HIGH FREQUENCY OF HYPOMORPHIC ALLELIC HAPLOTYPES OF THE *GH1* PROXIMAL PROMOTER IN PATIENTS WITH PROPORTIONAL UNDERGROWTH AND ISOLATED GH DEFICIENCY

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BACKGROUND AND AIM

- **Isolated GH deficiency (IGHD)** is one of the most frequent causes of postnatal proportional undergrowth (1/3000-4000).
- **85-90% of IGHD cases are still classified as idiopathic.**
- **A very high rate of interlocus gene conversion** (Sedman *et al.*, 2008) between the 5 highly homologous genes present in the chr. 17 GH cluster (**Fig. 1**), generates up to 40-60 different ***GH1*** proximal promoter haplotypes through the combination of 16 SNPs (**Table 1**) (Horan *et al*. 2003; Wolf *et al*., 2008).
- **GH gene (*GH1*) expression is highly influenced by the *GH1* proximal promoter haplotypes** (Horan *et al*., 2003).
- At least 12 of the generated proximal promoter haplotypes show **hypomorphic effects**, significantly affecting ***GH1*** **expression levels** in luciferase assays (**Fig. 2**) (Horan *et al*., 2003).

AIM

To investigate the frequency of ***GH1*** proximal promoter hypomorphic allelic haplotypes in a cohort of patients with IGHD.

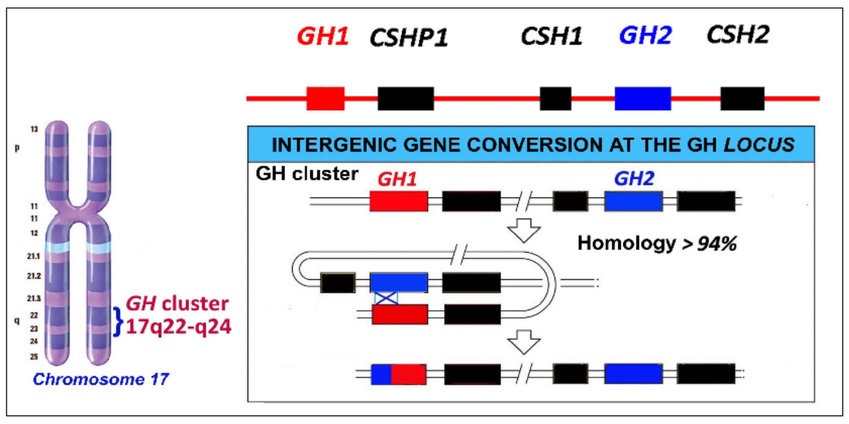

Fig. 1: Hyperactive intergenic gene conversion between the *GH2* (donor) and *GH1* acceptor) *loci* at the GH cluster in chr. 17

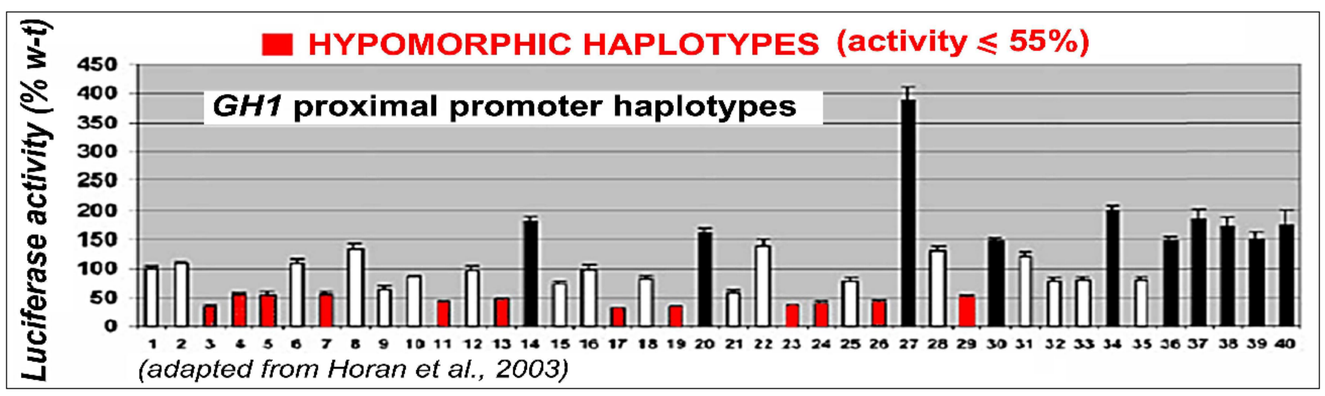

Fig. 2: Relative Luciferase activity of the 40 different *GH1* proximal promoter haplotypes according to Horan *et al*., (2003). Luciferase activity is expressed as % of the most frequent haplotype, i.e. H1.

METHODS

Subjects: 53 children (23 females, 30 males) with proportional undergrowth (heights <-2.5 SDS) and IGHD (peak GH <10ng/ml). ***Molecular studies***: Mutation screening/genotyping of the coding sequences, intron/exon boundaries and regulatory regions of *GH1*; *GH1* proximal promoter haplotype classification was performed according to Horan et al. (2003) **(Table 1)** and Wolf et al. (2009).

