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 [GH; 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 GH patient cohort (c.4664G>C, p.R1558E→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.K156E (V2-6, respectively)] 1 and determine their impact on the GH-IGF-I axis.

METHOD
- V1 identified in a GH 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 EUSA.
- Cell lysates from transfected (with V1, V2 & 5) & GH-stimulated (20 minutes) HepG2 cells subjected to WB analysis (anti-ERK/anti-pERK antibodies & anti-STATs/anti-pSTATs antibodies).

RESULTS
- All 6 subjects had characteristic facial features of NS and cardiac defects. Subjects V1 & 2 had features of SS & GH (height/IGF-I SDS of -2.3 & -2.1 respectively).
- All variants showed significantly reduced LZTR1 expression.
- Compared to WT (0.54±0.03), 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.
- IGF-I rise following GH stimulation in all 6 subjects correlated negatively with the subject's height SDS (p<0.001).
- 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. 6).

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.
- GH-causing LZTR2 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

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