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MUTATION UPDATE

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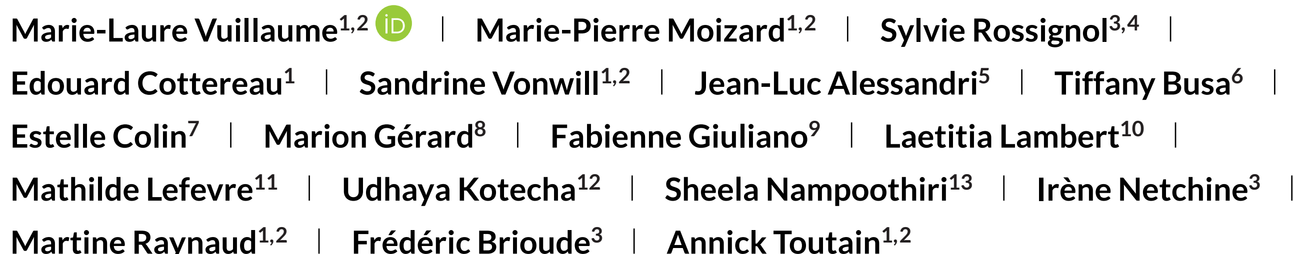
# Mutation update for the *GPC3* gene involved in Simpson-Golabi-Behmel syndrome and review of the literature



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## Abstract

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked multiple congenital anomalies and overgrowth syndrome caused by a defect in the glypican-3 gene (*GPC3*). Until now, *GPC3* mutations have been reported in isolated cases or small series and the global genotypic spectrum of these mutations has never been delineated. In this study, we review the 57 previously described *GPC3* mutations and significantly expand this mutational spectrum with the description of 29 novel mutations. Compiling our data and those of the literature, we provide an overview of 86 distinct *GPC3* mutations identified in 120 unrelated families, ranging from single nucleotide variations to complex genomic rearrangements and dispersed throughout the entire coding region of *GPC3*. The vast majority of them are deletions or truncating mutations (frameshift, nonsense mutations) predicted to result in a loss-of-function. Missense mutations are rare and the two which were functionally characterized, impaired GPC3 function by preventing GPC3 cleavage and cell surface addressing respectively. This report by describing for the first time the wide mutational spectrum of *GPC3* could help clinicians and geneticists in interpreting *GPC3* variants identified incidentally by high-throughput sequencing technologies and also reinforces the need for functional validation of non-truncating mutations (missense, in frame mutations, duplications).

KEYWORDS

GPC3, mutations, overgrowth, Simpson-Golabi-Behmel syndrome, X-linked disorder

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## 1 | INTRODUCTION

Simpson-Golabi-Behmel syndrome (SGBS) (MIM# 312870) is an X-linked disorder first reported by Simpson, Landey, New, and German (1975) and subsequently described by Golabi & Rosen (1984) and Behmel, Plöchl, and Rosenkranz (1984). Clinically SGBS is characterized by pre- and postnatal overgrowth, macrocephaly, dysmorphic facial features including coarse facies, extremities abnormalities, supernumerary nipples, organomegaly, cardiac, skeletal, gastrointestinal, and genitourinary malformations (Cottereau et al., 2013; Golabi, Leung, & Lopez, 2011). An increased risk of developing embryonal tumors, especially Wilms and liver tumors, is also associated with this syndrome and, in some cases, mild to moderate intellectual disability may be observed (Li et al., 2001; Neri, Gurrieri, Zanni, & Lin, 1998). This disorder is caused by loss-of-functional glypican-3 gene, *GPC3* (MIM# 300037). This gene which maps to Xq26.2, contains eight exons and has a full-length transcript of 2.568 kb (NM_004484), which codes for GPC3, a 70 kDa core protein of 580 amino acids. GPC3 is a member of the glypican family which includes six known mammalian heparan sulfate proteoglycans (HSPGs) that are bound to the exocytoplasmic surface of the plasma membrane through a covalent glycosylphosphatidylinositol (GPI) linkage. All glypicans share a characteristic structure with a conserved pattern of 14 cysteine residues and an heparan sulfate (HS) glycan chain in the C-terminal region close to the cell membrane. They regulate the signaling of WNTs, Hedgehogs, fibroblast growth factors, and bone morphogenetic proteins (Filmus, 2001; Filmus, Capurro, & Rast, 2008; Song & Filmus, 2002). GPC3, for its part, negatively regulates cell proliferation by inhibiting Hedgehog (Capurro et al., 2008) and by modulating WNT signaling pathways (Filmus & Capurro, 2013). In 2011, *GPC4* (MIM# 300138), a second gene coding for another member of the glypican family, was also suggested to be associated with SGBS. However, only one duplication of this gene has been reported in a family with SGBS by Golabi and Rosen (Waterson, Stockley, Segal, & Golabi, 2010). This duplication encompasses the whole *GPC4* gene with uncharacterized breakpoints as it was identified by multiplex ligation-dependent probe hybridization (MLPA). Moreover, no point mutations could be identified so far, questioning the exact role of this gene in the pathogenesis of SGBS (Cottereau et al., 2013). Finally, GPC4 deficient mice do not exhibit features suggestive of SGBS (The Jackson Laboratory, https://www.jax.org). Up to now, *GPC3* remains the principal monogenic contributor to SGBS.

From a clinical point of view, the SGBS has been well characterized. Indeed, the clinical features in a cohort of 42 patients with a molecularly confirmed diagnosis of SGBS were reviewed by our team in 2013, and compared with those of the literature in order to define specific clinical criteria for *GPC3* molecular testing. Nevertheless, the global genotypic spectrum of *GPC3* mutations has never been delineated even if isolated cases or small series of *GPC3* mutations were published. In this study, we review the 57 previously described *GPC3* mutations identified in 71 unrelated families. We significantly expand the mutational spectrum of *GPC3* with the description of 38 additional *GPC3* mutations, from which 29 were novel, in our patient cohort of 49 unrelated families.

