Abstract

The Hedgehog signalling pathway is involved in regulation of differentiation of embryonic cells, in body patterning, in development of brain, bone, muscle, gastrointestinal tract, lungs, and in maintenance and regeneration of adult tissues. The pathway includes more than 10 proteins: receptors, coreceptors, ligands, transcription effectors and repressors, linked in complex functional interactions. Disruption of the hedgehog signalling during embryogenesis can lead to a serious developmental disorder – to holoprosencephaly. Loss-of-function mutations of PTCH1 lead to Gorlin syndrome – a hereditary predisposition to basal cell carcinoma associated with anomalies of brain, scull, vertebrae, and ribs. Both holoprosencephaly and Gorlin syndrome have been shown to be genetically heterogeneous, both can be caused by germline mutations of several genes of the Hedgehog signalling pathway.

From 2006 to 2016, the PTCH1 gene was analysed for diagnostic purposes in 70 unrelated patients with suspicion of Gorlin syndrome (MIM 109400) referred to the Department of Biology and Medical Genetics, 2nd Medical School and University Hospital Motol, Prague. A pathogenic variant of the PTCH1 gene was detected in 35 (50%) of patients. No mutation was found in 35 patients, 10 of them fulfilled, and 25 of them did not fulfil diagnostic criteria.

The aim of this study was to expand diagnostic possibilities in Gorlin syndrome and in holoprosencephaly and to test the hypothesis that Gorlin syndrome in the PTCH1-negative patients might have been caused by a pathogenic variant of the SUFU gene, which is also involved in the Hedgehog signalling pathway. DNA analysis of the SUFU gene by Sanger sequencing and MLPA, and DNA analysis of the PTCH1 gene and the SUFU gene by an NGS gene panel have been introduced for this purpose.

In a pilot study, the SUFU gene was tested in twenty PTCH1-negative patients by Sanger sequencing. In one patient, both PTCH1 and SUFU genes were sequenced by Sanger sequencing, and in nine patients, both PTCH1 and SUFU genes were tested by the NGS gene panel.

In the group of patients studied, no pathogenic variant was detected in the SUFU gene. Two novel, highly likely pathogenic variants were detected in the PTCH1 gene in two female patients who fulfilled two major and several minor criteria: heterozygous c.3037dupT
p.(Tyr1013Leufs*132) in exon 18, and heterozygous c.3306+1G>T, p.(?) in a splice site of exon 19 (RefSeq. GenBank NM_000264.4).

The study has brought new data about the genetic basis of Gorlin syndrome and has helped improve and extend molecular genetic diagnostics in the Czech Republic. Germline mutations of the SUFU gene are probably a very rare cause of Gorlin syndrome in the Czech Republic. Implementation of NGS techniques has improved molecular genetic diagnostics of Gorlin syndrome and holoprosencephaly, disorders with possible disruption of the Hedgehog signalling pathway.

**Key words:** Hedgehog pathway, Gorlin syndrome, Holoprosencephaly, PTCH1 gene, SUFU gene, mutation analysis, mutation