

REVIEW ARTICLE

The Sonic Hedgehog–Patched–Gli Pathway in Human Development and Disease

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Introduction

Embryogenesis is regulated by a number of complex signaling cascades, which are critical for normal development. One such pathway begins with a secreted protein called "SONIC HEDGEHOG" (SHH [MIM 600725]), which sets off a chain of events in target cells, leading to the activation and repression of target genes by transcription factors in the Gli family. Dysregulation of the Sonic hedgehog–Patched–Gli (Shh-Ptch-Gli) pathway leads to several human diseases, including birth defects and cancers.

Elements of the Shh-Ptch-Gli pathway are highly conserved, indicating its essential role in development. However, Shh signaling is also apparently susceptible to evolutionary pressures. Thus, Shh signaling has been adapted to serve tremendously diverse functions in a wide array of animal models, in both embryonic and adult life. Shh and the downstream molecules involved in signal transduction vary, to some extent, between animals, as well. Although a great deal can be learned from the study of animal models, it is essential to not assume that what is true in one model system is necessarily true in another. Thus, this review focuses specifically on our current understanding of the Shh-Ptch-Gli pathway and its clinical significance in humans.

The Human SHH-PTCH-GLI Signaling Pathway

During the past few years, a great deal of information about the mechanisms and significance of human SHH-PTCH-GLI signaling has emerged. SHH induces cell proliferation in a tissue-specific manner during embryogenesis, and inherited or sporadic mutations in SHH-pathway genes have been implicated in a number of human birth defects. If the SHH-PTCH-GLI pathway is aberrantly activated during adult life, the resulting cel-

lular proliferation manifests as cancer. Thus, studying the human SHH-PTCH-GLI pathway is essential in the understanding of the mechanisms of human birth defects and cancer and, ultimately, in the identification therapeutic targets in SHH-pathway diseases.

Our current understanding of the human SHH-PTCH-GLI pathway and its role in human disease is outlined in figure 1. Since the Shh-pathway components, their tissue specificity, and their functions vary between species (Lee et al. 1997; Sasaki et al. 1997; Brewster et al. 1998; Keys et al. 1999; King and Brown 1999), only information from human genes and proteins is presented here. Interestingly, defects in any of several steps in the SHH-PTCH-GLI pathway lead to similar clinical phenotypes, presumably through functionally equivalent effects on downstream target genes.

Sonic Hedgehog

During development of the human embryo, *SONIC HEDGEHOG* (*SHH*) is expressed in the notochord, the floorplate of the neural tube, the brain, the zone of polarizing activity in the developing limbs, and the gut (Odent et al. 1999). The tissue-specific expression of other SHH-pathway members has not yet been determined in human embryogenesis, although *GLI1* (MIM 165220), *GLI2* (MIM 165230), and *GLI3* (MIM 165240) RNA expression is known to persist in some adult human tissues (Ruppert et al. 1988). Sporadic and inherited mutations in the human *SHH* gene have been shown to cause holoprosencephaly (HPE [MIM 236100]), a severe midline defect that includes cleft lip and palate, single maxillary incisor, impaired CNS septation, and phenotypes ranging in severity from hypotelorism to a single cyclopic eye (Belloni et al. 1996; Roessler et al. 1996, 1997; Nanni et al. 1999). HPE is a frequent cause of prenatal death in humans; it is detected in 1/250 induced abortions but in only 1/16,000 live births (Cohen 1989).

The SHH protein needs to be processed to an active form in order to function in developmental patterning. SHH is cleaved to an active N-terminal form, which is then modified by the addition of a cholesterol moiety (Pepinsky et al. 1998; Williams et al. 1999). Defects in cholesterol biosynthesis lead to the autosomal recessive