## 2 | MUTATIONAL SPECTRUM

Table 1 details *GPC3* mutations published between March 1996 and December 2017 in the international peer-reviewed literature (PubMed database) and The Human Gene Mutation Database (HGMD professional 2016.4) following HGVS nomenclature guidelines (www.HGVS.org) and the reference sequence GenBank entry NM_004484.3. We collected 57 distinct *GPC3* mutations detected in 71 unrelated families (Agatep et al., 2014; Das Bhowmik & Dalal, 2015; Day & Fryer, 2005; DiMaio, Yang, Mahoney, McGrath, & Li, 2017; Ganesamoorthy et al., 2013; Garavelli et al., 2012; Gertsch, Kirmani, Ackerman, & Babovic-Vuksanovic, 2010; Gurrieri et al., 2011; Halayem et al., 2016; Hughes-Benzie et al., 1996; Kehrer et al., 2016; Kosaki et al., 2014; Li et al., 2001; Lindsay et al., 1997; Magini et al., 2016; Mariani et al., 2003; Mateos et al., 2013; Mujezinović et al., 2016; Ochiai et al., 2013; Okamoto, Yagi, Imura, & Wada, 1999; Pilia et al., 1996; Rodríguez-Criado et al., 2005; Romanelli et al., 2007; Sakazume et al., 2007; Schmidt, Hollstein, Kaiser, & Gillessen-Kaesbach, 2017; Shimojima et al., 2016; Spencer, Fieggen, Vorster, & Beighton, 2016; Støve et al., 2017; Thomas et al., 2012; Vaisfeld, Pomponi, Pietrobono, Tabolacci, & Neri, 2017; Veugelers et al., 1998, 2000; Villarreal et al., 2013; Weichert et al., 2011; Xuan, Hughes-Benzie, & MacKenzie, 1999; Yano et al., 2011; Young, Wishnow, & Nigro, 2006). In this study, we also report 38 *GPC3* mutations in 63 additional male patients from 49 unrelated families (Cf. Table 2 and Supp. Table S1). These mutations were submitted to LOVD database (https://databases.lovd.nl/shared/genes/GPC3). The carrier status of the proband's mother has been ascertained for 33 out of the 49 (67%) unrelated probands. Twenty seven of these mutations were inherited from the mother (82%) and only six mutations occurred de novo (18%) as indicated in Supp. Table S1. Twenty nine of these mutations are reported for the first time (Cf. Table 2).

Overall, including our data and those of the literature, a total of 86 distinct mutations were found in 120 unrelated patients. In silico predictions show that the majority of these mutations (49 out of 86) lead to the occurrence of a premature stop codon. Although all types of mutation were found, the most prevalent type was large deletions (34.9%) followed by frameshift mutations leading to a stop premature codon (24.4%), nonsense mutations (16.3%), missense mutations (8.1%), large duplications (8.1%), splice site mutations (4.7%), translocations (2.3%), and one in frame mutation (1.2%) (Cf. Figure 1). Most mutations are unique: 86% of mutations (74 out of 86) have been found only in single families, 9.3% (eight out of 86) between 2 and 5 families and 4.6% (four out of 86) have been found in more than six families.

### 2.1 | Point mutations

Forty-seven distinct point mutations including one base substitutions and small insertions/deletions (54.6%) were reported in 61 out of the 120 unrelated probands. A schematic view of *GPC3* gene and the distribution of these point mutations are presented in Figure 2. Mutations were scattered along the entire coding sequence of *GPC3* (Figure 2). The distribution of point mutations was not uniform although they were found in every exon. Nearly half of the pathogenic

**TABLE 1** *GPC3* mutations associated with Simpson-Golabi-Behmel Syndrome previously reported by other groups

| Mutation type | Position | Nucleotide change | Amino-acid change | Variant reference (dbSNP, ClinVar) | Unrelated families in the literature | References |
|---|---|---|---|---|---|---|
| **Missense** | Exon 3 | c.886T>A | p.(Trp296Arg) | rs104894854, RCV000012453.23 | 1 | Veugelers et al. (2000) |
| **Nonsense** | Exon 2 | c.195T>A | p.(Cys65[a]) | | 1 | Veugelers et al. (2000) |
| | Exon 2 | c.256C>T | p.(Arg86[a]) | | 3 | Gertsch et al., 2010; Sakazume et al., 2007; Yano et al., 2011 |
| | Exon 3 | c.346G>T | p.(Glu116[a]) | | 1 | Magini et al., 2016 |
| | Exon 3 | c.595C>T | p.(Arg199[a]) | rs104894855, RCV000012455.15 | 1 | Veugelers et al., 2000 |
| | Exon 3 | c.691C>T | p.(Gln231[a]) | | 1 | Sakazume et al., 2007 |
| | Exon 3 | c.760C>T | p.(Arg254[a]) | | 1 | Sakazume et al., 2007 |
| | Exon 3 | c.999T>A | p.(Tyr333[a]) | | 1 | Kehrer et al., 2016 |
| | Exon 3 | c.1018A>T | p.(Lys340[a]) | | 1 | Veugelers et al., 2000 |
| | Exon 4 | c.1159C>T | p.(Arg387[a]) | rs122453121, RCV000012460.15 | 3 | Kosaki et al., 2014; Romanelli et al. 2007; Sakazume et al., 2007 |
| | Exon 5 | c.1276C>T | p.(Gln426[a]) | | 1 | Gurrieri et al., 2011 |
| | Exon 6 | c.1330C>T | p.(Gln444[a]) | | 1 | Shimojima et al., 2016 |
| | Exon 7 | c.1515del | p.(Cys505[a]) | | 1 | Okamoto et al., 1999 |
| **In frame with stop gain** | Exon 3 | c.780_785delinsAGC | p.(Trp260[a]) | | 1 | Sakazume et al., 2007 |
| **Frameshift** | Exon 1 | c.90_91dup | p.(Pro31Argfs[a]54) | | 1 | Shimojima et al., 2016 |
| | Exon 2 | c.194_206del | p.(Cys65Serfs[a]15) | RCV000012451.25 | 1 | Xuan et al., 1999 |
| | Exon 2 | c.240dup | p.(Tyr81Ilefs[a]36) | | 1 | Sakazume et al., 2007 |
| | Exon 3 | c.595_597delinsGG | p.(Leu205[a]) | | 1 | Garavelli et al., 2012 |
| | Exon 3 | c.758del | p.(Gly253Alafs[a]16) | | 1 | Shimojima et al., 2016 |
| | Exon 3 | c.767del | p.(Leu256Profs[a]13) | | 1 | Veugelers et al., 2000 |
| | Exon 3 | c.845dup | p.(Met282Ilefs[a]12) | | 1 | Kehrer et al., 2016 |
| | Exon 4 | c.1071_1074delinsCTT | p.(Arg358Phefs[a]16) | | 1 | Spencer et al., 2016 |
| | Exon 4 | c.1076delinsAT | p.(Ser359Tyrfs[a]6) | | 1 | Villarreal et al., 2013 |
| | Exon 8 | c.1692del | p.(Leu565Serfs[a]63) | RCV000256430.1 | 1 | Das Bhowmik et al. 2015 |
| **Splice** | Intron 2 | c.337+1G>A | p.? | RCV000012459.25 | 1 | Rodriguez-Criado et al. 2005 |
| | Intron 5 | c.1292+1G>T | p.? | RCV000012454.15 | 1 | Veugelers et al., 2000 |

(Continues)

