RESEARCH NOTE

Analysis of common *SHOX* gene sequence variants and ~4.9-kb PAR1 deletion in ISS patients

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Introduction

Defects of the *SHOX* gene (short stature homeobox-containing gene), localized in the pseudoautosomal region 1 (PAR1) have been associated with Léri–Weill dyschondrosteosis (LWD; MIM ID: 127300) (heterozygous microdeletion or causal point mutation), Langer syndrome (MIM ID: 249700) (homozygous defect), and idiopathic short stature (ISS; MIM ID: 300589). The same pathological phenotype can be caused by an aberration in flanking regulatory sequences of *SHOX* gene (f.e. Flanagan *et al.* 2002). Defects of the *SHOX* gene or its regulatory sequences are the origin of LWD in about 70% of patients (Kosho *et al.* 1999; Ogata *et al.* 2002; Hirschfeldova *et al.* 2012) and in about 3% of ISS patients (f.e. Rappold *et al.* 2002). What we do not know is the role of common *SHOX* gene sequence variants and a small common ~4.9-kb deletion (MLPA kit P018 SHOX, probe L05101) ~200 kb downstream of *SHOX* gene near its known regulatory sequences (f.e. Benito-Sanz *et al.* 2006; Hirschfeldova *et al.* 2012). Common variants could be responsible for common phenotypes such as ISS. A common variant can act as a predisposition and should be more frequent in patients but their presence in controls is not excluded. Deletion L05101 is quite common in ISS and LWD groups but was also detected in healthy people (Chen *et al.* 2009).

Materials and methods

The population group was 84 healthy individuals (51 women and 33 men). We used quantitative real-time PCR with a TaqMan probe specific for L05101 deletion (Custom TaqMan, Applied Biosystems, Prague, Czech Republic) for population sample analysis. We considered difference in threshold cycle number C_t between the locus examined by the L05101 probe and the control locus of the *GADPH* gene in one reaction. For L05101 locus we used forward primer 5′-CGGGAAATCGTAACCACTGTCA-3′, reverse primer 5′-GGAATTGGAGAATGCGGTTTGTTAA-3′ and FAM-labelled TaqMan probe 5′-CTGAGAGACCCAAATTG-3′. For the *GADPH* locus we used a probe that targeted exonic sequences (kindly provided by Ales Horinek). We expected that if C_t(L05101)–C_t(GADPH) = n go for people without deletion in L05101, than for people with heterozygous deletion go C_t(L05101)–C_t(GADPH) = $n + 1$. The frequency of L05101 deletion in the ISS group was obtained in well-characterized ISS group from our previous study as a part of MLPA analysis (Hirschfeldova *et al.* 2012). Only *SHOX*-defect-negative ISS patients were included ($N = 45$) for this purpose.

Direct sequencing of *SHOX* gene exon 1 and a noncoding part of exon 2 was conducted in the ISS group (primers on request). Exon one analysis was designed to cover adjacent 5′ end sequences. Analyses of coding part of exon 2 and of exons 3, 4, 5, 6a and 6b were conducted as a part of our previous study. In population sample only exons where polymorpic variants were detected in the ISS group were analysed (exons 1, 2, 6b).

Each polymorphic site was tested for Hardy–Weinberg equilibrium (HWE). To analyse an indirect effect of polymorphic variants we estimated common haplotypes. The linkage disequilibrium measurement between each pair of polymorphic sites (significance level = 0.05) and haplotype frequencies estimation based on a Gibbs sampling strategy were done using

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Keywords. *SHOX*; deletion; PAR1; idiopathic short stature; polymorphism.

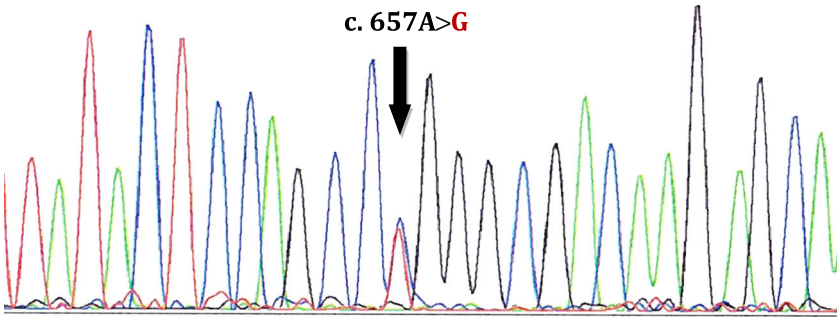

Figure 1. Polymorphic variant detected in exon 6b of *SHOX* gene (antisense strand).

