CD1 . A s 2 4 . s s s s A C s C s ss s s s s A C .

Glucose, Insulin, and Corticosterone Analysis. s s s s s (P s P B s β- s ; A s). s s s s. s s s s s E A (C s C). C s s s s ss (A; P B).

Quantification of Liver Glycogen. s s A (55) ss 20–100 ss 2 . A (2 C), ss s s s ( ) 55 C 15 . s s s s s ( ). s s - s . s , BCA (P). s, (s s) s - s .

Gene Expression Analyses. s s - A s s (50). A 2- - s s s s . - ss s s αs αs s - P C β- s s . C s - s s (56). P s, s, s .

Calcium, Phosphorus, and PTH Measurements. B , s s s, s P P 10 s s - s (50). ss s P s ss , s. . 50 /κ P (1–34). A P s fi s A (50), A P s s s A B κ .

Statistical Analyses. D s s s s fi s