**TABLE 1** (Continued)

| Mutation type | Position | Nucleotide change | Amino-acid change | Variant reference (dbSNP, ClinVar) | Unrelated families in the literature | References |
|---|---|---|---|---|---|---|
| **Deletions** | Exon 1 | c.(?_-1)_(175+1_176-1)del | p.? | | 5 | Hughes-Benzie et al., 1996 ( = Mariani et al., 2003); Li et al., 2001 ( = Mariani et al., 2003); Lindsay et al., 1997 ( = Mariani et al., 2003); Vaisfeld et al., 2017; Young et al., 2006 |
| | Exons 1–2 | c.(?_-1)_(337+1_338-1)del | p.? | | 3 | Lindsay et al., 1997 ( = Mariani et al., 2003); Mariani et al., 2003; Veugelers et al., 1998 ( = Veugelers et al., 2000 = Mariani et al., 2003) |
| | Exons 1–8 (+ *CCDC160,GPC4)* | c.(?_-1)_(ª1_?)del | p.? | | 1 | Weichert et al., 2011 |
| | Rearrangement of exon 1 + deletion of exon 2 | c.? | p.? | | 1 | Thomas et al., 2012 |
| | Deletion including the first 41 nucleotides of exon 2 | c.? | p.? | | 1 | Stove et al. 2017 |
| | Exon 2 | c.(175+1_176-1)_(337+1_338-1)del | p.(Gly59_Gln112del) | | 1 | Hughes-Benzie et al., 1996 ( = Pilia et al., 1996 = Mariani et al., 2003) |
| | Exons 2–3[a] | c.? | p.? | | 1 | Hughes-Benzie et al., 1996 ( = Mariani et al., 2003) |
| | Exon 3[a] | c.? | p.? | | 1 | Hughes-Benzie et al., 1996 ( = Mariani et al., 2003) |
| | Exon 3 (+ exons 3 to 9 of *GPC4, TFDP3*) | c.(337+1_338-1)_(1032+1_1033-1)del | p.(Glu113Aspfsª14) | | 1 | DiMaio et al., 2017 |
| | Exons 3–4 (size max exons 3–7) | c.? | p.? | | 1 | Magini et al., 2016 |
| | Exons 3–5 | c.(337+1_338-1)_(1292+1_1293-1)del | p.(Glu113Aspfsª11) | | 2 | Li et al., 2001 ( = Mariani et al., 2003); Li et al., 2001 ( = Mariani et al., 2003 = Agatep et al., 2014) |
| | Exons 3–6 | c.(337+1_338-1)_(1413+1_1414-1)del | p.(Glu113Alafsª11) | | 1 | Day et al. 2005 |
| | Exons 3–8 | c.(337+1_338-1)_(ª1_?)del | p.? | | 1 | Hughes-Benzie et al., 1996 ( = Mariani et al., 2003) |
| | Exon 4[a] | c.? | p.? | | 1 | Lindsay et al., 1997 ( = Mariani et al., 2003) |
| | Exons 4–5 | c.(1032+1_1033-1)_(1292+1_1293-1)del | p.(Gly346Glnfsª17) | | 1 | Li et al., 2001 ( = Mariani et al., 2003) |

(Continues)

**TABLE 1** (Continued)

| Mutation type | Position | Nucleotide change | Amino-acid change | Variant reference (dbSNP, ClinVar) | Unrelated families in the literature | References |
|---|---|---|---|---|---|---|
| | Exons 5–6 | c.(1166+1_1167-1)_(1413+1_1414-1)del | p.Arg389Serfs[a]3 | | 1 | Schmidt et al., 2017 |
| | Exons 5–7 | c.(1166+1_1167-1)_(1573+1_1574-1)del | p.(Glu390Thrfs[a]4) | | 1 | Li et al., 2001 ( = Mariani et al., 2003) |
| **Deletions** | Exon 6 | c.(1292+1_1393-1)_(1413+1_1414-1)del | p.(Arg431Serfs[a]3) | | 1 | Rodriguez-Criado et al. 2005 |
| | Exons 6–7 | c.(1292+1_1393-1)_(1573+1_1574-1)del | p.(Tyr432Thrfs[a]4) | | 1 | Li et al., 2001 ( = Mariani et al., 2003) |
| | Exons 6–8 | c.(1292+1_1393-1)_([a]1_?)del | p.? | | 2 | Halayem et al., 2016; Hughes-Benzie et al., 1996 ( = Pilia et al., 1996 = Mariani et al., 2003) |
| | Exon 7 | c.(1413+1_1414-1)_(1573+1_1574-1)del | p.(Leu472Asnfs[a]25) | | 2 | Veugelers et al., 2000 ( = Mariani et al., 2003) Sakazume et al., 2007 |
| | Exons 7–8 (+*GPC4*) | c.(1292+1_1393-1)_([a]1_?)del | p.? | | 1 | Hughes-Benzie et al., 1996 (Pilia et al. 1996 = Lindsay et al., 1997 = Veugelers et al., 1998 = Mariani et al., 2003) |
| | Exons 7–8 (+ *GPC4, TFDP3, USP26, HS6ST2, MBNL3*) | c.? | p.? | | 1 | Ganesamoorthy et al., 2013 |
| | Exon 8 | c.(1573+1_1574-1)_([a]1_?)del | p.? | | 2 | Li et al., 2001 ( = Mariani et al., 2003; Lindsay et al., 1997 ( = Mariani et al., 2003)) |

(Continues)

**TABLE 1** (Continued)

| Mutation type | Position | Nucleotide change | Amino-acid change | Variant reference (dbSNP, ClinVar) | Unrelated families in the literature | References |
|---|---|---|---|---|---|---|
| **Duplications** | Exon 2 | c.(175+1_176-1)_(337+1_338-1)del | p.? | | 1 | Ochiai et al. (2013) |
| | Exons 2–4 | c.(175+1_176-1)_(1166+1_1167-1)dup | p.Glu390Ilefs[a]9 | | 1 | Mateos et al. (2013) |
| | Exons 3–6 (+ *GPR101*, *SNORD61*, *RBMX*) | c.(337+1_338-1)_(1413+1_1414-1)dup | p.? | | 1 | Shimojima et al. (2016) |
| | Exons 3–7 | c.(337+1_338-1)_(1573+1_1574-1)dup | p.? | | 1 | Kehrer et al. (2016) |
| | exons 6–7(+ *GPC4*, *TFDP3*, *USP26*, *HS6ST2*) | c.(1292+1_1293-1)_(1573+1_1574-1)dup | p.? | | 1 | Mujezinovic et al. (2016) |
| **Translocations** | t(X,1) intron 2 | c.? | p.? | | 1 | Pilia et al. (1996) ( = Punnett et al., 1994) |
| | t(X,16) intron 7 | c.? | p.? | | 1 | Pilia et al. (1996) |

Numbering is according to the cDNA sequence (GenBank entry NM_004484.3).
[a]The exact location of the 3′ breakpoints of these deletions is unknown as exon 4 and 5 were not analyzed in Hugues Benzie et al.'s study and exon 5 was not analyzed in Lindsay et al.'s study.