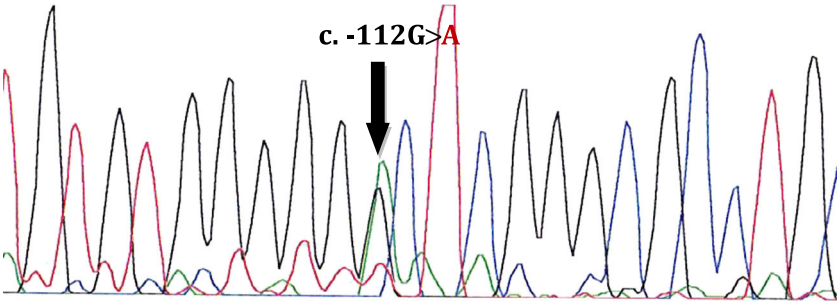

Figure 2. Polymorphic variant detected in exon 2 of *SHOX* gene (untranslated part).

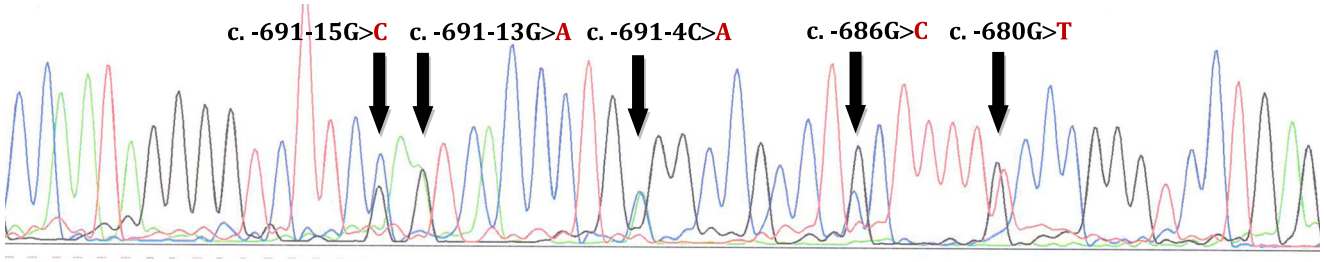

Figure 3. Polymorphic variants detected in the 5′ end and exon 1 of *SHOX* gene.

Arlequin software ver. 3.1. (Excoffier *et al.* 2005). Rare allele and haplotype frequencies were compared by chi-squared test (STATISTICA ver. 9.1) (StatSoft 2010).

Results

In population sample L05101 deletion was found in 11 individuals (nine women and two men) (13.1%). In ISS group we detected the L05101 deletion in six probands (13.3%). It reflects the L05101 deletion frequency is not significantly different between patiens with ISS and population sample ($P = 0.05$). No significant difference of the L05101 deletion frequnency was detected when comparing men and women in both ISS patients and in the population group.

Overall, 10 polymorphic variants were detected in the ISS group and population sample using the direct sequencing of the *SHOX* gene and adjacent 5′ regulatory sequences

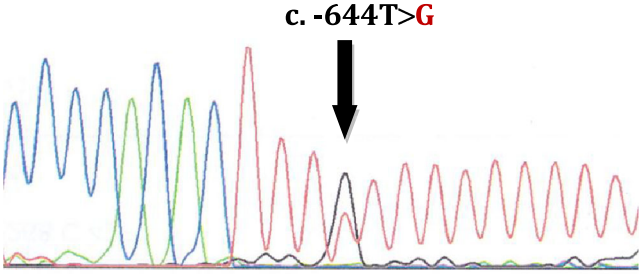

Figure 4. Polymorphic variants detected in exon 1 of *SHOX* gene.

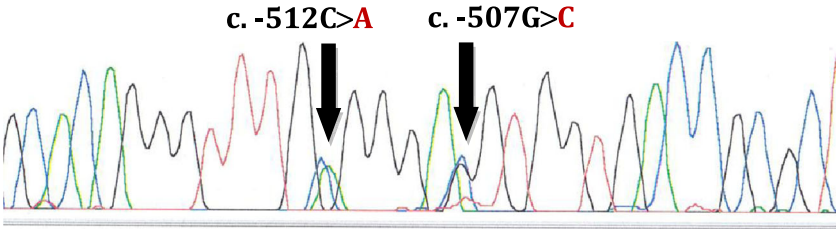

Figure 5. Polymorphic variants detected in exon 1 of *SHOX* gene.