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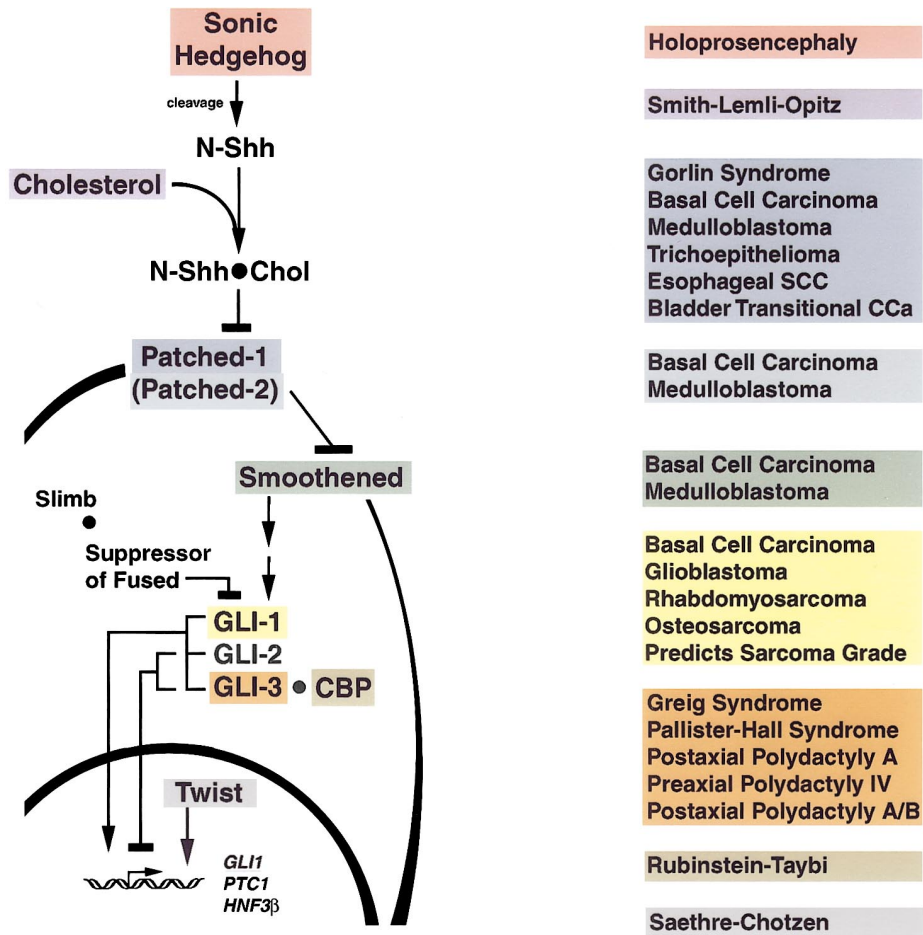


Figure 1 Human SHH-PTCH-GLI pathway (*left*) and its links to human diseases (*right*). The association of the human disease with malfunction of a given element in the pathway is indicated by the matching colors of the boxes. The placement of the elements of the pathway, as well as their role as a repressor (*bars*) or as an activator (*arrows*), has been demonstrated in humans. The exceptions are that interaction between human GLI3 and CBP was demonstrated using mouse CBP (Dai et al. 1999), human *GLI* genes were tested in frog and mouse cells (Ruiz i Altaba 1998; Dai et al. 1999), and mouse *gli2* protein function is shown for completeness, although human *GLI2* has not been fully characterized (Tanimura et al. 1998; Sasaki et al. 1999).

Smith-Lemli-Opitz (SLOS [MIM 270400]) syndrome in humans, which shares some features with SHH-pathway diseases. This suggests that some or all of the clinical features of SLOS may be due to defective SHH signaling (Donnai et al. 1987; Tint et al. 1994; Kelley et al. 1996). Patients with SLOS generally have syndactyly, anteverted nares, ptosis, cryptorchidism, CNS hypoplasia, failure to thrive, occasional polydactyly and HPE, and, in a subset of patients, death within the 1st year (Kelley et al. 1996; Jones 1997).

PATCHED-1, PATCHED-2, and SMOOTHENED

SHH functions by binding its 12-span transmembrane receptor proteins, PATCHED-1 (PTCH-1 [MIM 601309]) and PATCHED-2 (PTCH-2 [MIM 603673]), in target cells (Stone et al. 1996; Smyth et al. 1999).

PATCHED-1 and, possibly, PATCHED-2, normally inhibit downstream signaling through a physical interaction with a 7-span transmembrane protein, SMOOTHENED (SMOH [MIM 601500]). In model systems in which Shh is expressed nearby, it relieves the tonic inhibition of Smoothened by Patched and allows Shh-Ptch-Gli signaling to progress (Murone et al. 1999).

