

# qPCR Screening for Xp21.2 copy number variations in patients with elusive 46,XY DSD

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## background

Duplications of the dosage sensitive sex locus Xp21.2 have been associated with 46,XY gonadal dysgenesis (GD) for nearly 25 years. In the past, duplications have always included the *NR0B1* (nuclear receptor subfamily 0 group B, member 1, also known as DAX1) gene, a known antagonist of SF1 (Steroidogenic Factor 1) dependent *SOX9* (SRY Box 9) activation and the GD was attributed to its “double gene dose”. However, recent findings have questioned the necessity of *NR0B1* to be included in the duplications and identified upstream copy number variations (CNVs) associated with 46,XY GD. This has provoked us to put greater attention on this locus in patients with 46,XY GD and establish a time and cost effective screening for Xp21.2 CNVs.

## method and patients

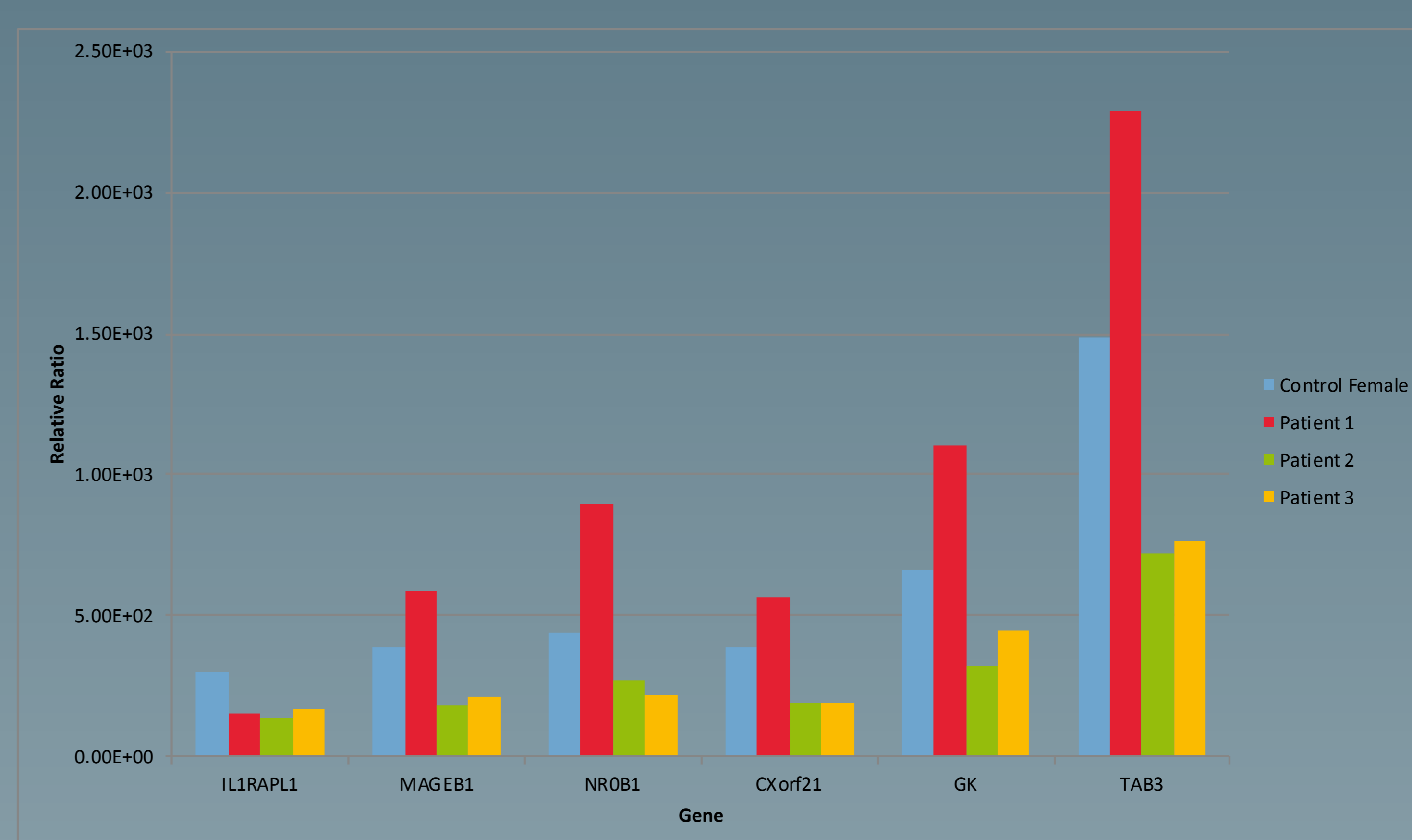
A real-time qPCR (quantitative PCR) routine for identifying CNVs at the Xp21.2 locus was established on a Roche LightCycler 96 and validated using samples with known Xp21.2 duplications identified by chromosomal microarray analysis (CMA).

## results

Thus far the qPCR routine was used to screen 93 patients with elusive genetic cause of 46,XY disorders/differences of sex development (DSD). This revealed two previously unidentified duplications and one unidentified triplication (Figure 1) of *NR0B1*. The use of more than one assay at the Xp21.2 locus (*IL1RAPL1*, *NR0B1*, *GK*, *CXorf21* and *TAB3*)

gives more confidence about the presence of a possible CNV and allows first insights into its size.

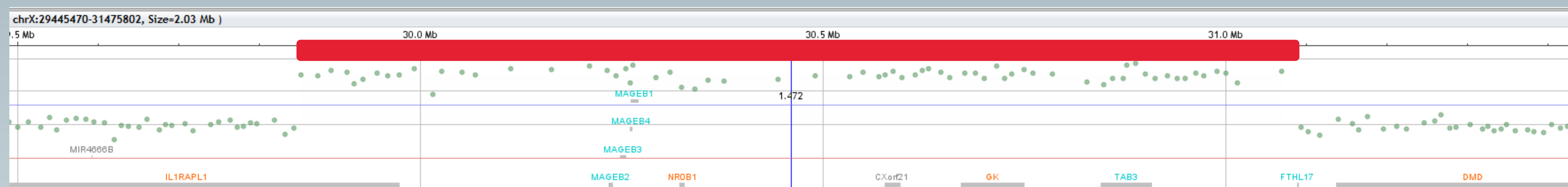
figure 1: RT-qPCR-Screening revealing a Xp21.2 triplication in distinction to a 46,XX control and two screened 46,XY patients



## conclusion

The number of published cases of non-syndromic *NR0B1* duplications associated with 46,XY GD is in the single digits and it remains a very rare diagnosis. Thus, Xp21.2 duplications are potentially underdiagnosed as cause of 46,XY DSD. The qPCR approach offers a short turnaround time at low consumable costs for identifying these patients. The CNVs could then be confirmed by other methods such as multiplex ligation mediated probe amplification, CMA (Figure 2) and studied in more detail, including their point of insertion through whole genome sequencing.

figure 2: CMA confirming Xp21.2 Triplication



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