# Heterozygous *NPR2* mutations cause disproportionate short stature, similar to Léri-Weill dyschondrosteosis (LWD)

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## INTRODUCTION

NPR-B (Natriuretic peptide receptor-B) is a transmembrane receptor that transduces CNP signals by increasing intracellular cGMP levels. It is encoded by the Natriuretic Peptide Receptor-2 gene (*NPR2*). Homozygous *NPR2* mutations have been shown to cause acromesomelic dysplasia Maroteaux type, a skeletal dysplasia with extreme disproportionate short stature. Recently, heterozygous *NPR2* mutations have been identified in patients with idiopathic short stature (ISS, 2-6%) (Vasques et al, 2013; Amano et al, 2014).

Patients with mutations in *NPR2* show a similar phenotype to that caused by *SHOX* mutations in Léri-Weill dyschondrosteosis (LWD) and Langer mesomelic dysplasia, and in ~2% of idiopathic short stature (ISS) cases. LWD, caused by SHOX haploinsuficiency, is characterized by disproportionate short stature and the characteristic Madelung deformity whilst the total absence of SHOX results in LMD, characterized by severe disproportionate short stature with marked mesomelic and rhizomelic limb shortening. *SHOX* mutations are detected in ~70% of LWD cases whilst the molecular defect in the remaining ~30% is unknown. We hypothesize that *NPR2* mutations could be present in LWD patients without *SHOX* defects.

## AIM

- To determine if *NPR2* mutations are the molecular defect in LWD and ISS patients with no known *SHOX* defect.

## COHORTS & METHODS

- Cohorts: 173 patients with LWD or suspected LWD and 95 with ISS, and with no known *SHOX* defect.
- Mutation screening: Custom-designed Skeletal Dysplasia panel (SKELETALSEQ.V3) or by traditional methods (HRM and Sanger sequencing).
- Functional analysis of the identified variants: 1) Intracellular localization by immunocytochemistry, and 2) Ability for the homozygous and heterozygous NPR-B variants to synthesize cGMP, determined by an ELISA.

## RESULTS

- Eight *NPR2* variants were detected in nine patients; seven with LWD and two with ISS.
- Functional analysis demonstrated that 6/8 variants were pathogenic:
- Variants p.D256Y, p.T421M and p.R1020W, are retained in the ER, whilst the rest are able to reach the cellular membrane (Fig. 1).
- A total of 6/8 variants had reduced GMP cyclase activity (Table 1, highlighted in red).
- Proband 8 is currently being treated with rhGH, showing an increase in height of +1 SDS (Fig. 2).

Figure 2. Growth chart of proband 8 (p.E991G), treated with rhGH.

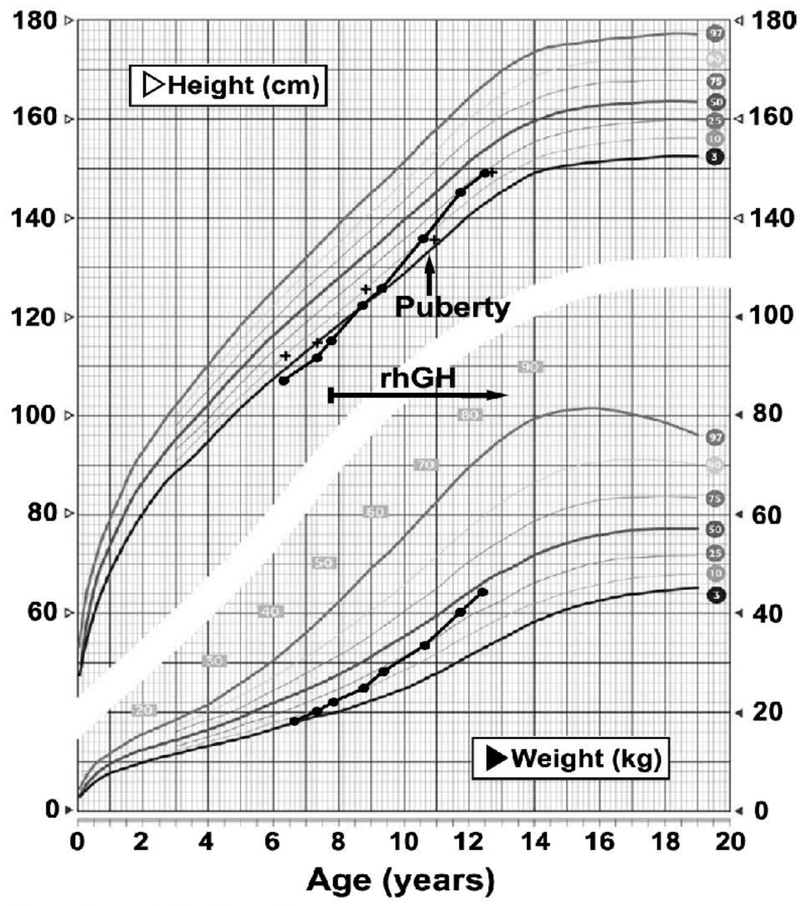


Figure 1. Subcellular localization of the NPR-B variants studied.