**TABLE 2** *GPC3* mutations identified in the two French laboratories in patients affected with Simpson-Golabi-Behmel Syndrome

| Mutation type | Position | Nucleotide change | Amino-acid change | Variant reference (dbSNP, ClinVar) | Unrelated families in our cohort | Unrelated families in the literature | References |
|---|---|---|---|---|---|---|---|
| **Missense** | Exon 2 | c.206G>A | p.(Gly69Asp) | | 1 | 0 | This study |
| | Exon 3 | c.461T>C | p.(Leu154Pro) | | 1 | 0 | This study |
| | Exon 3 | c.884A>G | p.(Tyr295Cys) | | 1 | 0 | This study |
| | Exon 6 | c.1413G>C | p.(Gln471His) | | 1 | 0 | This study |
| | Exon 8 | c.1666 G>A | p.(Gly556Arg) | rs2676060850, RCV000012461.22 | 3 | 0 | This study (One patient described in Pénisson-Besnier et al. (2008) |
| | Exon 8 | c.1667 G>T | p.(Gly556Val) | | 1 | 0 | This study |
| **Nonsense** | Exon 2 | c.256C>T | p.(Arg86*) | | 4 | 3 | This study; Gertsch et al. (2010); Sakazume et al. (2007); Yano et al. (2011) |
| | Exon 3 | c.271C>T | p.(Gln91*) | | 1 | 0 | Ratbi et al. (2010) |
| | Exon 3 | c.595C>T | p.(Arg199*) | rs104894855, RCV000012455.15 | 1 | 1 | This study; Veugelers et al. (2000) |
| | Exon 4 | c.1159C>T | p.(Arg387*) | rs122453121, RCV000012460.15 | 3 | 3 | This study; Kosaki et al. (2014); Romanelli et al. (2007); Sakazume et al. (2007) |
| | Exon 6 | c.1411C>T | p.(Gln471*) | | 1 | 0 | Baujat et al. (2005) |
| **Frameshift** | Exon 1 | c.80_81delinsT | p.(Pro27Leufs*57) | | 1 | 0 | This study |
| | Exon 1 | c.133_136delins AGGACTCTGGA | p.(Leu45Argfs*26) | | 1 | 0 | This study |
| | Exon 2 | c.205_206insC | p.(Gly69Alafs*48) | | 1 | 0 | This study |
| | Exon 3 | c.408_411del | p.(Ser136Argfs*27) | | 1 | 0 | This study |
| | Exon 3 | c.530del | p.(Val177Alafs*6) | | 1 | 0 | This study |
| | Exon 3 | c.591_610del | p.(Cys197*) | | 1 | 0 | This study |
| | Exon 3 | c.662del | p.(Lys221Serfs*13) | | 1 | 0 | Jedraszak et al. (2014) |
| | Exon 3 | c.674_688delinsA | p.(Val225Aspfs*9) | | 1 | 0 | This study |
| | Exon 3 | c.758_765del | p.(Gly253Alafs*17) | | 1 | 0 | This study |
| | Exon 3 | c.791dup | p.(Tyr264*) | | 1 | 0 | This study |
| | Exon 4 | c.1100_1101del | p.(Phe367Tyrfs*2) | | 1 | 0 | This study |
| **Splice** | Intron 1 | c.175+1G>A | p.? | RCV000255780.1 | 1 | 0 | This study |
| | Intron 1 | c.175+2T>C | p.? | | 1 | 0 | This study |
| **Deletions** | 5'UTR | c.-740_-49del | p.? | | 1 | 0 | This study |

(Continues)

**TABLE 2** (Continued)

| Mutation type | Position | Nucleotide change | Amino-acid change | Variant reference (dbSNP, ClinVar) | Unrelated families in our cohort | Unrelated families in the literature | References |
|---|---|---|---|---|---|---|---|
| | Exon 1 | c.(?_-1)_(175+1_176-1)del | p.? | | 1 | 5 | This study; Hughes-Benzie et al. (1996); Lindsay et al. (1997); Li et al. (2001); Vaisfeld et al. (2017) |
| | Exons 1–2 | c.(?_-1)_(337+1_338-1)del | p.? | | 1 | 3 | This study; Lindsay et al. (1997); Veugelers et al. (1998); Mariani et al. (2003) |
| | Exons 1–6 (+CCDC160) | c.(?_-1)_(1413+1_1414-1)del | p.? | | 1 | 0 | This study |
| | Exons 1–8 (+ *GPC4*, *TFDP3*, *USP26*, *HS6ST2*, *MBNL3*) | c.(?_-1)_(*1_?)del | p.? | | 1 | 0 | This study |
| | Exon 4 | c.(1032+1_1033-1)_(1166+1_1167-1)del | p.(Ile345Glyfs*14) | | 1 | 0 | This study |
| | Exons 6–7 | c.(1292+1_1393-1)_(1573+1_1574-1)del | p.(Tyr432Thrfs*4) | | 1 | 1 | This study; Li et al. (2001) |
| | Exon 7 | c.(1413+1_1414-1)_(1573+1_1574-1)del | p.(Leu472Asnfs*25) | | 2 | 2 | This study; Sakazume et al. (2007); Veugelers et al. (2000) |
| | Exons 7–8 | c.(1413+1_1414-1)_(*1_?)del | p.? | | 1 | 0 | This study |
| | Exons 7–8 (+ *GPC4*, *TFDP3*, *USP26*, *HS6ST2*) | c.(1413+1_1414-1)_(*1_?)del | p.? | | 1 | 0 | This study |
| | Exon 8 | c.(1573+1_1574-1)_(*1_?)del | p.? | | 4 | 2 | This study; Lindsay et al. (1997); Li et al. (2001) |
| **Duplications** | Exon 2 | c.(175+1_176-1)_(337+1_338-1)dup | p.Gln112_Glu113ins54 | | 1 | 1 | Cottereau et al. (2014); Ochiai et al. (2013) |
| | Exon 3–6 (+ *GPC4*, *TFDP3*, *USP26*) | c.(337+1_338-1)_(1413+1_1414-1)dup | p.Leu472Argfs*25 | | 1 | 0 | Vuillaume et al. (2018) |
| | Exon 7 | c.(1413+1_1414-1)_(1573+1_1574-1)dup | p.Glu525Alafs*11 | | 1 | 0 | Vuillaume et al. (2018) |

Numbering is according to the cDNA sequence (GenBank entry NM_004484.3).

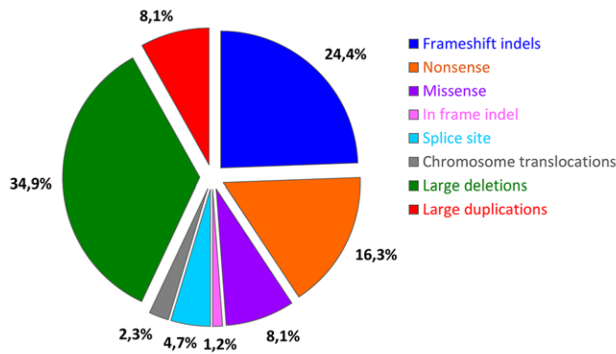


**FIGURE 1** Pie chart summarizing the distribution of the 86 *GPC3* mutations

point mutations were found in exon 3 (22 out of 47) (Supp. Figure S1A). However, when the number of mutations was corrected to the number of bases in each exon, there was no overrepresentation of mutations in this exon compared to the others, suggesting that exon 3 was not a hot-spot of mutation but that it was affected proportionally to its length (Supp. Figure S1B). Exon 2 showed the highest relative rate of mutations per base, whereas exon 7 appeared the less vulnerable to point mutations. Only 8.5% (4/47) of these point mutations were recurrent (Cf. Figure 2). The most frequent mutations were two nonsense mutations, c. 256C > T, p.(Arg86*) and c.1159C > T, p.(Arg387*) detected in seven and six unrelated families respectively. One missense mutation c.1666G > A, p.(Gly556Arg) was also detected in three unrelated families and one nonsense mutation, c.595C > T, p.(Arg199*) in two unrelated families.