Like all of the other components of the SHH-PTCH-GLI pathway, the *PATCHED* and *SMOOTHENED* genes are also clinically relevant. The *PTCH* gene functions as a tumor suppressor, and activating *SMOH* mutants have been shown to function as oncogenes (Stone et al. 1996; Xie et al. 1998). Mutations of human *PTCH-1*, *PTCH-2*, and *SMOH* have all been detected in basal cell carcinomas and in medulloblastomas (MIM 155255) and result in dysregulated GLI signaling. Sporadic inactivating *PTCH-1* mutations have also been

Table 1**Human *GLI* Genes and Associated Diseases**

Gene (Locus)	Associated Human Disease(s)	Presumed Mechanism(s)
<i>GLI1</i> (12q13)	Glioblastoma	Amplification
	Rhabdomyosarcoma	Amplification
	Osteosarcoma	Amplification
	B cell lymphoma	Amplification
	Basal cell carcinoma	Misexpression
	Predicts adult sarcoma grade	Misexpression
<i>GLI2</i> (2q14)	Unknown	Unknown
<i>GLI3</i> (7p13)	Greig cephalopolysyndactyly syndrome	Deletion, point mutation, or translocation
	Pallister-Hall syndrome	Frameshift or nonsense mutation
	Postaxial polydactyly type A	Frameshift
	Postaxial polydactyly type A/B	Frameshift, nonsense, or missense mutation
	Preaxial polydactyly type-IV	Frameshift
<i>GLI4</i> (<i>HKR4</i>) ^a (8q24.3)	Unknown	Unknown

^a Gene encodes a Krüppel-like protein and was named “*GLI4*” by the Human Gene Mapping Workshops.

reported in trichoepitheliomas (Vorechovsky et al. 1997), esophageal squamous cell carcinomas (Maesawa et al. 1998 [MIM 133239]), and transitional cell carcinomas of the bladder (McGarvey et al. 1998 [MIM 109800]). Furthermore, *PTCH-1*-inactivating mutations cause the genetic disease nevoid basal cell carcinoma syndrome (NBCCS), also called “Gorlin syndrome” or “basal cell nevus syndrome” (BCNS [MIM 109400]), which is an autosomal dominant disorder characterized by basal cell nevi with a high rate of malignant transformation, an increased risk of medulloblastomas and rhabdomyosarcomas, generalized overgrowth, and developmental defects including short metacarpals, rib defects, broad facies, and dental abnormalities (Bale et al. 1991; Gorlin 1995; Jones 1997).

The *GLI* Family

The *GLI1* gene was originally identified as an amplified gene in a malignant glioma (Kinzler et al. 1987). It was the first member described in the human *GLI* gene family. During the past decade, four human genes have been designated “*GLI1*”–“*GLI4*”, and these genes have been mapped to separate loci by FISH (Arheden et al. 1989; Ruppert et al. 1990; Kas et al. 1996; Matsumoto et al. 1996).

GLI1, *GLI2*, and *GLI3* encode transcription factors that share five highly conserved tandem C₂-H₂ zinc fingers and a consensus histidine-cysteine linker sequence between zinc fingers (Ruppert et al. 1988). These features distinguish between the similar Gli and Krüppel protein families (Kinzler et al. 1988). The *GLI1* and *GLI3* proteins recognize a conserved GACCACCCA sequence in the promoters of target genes (Kinzler and Vogelstein 1990; Ruppert et al. 1990; Pavletich and Pabo 1993; Vortkamp et al. 1995), and *GLI2* recognizes a nearly identical GAACCACCCA motif (Tanimura et al. 1998). *GLI4*, which is also known as “*HKR4*” (hu-

man Krüppel-related gene 4), is actually most similar to the *Drosophila* Krüppel family of zinc-finger proteins (Ruppert et al. 1988) and was misclassified as a member of the human *GLI* gene family (*GLI4* [MIM 165280]) (Kas et al. 1996). This nomenclature issue has caused confusion in the Gli literature during recent years. Throughout the present report, the human *GLI* family of genes refers to *GLI1*–*GLI3*. The chromosomal locations of human *GLI1*–*GLI4* are summarized in table 1.

GLI1

In addition to its amplification in glioblastomas (Kinzler et al. 1987), *GLI1* has also been detected as an amplified gene in some osteosarcomas, rhabdomyosarcomas, and B cell lymphomas (Roberts et al. 1989; Werner et al. 1997), as shown in table 1. The clinical significance of *GLI1* amplification in tumors is unknown, since the *MDM2* (MIM 164785) and *CDK4* (MIM 123829) genes are usually coamplified. *GLI1* is also overexpressed in basal cell carcinomas, compared with that in normal human skin (Dahmane et al. 1997; Ghali et al. 1999). The degree of *GLI1* overexpression (whether by amplification or mutation) correlates with tumor grade in adult bone and soft-tissue sarcomas (Stein et al. 1999). *GLI1* has been classified as an oncogene on the basis of its ability to transform cells in cooperation with adenovirus E1A (Ruppert et al. 1991).

