Paternal loss-of-function mutations of *GNAS* and growth retardation in a mice model: a specific placental transcriptomic signature ?

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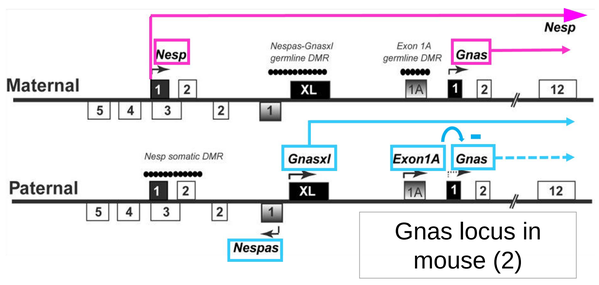
OBJECTIVES

GNAS is a complex imprinted locus which leads to different transcripts characterized by one specific exon 1 and shared exons 2-13, with monoallelic (*XL*, *NESP55*) or biallelic ($G_s\alpha$) expression. A severe intrauterine growth retardation (IUGR) associated with placental hypotrophy has been observed in patients presenting pseudo-pseudohypoparathyroidism due to paternal *GNAS* mutations (1).

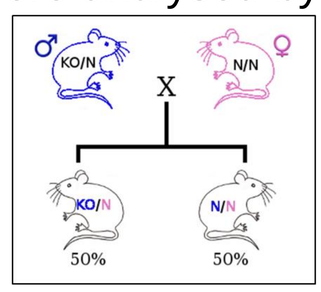


Gnas locus in mouse (2)

Given the role of placenta in fetal growth, we suspected transcriptomic alterations due to *Gnas* loss-of-function mutations.

METHODS

WT female mice were crossed with male mice harboring heterozygote mutation in exon 1 of *XL*, of $G_s\alpha$ (E1) or in E2 (exon 2, shared by $G_s\alpha$ and *XL)*. Placentas were obtained at E18 for each litters and the fetus genotyped. After extraction, placental RNA were hybridized on a microarray (GeneChip® Mouse Transcriptome Assay 1.0.), and data were analysed by bioinformatics (Gene Set Enrichment Analysis, Webgestalt, String db, Venny Venn). We chose to focus our analysis on modified biological pathways associated with growth.



XL KO	XL	E1	1A	Nesp55
Theory	**0.00**	1.00	1.00	1.00
RQ	**0.20**	0.97	0.83	0.91

E1 KO	XL	E1	1A	Nesp55
Theory	**1.00**	0.50	1.00	1.00
RQ	**1.70**	0.58	0.77	0.89

E2 KO	XL	E1	1A	Nesp55
Theory	**0.00**	0.50	0.00	1.00
RQ	**0.21**	0.52	0.09	0.97

Fig 1

Figure 1. Relative quantification (RQ) of the different transcripts. Results are expressed compared to WT (considered as value of 1).

Protein-protein interaction networks : known (red circle) and predicted protein interactions built using String db

Figure 2 "Prenatal growth retardation"
18 genes, ajusted P-values (adjP)= 3.1 x10^{-22}