### 2.1.1 | Missense mutations

The seven missense mutations, among which six were detected in our cohort, were interpreted in silico with Alamut v2.8.0 software (Interactive Biosoftware, Rouen, France; https://www.interactivebiosoftware.com). Polyphen2, SIFT, and Mutation Taster prediction programs were used to assess the predicted pathogenicity of each variation (Cf. Supp. Table S2). All these mutations were predicted to be "disease causing" or "probably damaging" by Mutation Taster and Polyphen-2, respectively, whereas only one of these mutations, c.886T > A, p.(Trp296Arg) was classified as deleterious by SIFT analysis. Functional studies were performed for two missense mutations, c.886T > A, p.(Trp296Arg) and c.1666G > A, p.(Gly556Arg) (Shi & Filmus, 2009; Veugelers et al., 2000). These mutations both altered a conserved amino acid found in all glypicans and resulted in a loss-of-function. For the c.886T > A, p.(Trp296Arg) mutation, functional studies showed that the mutant protein was poorly processed and failed to increase the cell surface expression of HS (Veugelers et al., 2000).

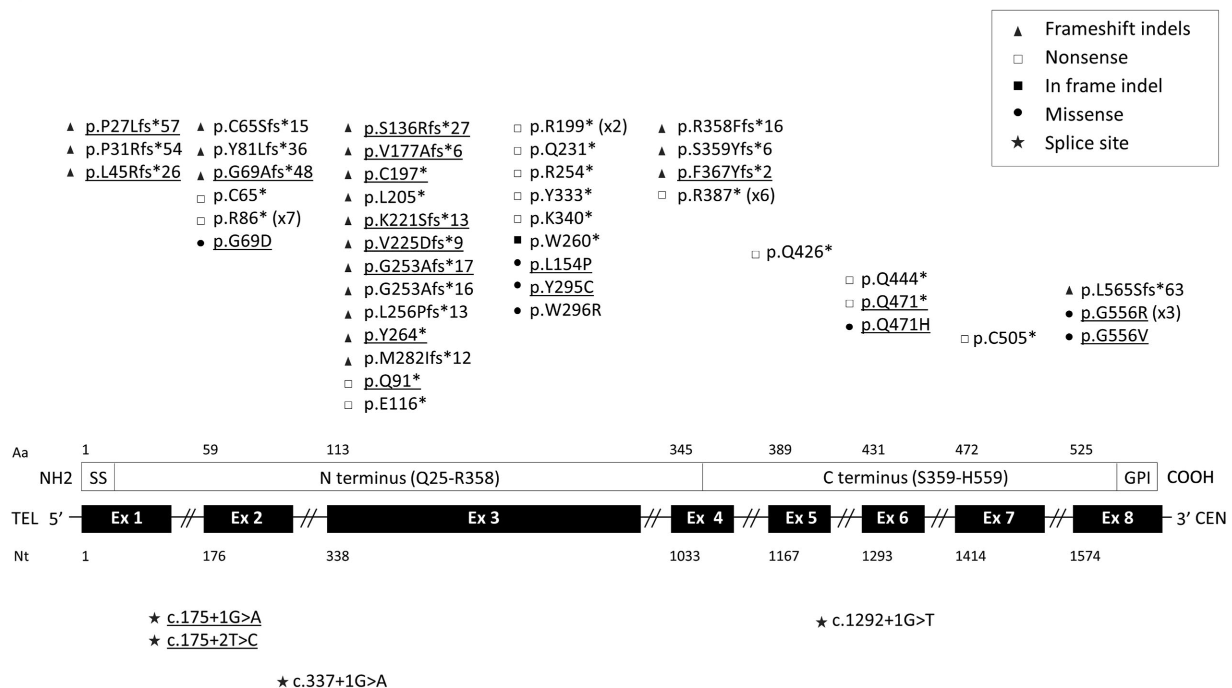


**FIGURE 2** Distribution of *GPC3* previously described and novel point mutations. Genomic structure of *GPC3* gene including 8 exons (black boxes) and introns (black horizontal lines) is represented. cDNA numbering below exons is according to NM_004484.3 and uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. GPC3 protein structure is represented above *GPC3* genomic structure. N-terminal signal peptide (SS = Signal Sequence for secretion; residues 1–24); N terminus (residues 25–358), C-terminus (residues 359–559) and C terminal GPI anchor addition signal (GPI: glycosylphosphatidylinositol; residue 560–580) are represented. Amino acid numbers are indicated above GPC3 protein structure. Mutations above GPC3 protein represent exonic mutations and are displayed as changes at protein level (p). Mutations below *GPC3* gene represent intronic mutations affecting the splice sites and are displayed as changes at DNA level (c). Underlined mutations correspond to novel mutations reported for the first time by our group. In case of recurrence, number of occurrence is indicated between parentheses next to the mutation. Black triangles, white squares, black square, black circle, and black stars, respectively, indicate frameshift mutations, nonsense mutations, in frame mutation, missense mutations, and splice site mutations. Aa amino acid, Nt nucleotide