Expression of human *GLI1* in transgenic mice results in a range of developmental defects, including failure to thrive and Hirschsprung-like dilatation of the gastrointestinal (GI) tract (Yang et al. 1997). The GI-tract phenotype is consistent with endogenous mouse *gli1* expression patterns (Walterhouse et al. 1993) and with recent evidence that, in the mouse, Shh is responsible for radial patterning of the intestine (Sukegawa et al. 2000).

GLI2

Although no defects in *GLI2* have been correlated, to date, with human diseases, studies in mice suggest that associations with basal cell carcinomas, skeletal defects, and other disorders are likely (Sasaki et al. 1999; Grachtchouk et al. 2000; Park et al. 2000).

GLI3

Mutations in human *GLI3* have been implicated in several types of birth defects. Translocations, deletions, and point mutations throughout the *GLI3* gene cause Greig cephalopolysyndactyly syndrome (GCPS [MIM 175700]), which is characterized by syndactyly, predominantly preaxial polydactyly, broad thumbs and first toes, and facial anomalies such as hypertelorism and frontal bossing (Vortkamp et al. 1991; Jones 1997; Wild et al. 1997; Kalff-Suske et al. 1999). *GLI3* frameshift and nonsense mutations have also been linked to Pallister-Hall syndrome (PHS [MIM 146510]), an autosomal dominant disease involving hypothalamic hamartoma, central or postaxial polydactyly, syndactyly, imperforate anus, anteverted nares and other facial abnormalities, and, occasionally, associated HPE and malformations of the axial skeleton (Jones 1997; Kang et al. 1997). Recently, frameshift, nonsense, and missense *GLI3* mutations were detected in the human polydactyly syndromes postaxial polydactyly type A (PAPA [MIM 174200]), autosomal dominant preaxial polydactyly type IV (MIM 174700), and postaxial polydactyly type A/B (Radhakrishna et al. 1997, 1999). Contrary to early reports, the type and location of *GLI3* mutations do not appear to correlate with clinical disease phenotype (Kalff-Suske et al. 1999). Table 1 summarizes the *GLI* family's associations with human birth defects and cancers.

Suppressor of Fused

Little is known, in human cells, about how information gets from the cell surface to the human GLI proteins, which are downstream effectors of SHH via PTCH and SMOH (Sasaki et al. 1999). In the homologous *Drosophila* system, this process involves the serine-threonine kinase Fused (Fu), the kinesin-related protein Costal-2 (Cos-2), and a physical interaction with microtubules (Robbins et al. 1997; Sisson et al. 1997). However, Cos-2 and Fu have not yet been demonstrated in vertebrates. The only cytoplasmic element that has been shown to function upstream of the *GLI* genes in human cells is Suppressor of Fused (Su[*fu*]), which cooperates with the F-box-containing protein Slimb to inhibit transactivation by GLI1 (Stone et al. 1999). Su(*fu*) can inhibit SHH-induced osteogenic differentiation and can sequester

GLI1 in the cytoplasm (Kogerman et al. 1999), but it is currently unclear whether Su(*fu*) acts immediately downstream of *PATCHED* within the SHH pathway or, rather, as an independent regulator of GLI-mediated signaling. No human diseases have been associated, to date, with defects in Su(*fu*).

CREB-Binding Protein (CBP)

Studies of human *GLI3* protein have shown that it is bound and coactivated by mouse CBP (MIM 600140), which is a common transcriptional coactivator (Akimaru et al. 1997; Dai et al. 1999). Several studies have shown that human mutations leading to haploinsufficiency of CBP cause Rubinstein-Taybi syndrome (RTS [MIM 180849]) (Petrij et al. 1995; Blough et al. 2000). Rubinstein-Taybi syndrome is a developmental disorder with symptoms that include broad thumbs and first toes, syndactyly, short stature, delayed osseous maturation, vertebral anomalies, mental retardation, dysmorphic facies, cryptorchidism, and an increased risk of neural and developmental tumors (Rubinstein 1990; Miller and Rubinstein 1995; Jones 1997). The similarity between the Rubinstein-Taybi phenotype and diseases caused by *GLI3* mutations suggests that the biochemical interaction between CBP and *GLI3* may significantly influence SHH signaling.