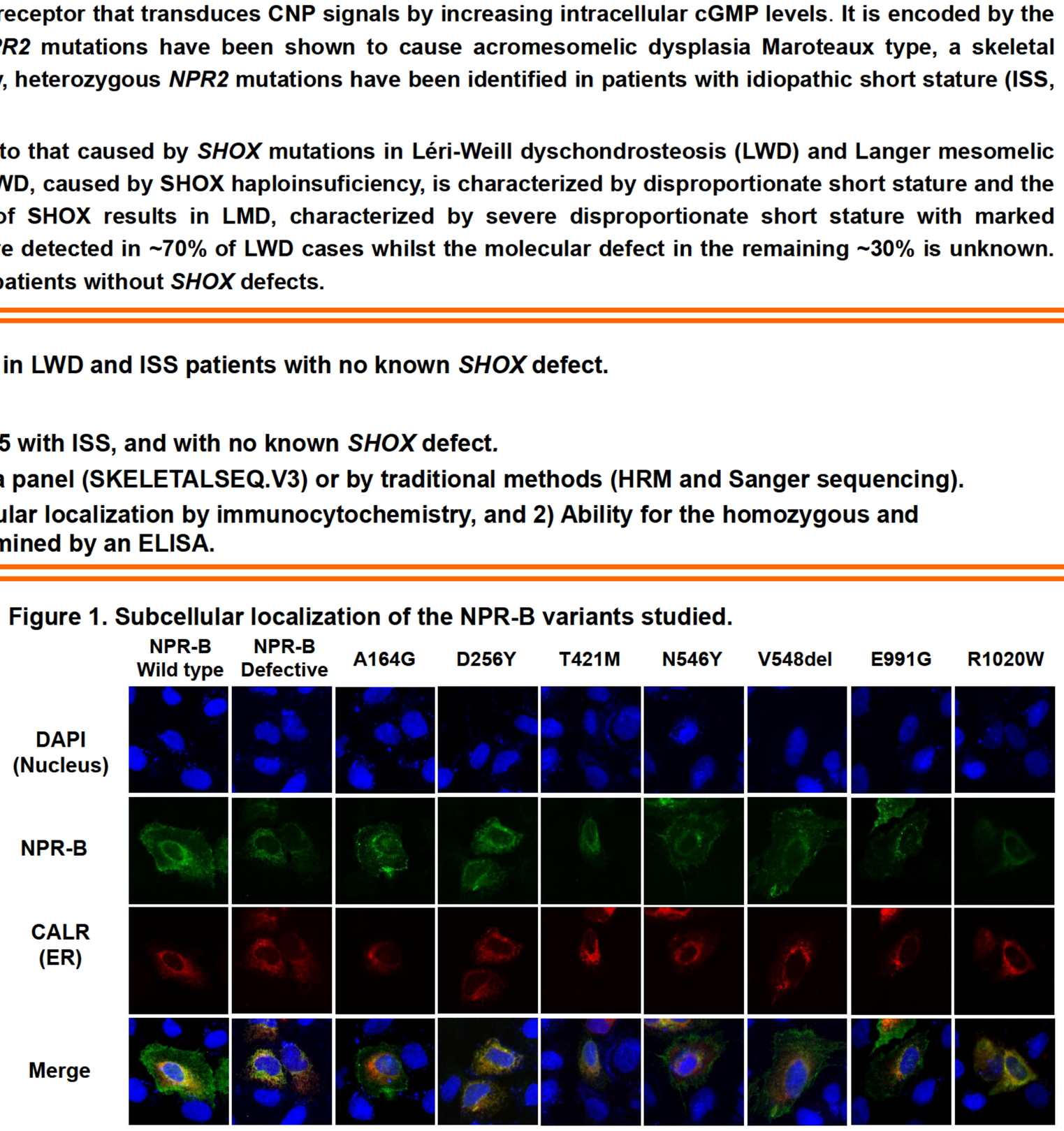


Table 1. Characteristics of the *NPR-B* variants detected in LWD and ISS patients.

| Prob. | Variant | Domain | Cosegregation | cGMP activity† | |
|---|---|---|---|---|---|
| | | | | Homozygous | Heterozygous |
| 1 (ISS) | p.A164G* | Ligand binding | No | 81.0±2.2% | 89.2±6.2% |
| 2 (LWD) | p.D256Y | Ligand binding | Yes | 1.5±0.1% | 40.8±4.4% |
| 3 (LWD) | p.T421M* | Ligand binding | Not determined | 39.0±4.7% | 52.0±1,7% |
| 4 (LWD) | p.N546Y | Kinase | Non-informative | 69.3±1.0% | 91.5±2.2% |
| 5 (ISS) | p.N546Y | Kinase | Not determined | | |
| 6 (LWD) | p.V548del | Kinase | Yes | 0.2±0.1% | 36.8±3.0% |
| 7 (LWD) | p.R819C* | -- | Yes | Pathogenic** | |
| 8 (LWD) | p.E991G | GMP cyclase | Yes | 0.0±0.0% | 54.6±6.2% |
| 9 (LWD) | p.R1020W | GMP cyclase | Not determined | 0.1±0.0% | 35.2±6.0% |

*Variants found in control populations (ExAc) at a very low frequency (<0.0001). †GMP cyclase activity represents % of NPR-B WT activity (100%). **Functional analysis undertaken by Amano et al, 2014.

## CONCLUSIONS

- We identified six *NPR2* pathogenic mutations in patients referred for LWD (~3.5%), none of whom actually presented the Madelung deformity but all had disproportionate short stature and secondary LWD characteristics. All the variants identified in ISS patients were determined to be non-pathogenic variants. Thus, functional analyses are essential to determine the pathogenicity of novel *NPR2* variants.
- Interestingly, one of the *NPR2* mutation carriers is currently being treated with rhGH, and in contrast to previous reports, is showing a positive response.
- In summary, *NPR2* mutations are a cause of disproportionate short stature, thus we recommend *NPR2* mutation screening analysis in patients with disproportionate short stature, in which *SHOX* defects have been already excluded.

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