Table 1. Frequency and localization of common *SHOX* gene polymorphic sites in the ISS group and population sample. Sequence variants are described according to HGVS recommendations based on the coding DNA reference sequence (NM_006883.2) (den Dunnen and Antonarakis 2000).

Variant	Localization		Frequency ± SD		SHOX	
			ISS group	Population	Database ID[1]	refSNP
c. −691 -15G>C	5′ end	Promoter	0.244 ± 0.047	0.253 ± 0.032	_	_
c. −691 -13G>A	5′ end	Promoter	0.244 ± 0.047	0.253 ± 0.032	_	_
c. −691 -4C>A	5′ end	Promoter	0.268 ± 0.049	0.242 ± 0.031	_	_
c. −686 G>C	Exon 1	Promoter	0.244 ± 0.047	0.242 ± 0.031	SHOX_00163	rs28475683
c. −680 G>T	Exon 1	Promoter	0.256 ± 0.048	0.258 ± 0.032	SHOX_00216	rs3813940
c. −644 T>G	Exon 1	Promoter	0.354 ± 0.053	0.300 ± 0.033	_	_
c. −512 C>A	Exon 1	Promoter	0.025 ± 0.017	0.042 ± 0.015	SHOX_00365	rs113313554
c. −507 G>C	Exon 1	Promoter	0.325 ± 0.052	0.363 ± 0.035	SHOX_00366	rs111549748
c. −112 G>A	Exon 2	Promoter	0.110 ± 0.035	0.135 ± 0.025	_	_
c. 657 A>G	Exon 6b	p.Pro219Pro	0.452 ± 0.054	0.422 ± 0.036	SHOX_00135	Flanagan *et al.* (2002)

[1]SHOX @ http://www.hd-lovd.uni-hd.de/.

(figures 1–5) (table 1). Five polymorphisms were already described in the *SHOX* database. The position of polymorphic variants and corresponding rare allele frequencies in both study groups are summarised in table 1. Allele frequencies were in HWE in both ISS group and population sample. There was no statistically significant difference in the rare allele frequencies between the ISS group and population sample. Linkage disequilibrium between each pair of polymorphic sites was analysed. Strong linkage disequilibrium was only preserved among polymorphic sites from 5′ end sequences and exon 1. The c. − 512C > A polymorphism was excluded from further analysis because of low frequency. Nine-pol haplotypes were estimated in both study groups and frequencies were compared. Only haplotypes estimated to have frequency of at least 2% in the population sample were included for association analyses. Overall 14 9-pol haplotypes were analysed. There was no statistically significant difference in haplotype frequencies between the ISS group and the population sample ($P = 0.05$).

No linkage disequilibrium was detected between the *SHOX* gene polymorphic sites and the L05101 deletion.

Conclusion

The frequency of common L05101 deletion (~4,9 kb) is not significantly different between the ISS group and the population sample. Absence of linkage disequilibrium between the *SHOX* gene polymorphic sites and the L05101 deletion is in compliance with high recombination rate in the area (May *et al.* 2002). Our results correspond with conclusions published by Benito-Sanz *et al.* (2011), who found L05101 deletion in 11.5% of patients with ISS and in 12.1% of healthy people. Further, comparing our data and data from Benito-Sanz, we found no significant difference in the L05101 deletion frequency between men and women in ISS patients as well as in the population group. We are convinced that we can confirm the thesis of Benito-Sanz *et al.* (2011) that this small PAR1 deletion represents a nonpathogenic polymorphism.

Common sequence variants were detected in the *SHOX* gene coding sequences including 5′ end region. Especially the 5′ end and exon 1 seem to be quite polymorphic. A strong linkage disequilibrium between corresponding polymorphic sites is comprehensible. Nonsignificant linkage disequilibrium between these sites and more distant *SHOX* gene polymorphic variants seems to be due to a high local recombination rate and the different age. No association was detected for common sequence variants nor single nor 9-pol haplotypes with the ISS phenotype.

Acknowledgement

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