TWIST

A novel interaction between the SHH-PTCH-GLI pathway and TWIST (MIM 601622), the developmental regulatory gene and potential oncogene (Gitelman 1997; Bourgeois et al. 1998; Maestro et al. 1999), has been demonstrated (authors' unpublished observation). Mouse Twist protein can activate human *GLI1* at the transcriptional level by interacting with E-boxes in *GLI1*'s first intron. Nonsense, missense, deletion, and insertion mutations in several regions of the human *TWIST* gene have been shown to cause Saethre-Chotzen syndrome (SCS [MIM 101400]), an autosomal dominant disease characterized by craniosynostosis, asymmetrical facies, syndactyly, broad thumbs and first toes with or without partial reduplication, ossification defects, and prominent ear crura (El Ghouzzi et al. 1997; Howard et al. 1997). Alternatively, some patients with Saethre-Chotzen have *TWIST* mutations that appear to disrupt either the stability of TWIST protein or its ability to correctly localize to the nucleus (El Ghouzzi et al. 2000). The clinical phenotype of Saethre-Chotzen syndrome partially overlaps with those of other Shh-pathway-related human diseases discussed above, indicating that dysregulation of Gli-family transcription factors by either Shh-signaling defects or *TWIST* mutations causes similar downstream effects. It has not yet been deter-

mined whether TWIST is regulated by SHH signaling, but TWIST does appear to be linked to SHH signal transduction.

GLI Research: Progress and Controversies

As the most downstream elements within the SHH-PTCH-GLI signaling cascade, GLI proteins are the key regulators of target-gene expression in response to SHH signaling. Although it is known that the three *GLI* genes encode zinc-finger transcription factors in the SHH-PTCH-GLI pathway, there are many controversies and unanswered questions.

There has been a great deal of discussion in recent years as to whether the Gli proteins function as transcriptional activators, repressors, or both. In several experimental systems, human GLI1 has been shown to contain a VP16-like, C-terminal activation domain and to be a transcriptional activator of both endogenous genes such as *HNF-3 β* (MIM 600288) and reporter constructs containing the GLI1 binding site. GLI1 repressor function has never been observed (Lee et al. 1997; Yoon et al. 1998; Dai et al. 1999).

Different groups have reported strong data suggesting that GLI3 either activates (Dai et al. 1999) or represses (Wang et al. 2000) *GLI1* transcription or protein activity. Human GLI3 appears to contain the C-terminal activation domain, in addition to an N-terminal repression domain, and can, in model systems, apparently function as either an activator or repressor of downstream genes such as *HNF-3 β* (Sasaki et al. 1999), *Gli1*, and *Ptch-1*. The mechanism of switching between activation and repression has not been unequivocally determined, although cleavage of the GLI3 protein to release activating or repressing fragments has been suggested as one possibility. Current data suggest that human GLI2 works only as an activator, whereas mouse *gli2* can function as either an activator or a repressor (Lee et al. 1997; Sasaki et al. 1997, 1999; Tanimura et al. 1998; Dai et al. 1999; Ruiz i Altaba 1999; Shin et al. 1999; von Mering and Basler 1999).

The homologue of GLI in *Drosophila*, *cubitus interruptus* (*ci*), is localized in the cytoplasm, and its translocation to the nucleus, where it functions as a transcription factor, is part of the Hedgehog-signaling response. The subcellular localization of Gli proteins within the cell is a matter of debate. Immunohistochemistry of several human tumor cell lines has demonstrated GLI1 protein in both the nucleus and the cytoplasm, in varying proportions in different cell lines (Stein et al. 1999). Other reports have noted GLI1 only in the nucleus of Tera-1 and D259MG cells (Kinzler and Vogelstein 1990) or mainly in the cytoplasm in basal cell carcinomas (Ghali et al. 1999).