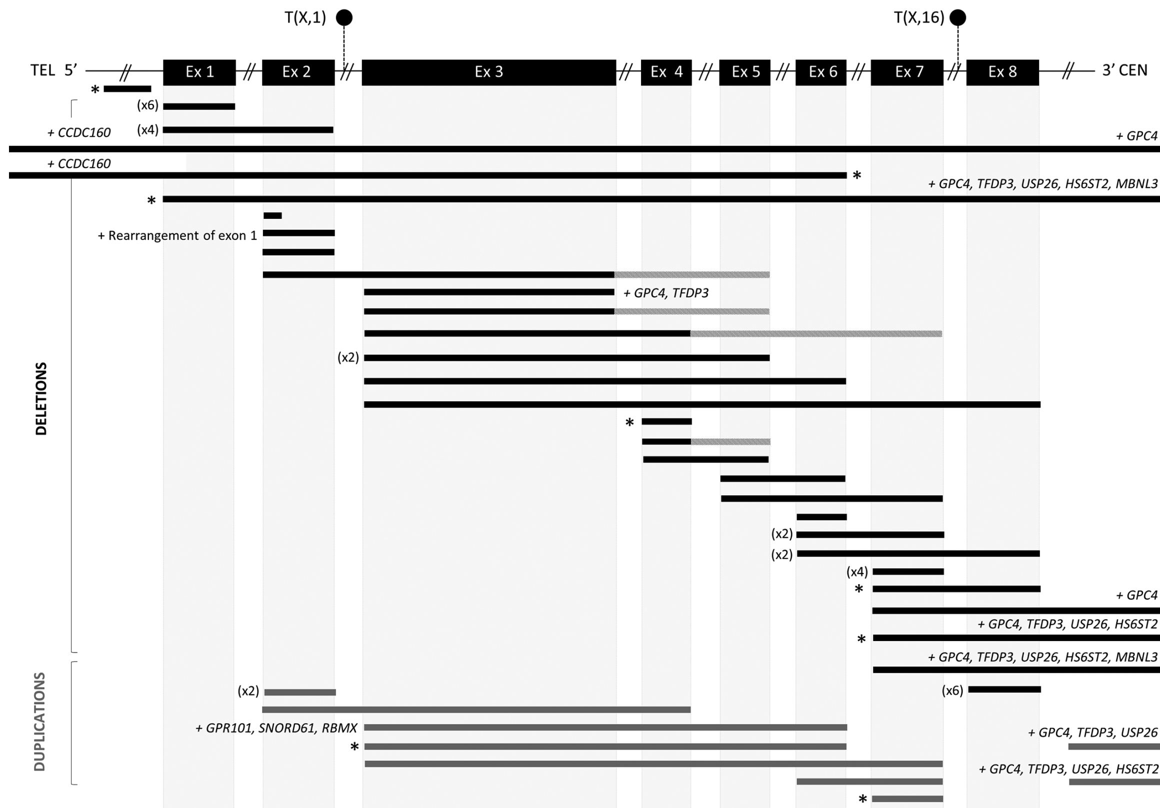


**FIGURE 3** Graphical representation of *GPC3* intragenic rearrangements. An * indicates a novel rearrangement reported for the first time by our group. Black circles indicate translocations. Black rectangles correspond to deletions and gray rectangles to duplications. Dotted rectangles indicate a deletion or duplication for which the exact location of the 3′ breakpoint remains uncertain. In case of recurrence, number of occurrence is indicated between parentheses next to the rearrangement

The second missense mutation, c.1666G > A, p.(Gly556Arg), occurred in a region critical for cleavage of GPC3, which is necessary for GPC3 to be anchored to the plasma membrane via GPI linkage. Western blot analysis and immunostaining showed that the mutant protein was not glycanated and was present in the cell lysate and the conditioned medium instead of being attached to the cell surface. This defect was shown to impair the Hedgehog inhibitory activity of GPC3 (Shi & Filmus, 2009).

### 2.1.2 | In frame mutation

Only one in frame mutation was identified in *GPC3* gene: c.780_785delinsAGC, p.(Trp260*). This mutation, by inserting three nucleotides and deleting six nucleotides, changes the amino acid Tryptophan to a stop codon at position 260 leading to a premature truncation of GPC3.

### 2.1.3 | Nonsense and frameshift mutations

Fourteen nonsense and 21 frameshift mutations have been identified in *GPC3* including the two novel nonsense mutations and 11 novel frameshift mutations of our cohort. All these mutations introduced a premature stop codon except a mutation, c.1692del, p.(Leu565Serfs*63), recently identified by whole exome sequencing in a child with an unknown overgrowth syndrome (Das Bhowmik & Dalal, 2015). This hemizygous single base pair deletion in exon 8 of *GPC3* results in a frameshift which increases the length of the protein by adding 46 news amino acids towards the 3′UTR region instead of causing a premature truncation. As exon 8 encodes the signal sequence for the HS attachment and GPI anchorage at the cell surface, this mutation could impair GPC3 function by disrupting the attachment of GPC3 to the exocytoplasmic surface of the plasma membrane.

### 2.1.4 | Splice site mutations

Two splice site mutations were previously described, c.337+1G > A and c. 1292+1G > T, and two were identified in our cohort, c.175+1G > A and c.175+2T > C. These four mutations disrupted the consensus GU donor site. As expected, splice-site prediction programs (MaxEntScan, NNSPLICE and Human Splicing Finder programs) available on Alamut v2.8.0 software predicted a decrease of splice site score of 100% leading to a probable exon 1 (mutations c.175+1G > A and c.175+2T > C), exon 2 (mutation c.337+1G > A) or exon 5 (c.1292+1G > T) skipping (Cf. Supp. Table S3).

## 2.2 | GPC3 large rearrangements

Thirty-seven *GPC3* rearrangements including 30 deletions and seven duplications were reported in 57 out of the 120 unrelated probands suggesting that large-scale rearrangements may be responsible for

almost half of the cases of SGBS. These rearrangements are listed in Tables 1 and 2 and represented in Figure 3.

Deletions and duplications were described for each exon with ten rearrangements (27%) encompassing only one exon. Exon 5 is the only exon which was not found deleted or duplicated alone (Cf. Figure 3).

Eight of these rearrangements (21.6 %) were recurrent. Deletion of exon 1 and deletion of exon 8 were the most common, each detected in six unrelated families. Deletion of exon 7 and deletion of exon 1 to exon 2 were also frequent and each detected in four unrelated families. Four other rearrangements, deletion of exon 3 to 5, deletion of exon 6 to 7, deletion of exon 6 to 8 and duplication of exon 2 were detected twice (Cf. Figure 3).

At least 13 out of 37 (35%) of these rearrangements alter the open reading frame by introducing a premature stop codon and 5/37 (13%) remove the proper start codon. Five rearrangements, one deletion and four duplications, were studied at the cDNA level. cDNA analysis confirmed the in silico predictions for deletion of exons 5 to 6 (Schmidt et al., 2017) and showed that duplications of exon 2 to 4, exon 3 to 6, and exon 7 also lead to the truncation of the protein with a complete absence of GPI anchoring domain ((Mateos et al., 2013; Vuillaume et al., 2018), whereas duplication of exon 2 maintains the open reading frame with an insertion of 54 amino acids which probably disrupts the conserved glypican three-dimensional structure (Cottereau et al., 2014).

