Novel *LZTR1* mutations in subjects with features of Noonan Syndrome and GH insensitivity negatively regulate GH-induced IGF-I production and hyperactivate GH-induced ERK1/2 activation in response to GH *in vitro*

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INTRODUCTION

Noonan Syndrome (NS) can overlap clinically and biochemically with growth hormone insensitivity [GHI; short stature (SS), low IGF-I and normal/elevated GH levels]. Mutations in multiple genes regulating RAS-MAPK pathway have been identified in NS including *LZTR1* variants. Function of LZTR1 is poorly understood and it's role in growth retardation is unknown.

AIM

To functionally characterise 6 novel *LZTR1* variants -1 identified in our GHI patient cohort (c.466A>G; p.K156E –V1) and 5 previously published [c.742G>A;p.G248R, c.850C>T;p.R284C, c.740G>A;p.S247N, c.356A>G;p.Y119C and c.859C>T;p.H287Y (V2-6,respectively)] [1] and determine their impact on the GH-IGF-I axis.

METHOD

- V1 identified in a GHI subject by our SS whole genome panel. 5 previously published NS-associated heterozygous inactivating missense *LZTR1* variants (V2-6) also studied.
- V1-6 LZTR1 vectors generated by site-directed mutagenesis and verified by Sanger sequencing.
- Western blot (WB) analysis of transfected HEK293T cell lysates performed using anti-c-Myc & anti-ERK/anti-pERK antibodies (anti-beta actin antibody as control).
- Supernatant from transfected & GH-stimulated (24 hours) HepG2 cells assessed by ELISA.
- Cell lysates from transfected (with V1, 2 & 5) & GH-stimulated (20 minutes) HepG2 cells subjected to WB analysis (anti-ERK/anti-pERK antibodies & anti-STAT5/anti-pSTAT5 antibodies).

RESULTS

- All 6 subjects had characteristic facial features of NS and cardiac defects. 2 subjects (V1 & 2) had features of SS & GHI (height/IGF-I SDS of -2.3/-2.3 and -2.1/-2.2 respectively).
- All variants showed significantly reduced LZTR1 protein expression (Fig. 1) and increase in p-ERK/total ERK ratios compared to WT (Fig. 2), latter suggesting up-regulation of RAS-MAPK pathway.
- Compared to WT (0.54±0.12), GH-induced mean IGF-I levels were significantly lower in V1 & 2 (0.28±0.03 & 0.29±0.07, respectively; both p <0.05), but not in V3-6 (Fig.3).
- IGF-I rise following GH stimulation in all 6 subjects correlated negatively with the subject's height SDS (p<0.001) (Fig.4).
- Following GH stimulation, as compared to WT, a significant increase in p-ERK/total ERK ratios but no difference in p-STAT5/total STAT5 ratios were observed in V1 & 2 (Fig. 5 & 6). This suggests GH-induced ERK1/2 hyperactivation and upregulation of RAS-MAPK pathway in variants causing SS.

Fig. 1. Immunoblotting of LZTR1 levels in WT and mutant constructs.

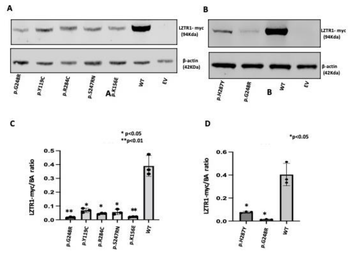


Fig. 1A & 1B. WB showing attenuated levels of LZTR1 in mutant constructs. Fig. 1C & 1D. Densitometry analysis *p<0.05 **p<0.01

Fig.2. Immunoblotting of pERK in WT and mutant *LZTR1* constructs

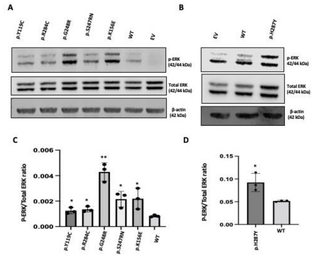
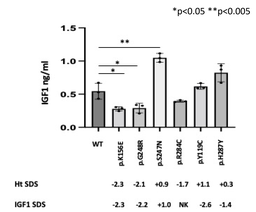


Fig. 2A & 2B. WB showing increased expression of p-ERK in mutant constructs. Fig. 2C & 2D. Densitometry analysis. *p<0.05 **p<0.001

Fig. 3. Scatter dot plot showing rise in IGF-I level upon GH stimulation in WT and mutant *LZTR1* constructs



Ht SDS	-2.3	-2.1	+0.9	-1.7	+1.1	+0.3
IGF1 SDS	-2.3	-2.2	+1.0	NK	-2.6	-1.4

Fig.4. Scatterplot showing correlation between the change in IGF-I level with GH treatment *in vitro* and the height SDS of the patients

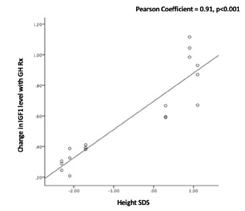


Fig. 5. Immunoblotting of pERK in WT and mutant LZTR1 constructs following GH stimulation

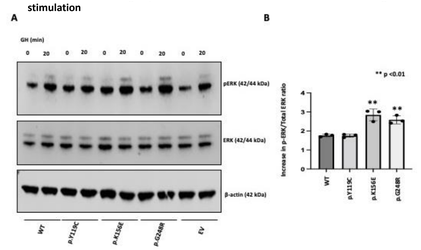
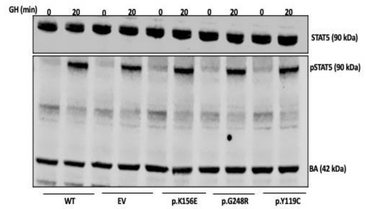


Fig. 5A. WB showing increased expression of p-ERK in mutant constructs p.K156E and p.G248R upon GH stimulation. EV, Empty Vector Fig. 5B. Densitometry analysis **p<0.01

Fig. 6. Immunoblotting of pSTAT5 in WT and mutant *LZTR1* constructs following GH stimulation



CONCLUSIONS

- Novel LZTR1 variants in NS cause reduced LZTR1 protein expression.
- They also result in enhanced RAS-MAPK signalling, similar to that observed in *PTPN11* and *SOS1* mutations.
- GHI-causing *LZTR1* mutants negatively regulate GH-induced IGF-I production and hyperactivate ERK1/2 activation in response to GH in vitro.
- This suggests that dysregulation of GH-induced RAS-MAPK pathway could contribute to growth retardation.

REFERENCES

[1] Yamamoto GL, Aguena M, *et al*. Rare variants in SOS2 and LZTR1 are associated with Noonan syndrome. *J. Med. Genet*. 2015. 52: 413-421.

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