Table 1: GH1 proximal promoter haplotypes defined by genetic variation at 16 locations (Horan et al., 2003)

No.	SNP position relative to *GH1* gene transcriptional start site														
	−476	−364	−339	−308	−301	−278	−168	−75	−57	−31	−6	−1	+3	+16	+25	+59
1	G	G	G	G	G	G	T	A	T	G	A	A	G	A	A	T
2	G	G	G	G	T	T	T	A	G	G	A	A	G	A	A	T
3[b]	G	G	G	T	G	T	T	A	G	G	A	A	G	A	A	T
4[b]	G	G	G	T	G	T	T	A	G	G	A	A	G	A	A	G
5[b]	G	G	G	T	G	T	T	A	G	G	A	A	G	A	A	T
6[b]	G	G	G	T	G	T	T	A	G	G	A	A	G	A	A	T
7[b]	G	G	G	T	G	T	T	A	G	G	A	A	G	A	A	T
8	G	G	G	G	G	G	T	A	G	G	A	A	G	A	A	T
9	G	G	G	G	G	G	T	A	G	G	A	A	G	A	A	T
10	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
11[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
12	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
13[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
14	G	G	G	G	G	T	C	T	T	T	A	A	G	A	A	T
15	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
16[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
17[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
18	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
19[b]	G	A	G	G	G	T	T	A	G	G	A	A	G	A	A	T
20	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
21	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
22	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
23[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
24[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
25	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
26[b]	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
27	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
28	G	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
29[b]	G	A	G	G	G	T	T	A	G	G	A	A	G	A	A	T
30	G	G	G	-	G	T	T	A	G	G	A	A	G	A	A	T
31	G	G	G	-	G	T	T	A	G	G	A	A	G	A	A	T
32	G	G	G	-	G	T	T	A	G	G	A	A	G	A	A	T
33	G	G	G	-	G	T	T	A	G	G	A	A	G	A	A	T
34	G	G	G	-	G	T	T	A	G	G	A	A	G	A	A	T
35	G	G	G	-	G	T	T	A	G	G	A	A	G	A	A	T
36	G	G	G	-	G	T	C	T	T	T	A	A	G	A	A	T
37[c]	A	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
38[c]	A	G	G	G	G	T	T	A	G	G	A	A	G	A	A	T
39[c]	A	G	G	G	T	G	T	A	G	G	A	A	G	A	C	T
40	G	G	G	G	G	T	C	A	G	G	A	A	G	A	A	T

[a]Haplotypes exhibiting a significantly reduced level (≤55% that of haplotype 1) of luciferase activity in GC cells.
[b]Only found in solitary cases of GH deficiency.
–,denotes the absence of the base in question.

RESULTS

1. **19 out of 53 (35.8%) patients presented with hypomorphic allelic haplotypes of the *GH1* proximal promoter. Their main clinical characteristics are summarized in the enclosed table.**
2. **Only three out of 53 (5.7%) patients presented with three previously described heterozygous *GH1* mutations: c.291+1G>A, p.Arg42Cys, and p.Arg209His.**

Patient	Gender	IGF1 (SDS)	Response to rhGH	Height (SDS)	GH test (peak) (ng/ml)		*GH1* promoter haplotypes (¥)		*GH1* mutations	Other characteristics
					HI	CL				
1	M	-2.5	++	-3.4	0.3	0.5	H3	H2	c.291+1G>A	Pituitary hypoplasia
2	H	-3.8	++	-2.9	2.8	1.9	H3	H15	-	
3	H	-2.6	++	-3.0	4.9	3.9	H26	H1	-	
4	M	-	++	-2.7	4.9	5.8	H11	H7	-	
5.1	H	-3.7	++/-	-3.6	0.5	4.4	H5	H2	p.Arg42Cys	SGA
5.2	M	-3.5	++	-2.7	4.0	3.1	H5	H2	p.Arg42Cys	SGA
6	M	-3.5		-3.5	7.8	3.3	H5	H1	-	SGA
7	H	-1.51		-2.5	1.3	2.2	H13	H2	-	Pituitary hypoplasia BA -2 yrs vs. CA
8	H			-3.2	2.7	2.0	H3	H2	-	
9	H	-4.6	+	-3.3	2.2	1.4	H7	H1	-	
10	H	-2.4	+	-2.7	2.8	3.9	H13	H1	-	
11	M	-2.3		-2.6	-	9.5	H3	H59	-	
12	H	-2.4		-3.9	2.7	0.9	H11	H2	-	BA -2 yrs vs. CA
13	M	-2.1	++	-2.6	3.7	2.7	H3	H12	-	Pituitary hypoplasia
14	M	-3.8		-4.3	2.1	3.2	H19	H1	-	
15	H	-3.8		-2.6	3.5	4.0	H11	H2	-	Pituitary hypoplasia
16	H	-		-2.6	3.4	5.2	H7	H2	-	
17	H	-2.5		-3.5	5.0	5.9	H13	H8	-	SGA
18	H	-	++	-2.7	8.8	-	H13	H4	-	
19	M	-		-3.4	2.5	4.3	H7	H1	-	

(¥): Haplotype classification according to Horan et al., (2003) and Wolf et al., (2008); BA: bone age; CA: chron. age.

Red box: Hypomorphic allelic haplotypes

CONCLUSIONS

- **Up to 35.8% of the examined IGHD patients presented with hypomorphic allelic haplotypes of the *GH1* proximal promoter.**
- **The associated clinical phenotype is very similar to that presented by patients with type II IGHD** (height < -2.5 SDS; GH peak < 10 ng/ml; low IGF-I; SGA; pituitary hypoplasia; delayed bone age; good response to rhGH treatment).
- **Hypomorphic allelic haplotypes of the *GH1* promoter may represent an important causative or contributing factor to IGHD, which has been underestimated so far.**

References

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