*GPC3* rearrangements were initially detected by Southern Blot and PCR in case of deletions (Hughes-Benzie et al., 1996; Li et al., 2001; Lindsay et al., 1997; Rodríguez-Criado et al., 2005; Veugelers et al., 1998, 2000) and by MLPA in case of duplications (Kehrer et al., 2016; Mateos et al., 2013; Ochiai et al., 2013; Vaisfeld et al., 2017). Recently, array-CGH also allowed the detection of *GPC3* rearrangements (Cf. Table 3) in eight cases of prenatal diagnosis (DiMaio et al., 2017; Ganesamoorthy et al., 2013; Magini et al., 2016; Støve et al., 2017; Vuillaume et al., 2018; Weichert et al., 2011) and, in three postnatal cases of SGBS (Schmidt et al., 2017; Shimojima et al., 2016). In our cohort, chromosomal microarray was performed, after PCR, in order to fine-map rearrangement breakpoints when they occurred at the 5′ and/or 3′end of *GPC3* (Cf Table 3). Overall, 21 rearrangements associated with SGBS, ranging from 30 kb to 1.7 Mb, were identified or fine-mapped by array-CGH. Nine of these rearrangements encompassed *GPC3* neighboring genes (Cf Supp. Figure S2) but did not seem to have a more pronounced effect on the phenotype. However, given the fact that only few breakpoints occurring at the 5′ or 3′ end of *GPC3*, have been precisely characterized, it remains difficult to establish genotype/phenotype correlations according to the gene content of these rearrangements. The contribution of other genes is still questionable as illustrated by the patient reported by Young et al. (2006) who had an unusual facial appearance with an external ear malformation and a deletion of which the 5′ breakpoint is not determined.

Of note, a large number of deletions and duplications encompassing the whole *GPC3* gene and many other genes are found in public databases (DECIPHER, ISCA, and dbVar databases). All *GPC3* deletions are expected to be associated with SGBS.

As discussed previously (Vuillaume et al., 2018), six small duplications with a size ranging from 126 kb to 1.035 Mb (nssv584468, nssv13650346, DECIPHER 258050, DECIPHER 326611, nssv1415234, nssv13644225) could lead to a GPC3 loss-of-function by disrupting the reading frame even if features consistent with SGBS were documented in only two of them (DECIPHER 258050, nssv13644225) and no functional molecular analysis was performed.

### 2.3 | Translocations

Two X/autosomes translocations t(X,1) and t(X,16) were the first mutations described as associated with SGBS (Pilia et al., 1996; Punnett, 1994) allowing the recognition of *GPC3* as responsible for the disease. These translocations were identified in two unrelated female patients with SGBS. The use of STSs derived from different portions of *GPC3* showed that t(X,1) translocation interrupted the gene in the second intron, whereas t(X,16) translocation interrupted the gene in the seventh intron, both translocations leading to a probable loss-of-functional *GPC3*.

## 3 | FUNCTIONAL IMPACT OF *GPC3* MUTATIONS

*GPC3* mutations are dispersed throughout all the coding regions of the gene with no obvious mutation hotspots. The vast majority of these mutations are deletions or truncating mutations (frameshift, nonsense mutations) predicted to result in a loss-of-function. Other types of mutations such as missenses mutations or duplications are less frequent and were also shown to alter GPC3 function when they were functionally characterized (Cottereau et al., 2014; Mateos et al., 2013; Veugelers et al., 2000; Vuillaume et al., 2018). These observations should be taken into account for the interpretation of novel *GPC3* variants as a lot of variants are nowadays identified by next-generation sequencing.

## 4 | GENOTYPE/PHENOTYPE CORRELATIONS

Supp. Table S1 summarizes the main clinical features associated with each mutation found in our cohort of 63 patients belonging to 49 unrelated families. Clinical features of 42 of these male cases were previously reviewed by our team (Baujat et al., 2005; Cottereau et al., 2013; Jedraszak et al., 2014; Pénisson-Besnier et al., 2008; Ratbi, Elalaoui, Moizard, Raynaud, & Sefiani, 2010). We did not find any link between specific clinical symptoms and specific mutations, mutation type or location in *GPC3*. Our observations are in line with previous studies (Cottereau et al., 2013; Hughes-Benzie et al., 1996; Mariani et al., 2003), which have not found a robust genotype–phenotype correlation in SGBS.

**TABLE 3** Microarray results for *GPC3* deletions/duplications identified or fine-mapped by array-CGH

| Type of rearrangement | Pre- or postnatal diagnosis? | Exons rearranged in *GPC3* | Min sequence coordinates Hg19 | Size (kb) | RefSeq genes in interval? | Array-CGH platform | Reference study |
|---|---|---|---|---|---|---|---|
| ***Deletions/duplications identified by array-CGH*** | | | | | | | |
| Deletion | Prenatal diagnosis | Exons 1–8 | 132,363,525-133,429,657 | 1 066 | *CCDC160, GPC3, GPC4* | 105K (Agilent) | Weichert et al. (2011) |
| | Prenatal diagnosis | Deletion including the first 41 nucleotides of exon 2 | 132,996,180-133,087,198 | 91 | *GPC3* | 180K (Agilent) | Stove et al. (2017) |
| | Prenatal diagnosis | Exon 3 + duplication downstream *GPC3* | 132,887,828-132,972,199 132,300,487-132,454,025 | 84.3 | *GPC3 GPC4 (exons 3–9), TFDP3* | 180K (Agilent) | DiMaio et al. (2017) |
| | Prenatal diagnosis | Exons 3–4 | 132,834,006-132,986,815 | 152.8 | *GPC3* | 60K (Agilent) | Magini et al. (2016) |
| | Postnatal diagnosis | Exons 5–6 | 132,796,480-132,826,558 | 30 | *GPC3* | 105K (Agilent) | Schmidt et al. (2017) |
| | Prenatal diagnosis | Exons 7–8 | 131,508,464-132,785,077 | 1 276,5 | *GPC3, GPC4, TFDP3, USP26, HS6ST2, MBNL3* | Affimetrix or Illumina | Ganesamoorthy et al. (2013) |
| | Postnatal | Exon 8 | 132,579,767-132,695,101 | 115,3 | *GPC3* | 180K (Agilent) | This study (case No.46) |
| Duplication | Prenatal diagnosis | Exons 3–6[a] | 132,161,510-132,986,815 | 825,3 | *GPC3, GPC4, TFDP3, USP26* | 60K (Agilent) | This study (case No.48) |
| | Postnatal diagnosis | Exons 3–6 + duplication upstream *GPC3* | 132,742,336-132,933,597 135,873,188-136,230,169 | 191 357 | *GPC3 GPR101, SNORD61, RBMX* | Custom Array (Agilent) | Shimojima et al. (2016) |
| | Prenatal diagnosis | Exons 6–7 + duplication downstream *GPC3* | 132,705,816-132,821,373 132,076,106-132,650,019 | 115,56 573,91 | *GPC3 GPC4, TFDP3, USP26, HS6ST2* | 180K (ISCA) | Mujezinovic et al. (2016) |
| | Prenatal diagnosis | Exon 7 | 132,717,085-132,769,148 | 52 | *GPC3* | 180K (Agilent) | This study (case No.49) |
| ***Deletions fine-mapped by array-CGH*** | | | | | | | |
| Deletion | Postnatal diagnosis | Exon 2 | 133,051,023-133,119,079 | 68 | *GPC3* | NA | Thomas et al. (2012) |
| | | 5'UTR | Not detected | Not detected | *GPC3* | 180K (Agilent) | This study (case No.32) |
| | | Exon 1 | Not detected | Not detected | *GPC3* | 180K (Agilent) | This study (case No.33-1) |
| | | Exons 1–2 | 132,971,999-133,128,643 | 156.6 | *GPC3* | 180K (Agilent) | This study (case No.34) |
| | | Exons 1–6 | 132,761,685-133,455,720 | 694 | *CCDC160, GPC3* | 180K (Agilent) | This study (case No.35) |
| | | Exons 1–8 | 131,540,134-133,305,858 | 1765.7 | *GPC3, GPC4, TFDP3, USP26, HS6ST2, MBNL3* | 180K (Agilent) | This study (case No.36) |
| | | Exons 7–8 | 131,872,895-132,761,744 | 888.8 | *GPC3, GPC4, TFDP3, USP26, HS6ST2* | 180K (Agilent) | This study (case No.41) |
| | | Exons 7–8 | 132,588,444-132,744,654 | 156.2 | *GPC3* | 180K (Agilent) | This study (case No.42) |
| | | Exon 8 | Not detected | Not detected | *GPC3* | 180K (Agilent) | This study (case No.44) |
| | | Exon 8 | Not detected | Not detected | *GPC3* | 180K (Agilent) | This study (case No.45) |