Figure 3. "Decreased placenta weight"
5 genes, adjP = 2.4 x10^{-10}

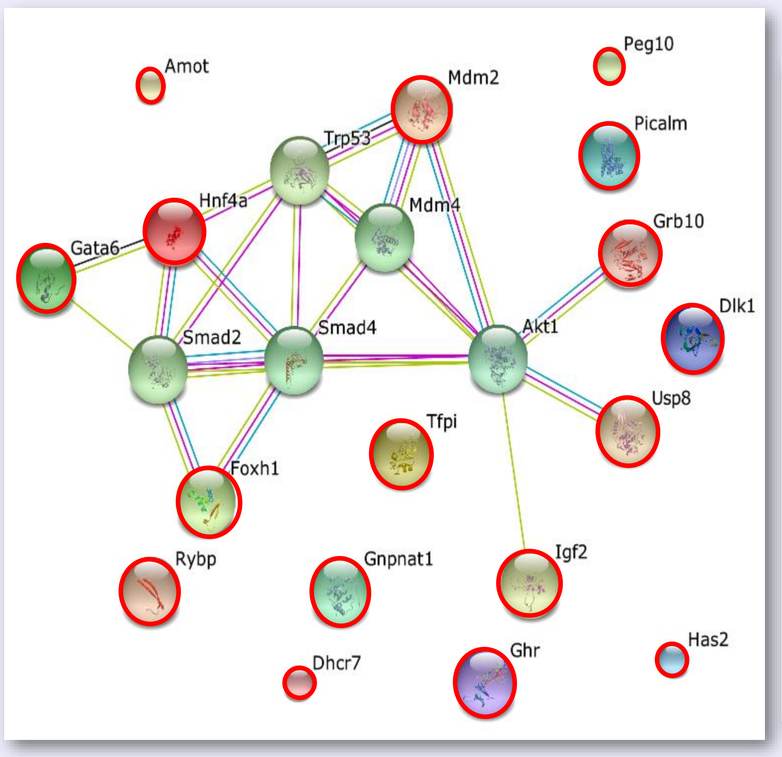


Fig 2

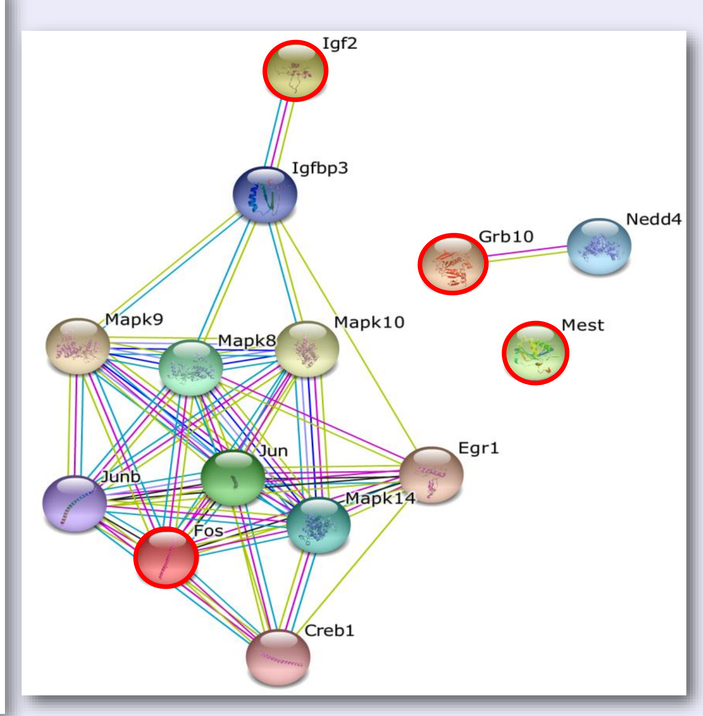


Fig 3

RESULTS

XL expression was dramatically decreased in $XL^{m+/p-}$ mice (relative quantification [RQ] *versus* WT: 0.20, $P < 1.00 \times 10^{-4}$). As expected in theory, $G_s\alpha$ was biallelically expressed in mice placentas, with its RQ halving in $E1^{m+/p-}$ mice (specific to $G_s\alpha$) and in $E2^{m+/p-}$ mice (shared by the 3 other transcripts). However, *XL* was overexpressed in $E1^{m+/p-}$ mice : to explain this result, we assumed there was a promoter competition between *XL* 'weak' promoter, expressing more while E1 'strong' promoter is disrupted.
We showed significant variations in gene networks involved in phenotypes and pathways, such as "Prenatal growth retardation" ($P = 3.1 \times 10^{-22}$) and "Decreased placenta weight" ($P = 2.4 \times 10^{-10}$). Down-regulated transcripts such as *Meg3*, *Mest* and *Igf2* have already been described in a human model of IUGR (3).

CONCLUSIONS

XL showed a preferential placental expression from the paternal allele.
XL and *E1* promoters may compete and show an overexpression of *XL* in $E1^{m+/p-}$ mice.
Subtle variations of gene networks involving *XL* would be implicated in the pathogenesis of placental hypotrophy and IUGR associated with *GNAS* paternal mutation.

(1) Richard et al. JCEM 2013 Sep;98(9):E1549–56. (2) Mehta et al. Transcription driven somatic DNA methylation within the imprinted Gnas cluster. PloS One. 2015;10(2):e0117378. (3) Kappil et al. Placental expression profile of imprinted genes impacts birth weight. Epigenetics. 2015;10(9):842–9 . The authors have been funded by the Société Française d'Endocrinologie et Diabétologie Pédiatrique.

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