Nonhuman experimental systems have also been used

to study the localization of human GLI proteins, with varying results. In transfection studies of monkey COS cells, GLI1 accumulated primarily in the nucleus and, to a limited extent, in the cytoplasm (Dahmane et al. 1997; Lee et al. 1997). However, in transfected 10T1/2 mouse cells, human GLI1 localized primarily to the cytoplasm, particularly in the presence of transfected Su(fu) (Stone et al. 1999). GLI3 localization also differed between cell lines, with mainly cytoplasmic labeling in COS cells (Lee et al. 1997) and nuclear staining in 10T1/2 cells (Ruiz i Altaba 1999). In one study, transfected HeLa cells showed full-length GLI3 in the cytoplasm, GLI3 with a PHS-type frameshift in the nucleus, and GLI3 with a Greig syndrome-type frameshift in both the cytoplasm and nucleus (Shin et al. 1999). It has been suggested, on the basis of experiments with mouse fibroblasts and frog tissues, that N-terminal GLI-protein motifs target proteins to the nucleus, whereas the C-terminal regions of GLI proteins contain a cytoplasmic tethering domain (Ruiz i Altaba 1999). The discrepancies between these experiments may reflect differences in localization of GLI that are based on different tissues or different cell-cycle states. Furthermore, it is unclear whether the subcellular localization of Gli proteins observed in nonhuman transfected cells accurately reflects the subcellular localization of human GLI in wild-type human cells and whether human tumor cells localize GLI in the same manner as do wild-type cells. Another unresolved issue in human GLI studies is whether any of the GLI proteins are cleaved, since GLI's homologue in *Drosophila*, *ci*, requires cleavage into separate activator and repressor forms (Methot and Basler 1999).

There has been little convincing evidence to suggest that human GLI1 is cleaved in vivo. GLI1 antibody has demonstrated a major 150-kD band in human Tera-1 (teratocarcinoma) and human RMS (rhabdomyosarcoma) cells and, independently, a single 150-kD band in human D259 (glioblastoma) cells (Kinzler and Vogelstein 1990; Ruppert et al. 1991). Mouse-embryo extracts have demonstrated a single *gli1* protein band (Dai et al. 1999), but frog *gli1* has yielded multiple protein bands when transfected into human cells (Ruiz i Altaba 1999).

Reports from different groups studying different cell types have yielded conflicting data regarding GLI3. Human *GLI3* transfected into 10T1/2 cells has produced a single full-length band, whereas the same construct transfected into COS cells has shown a full-length band, as well as an ~100-kD band (Ruiz i Altaba 1999). In mouse cells, human GLI3 also produced a full-length band at all times, but additional processed forms were observed in the absence of Shh (Dai et al. 1999). Recently, PKA-dependent cleavage of human GLI3 to an 83-kD form was reported in transfected COS, mouse

limb, and chick limb cells, as well as in endogenous mouse and chick limb buds. Phosphorylation of full-length *GLI3* was apparently necessary for cleavage. No cleavage or phosphorylation of *GLI2* was observed (Wang et al. 2000).

Conclusions

Normal development is regulated by the complex interaction of gene hierarchies, and the central role of signal-transduction pathways in these gene hierarchies is well established. The Shh-Ptch-Gli pathway is one of the most critical of these during early development. Although some components and functions of the Shh-Ptch-Gli pathway are highly conserved, the implications of species-specific differences should not be overlooked. In humans, genetic disruption of SHH-PTCH-GLI-pathway components leads to a range of developmental defects, and aberrant SHH signaling in postnatal life leads to dysregulated cell growth, as in basal cell carcinomas. As such, study of the human SHH-PTCH-GLI pathway is critical for the understanding of the pathogenesis of SHH-related human diseases, as well as in the determination of potential therapeutic targets.

Electronic-Database Information

The URL and accession numbers for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for Shh [MIM 600725], *GLI1* [MIM 165220], *GLI2* [MIM 165230], *GLI3* [MIM 165240], HPE [MIM 236100], SLOS [MIM 270400], PTCH-1 [MIM 601309], PTCH-2 [MIM 603673], SMOH [MIM 601500], medulloblastomas [MIM 155255], esophageal squamous cell carcinomas [MIM 133239], transitional cell carcinomas of the bladder [MIM 109800], BCNS [MIM 109400], *GLI4* [MIM 165280], *MDM2* [MIM 164785], *CDK4* [MIM 123829], GCPS [MIM 175700], PHS [MIM 146510], PAPA [MIM 174200], preaxial polydactyly type IV [MIM 174700], CBP [MIM 600140], RTS [MIM 180849], Twist [MIM 601622], and SCS [MIM 101400])

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