[a]This rearrangement initially identified by Array-CGH as a single duplication was in fact a complex chromosome rearrangement composed of two adjacent duplications with a DUP-NML-DUP pattern. The first duplication involves *USP26*, *TFDP3*, and *GPC4* genes, whereas the second involves exon 3 to 6 of GPC3.

## 5 | CLINICAL AND DIAGNOSTIC RELEVANCE

The diagnosis of classical SGBS is based on several criteria including typical clinical symptoms, family history consistent with X-linked inheritance and molecular genetic testing for *GPC3* mutations. Typical clinical symptoms, especially in males, mostly include overgrowth (macrosomia, macrocephaly, and/or pre-and postnatal overgrowth), typical facial features, hand anomalies, supernumerary nipples, congenital malformations, and tumor predisposition. Variable degree of intellectual disability may be present. The identification of a *GPC3* mutation confirms the clinical diagnosis allowing a more appropriate management, and allows a reliable genetic counseling and prenatal diagnosis if desired. In France, two laboratories (Tours and Paris) offer molecular genetic testing for *GPC3* mutations since the early 2000′s with a combined approach based on PCR, direct sequencing and MLPA analysis. Management of SGBS includes treatment of neonatal hypoglycemia and requires a multidisciplinary approach with pediatric cardiologists, neurologists, orthopedics, and speech therapists. Specific management and follow-up of tumors especially Wilms tumors, liver tumors and gonadoblastoma should be performed in all individuals with SGBS. In families at risk, prenatal diagnosis may be proposed with careful ultrasound follow up in order to detect disproportionate fetal overgrowth often associated with elevated maternal serum alpha-fetoprotein.

## 6 | ANIMAL MODELS

Currently, two glypican-encoding genes have been identified in *Drosophila melanogaster*, and were shown to play an important role in development. The best-characterized gene is *dally* (division abnormally delayed), a gene involved in cell division patterning in the visual system. In *Dally* mutants, cell cycle progression is impaired leading to morphological and developmental defects in several tissues including the eyes, brain, antenna, wings, and genitalia (Nakato, Futch, & Selleck, 1995). Interestingly, a decrease of *dally* expression is associated with segment polarity defects similar to the ones caused by the loss of Wingless (Wg) activity (Lin & Perrimon, 1999; Tsuda et al., 1999). The second glypican gene found in *D. melanogaster* is *dally-like (dly)*, a gene also implicated in the Wingless mediated patterning of the developing embryo. This gene regulates extracellular growth factor distribution and, in some cases, may block growth factor signaling (Baeg, Lin, Khare, Baumgartner, & Perrimon, 2001). This gene is also required for the reception of Hedgehog (Hh) signal known to promote embryonic growth (Desbordes & Sanson, 2003).

In mice targeted deletion of *Gpc3* results in some of the typical SGBS abnormalities including developmental overgrowth, perinatal death, renal dysplasia, accessory spleens, impaired lung development, polydactyly, and placentomegaly (Cano-Gauci et al., 1999). This knock-out phenotype, in accordance to the human overgrowth phenotype, suggests that GPC3 can act as a negative regulator of cell proliferation. It was first suggested that GPC3 acts as an inhibitor of IGF-II (Pilia et al., 1996) given the critical role of insulin-like-growth factor II (IGF-II) in the regulation of embryonic growth. However, this hypothesis was ruled out after several experiments showing that GPC3 does not interact with IGF-II. Furthermore, GPC3-null embryos display normal levels of IGF-II without any genetic interaction when they are crossed with various mouse strains lacking critical components of the IGF signaling pathways (Cano-Gauci et al., 1999; Chiao et al., 2002; Song, Shi, & Filmus, 1997). In humans, the overgrowth phenotype could be due, at least in part, to the hyperactivation of Hedgehog signaling. This latter hypothesis was strongly supported by the fact that Hedgehog signaling was elevated in GPC3-null mice (Capurro et al., 2008) and that GPC3-null embryos display higher levels of Sonic Hedgehog and Indian Hedheog proteins than normal littermates (Capurro et al., 2008; Capurro, Li, & Filmus, 2009). Moreover, GPC3 knock-out mice also exhibit alterations in the Wnt signaling pathway (Song, Shi, Xiang, & Filmus, 2005).

## 7 | CONCLUSION AND FUTURE PROSPECTS

In this mutation update, we review the current state of our knowledge on human *GPC3* mutations. We have compiled previously reported and novel mutations of *GPC3* gene responsible for classical SGBS and collected 86 distinct *GPC3* mutations in 120 unrelated families. These mutations ranging from single nucleotide variations to complex genomic rearrangements involve the entire coding region of *GPC3*. Most of them are unique, inherited and are predicted to result in a lack of functional GPC3. Only 18% of these mutations occurred de novo. Missense mutations are rare and those which were functionally characterized also impaired GPC3 function. In most cases, mutations were identified by a targeted analysis of *GPC3* in patients clinically diagnosed with SGBS. Recently, eleven *GPC3* variants were found by high-throughput technologies without a preliminary established clinical diagnosis of SGBS. Ten of these variants are clearly associated with SGBS phenotype. Nine were detected prenatally by chromosomal microarray (DiMaio et al., 2017; Ganesamoorthy et al., 2013; Magini et al., 2016; Mujezinović et al., 2016; Støve et al., 2017; Weichert et al., 2011) or whole exome sequencing (Magini et al., 2016) in fetuses with abnormal ultrasound findings. In these fetuses, clinical features including notably fetal overgrowth, craniofacial abnormalities (DiMaio et al., 2017; Magini et al., 2016; Mujezinović et al., 2016; Støve et al., 2017; Weichert et al., 2011), and congenital diaphragmatic hernia (Ganesamoorthy et al., 2013) were retrospectively in line with SGBS diagnosis. Two other variants were detected postnatally by next-generation sequencing. The first variant was found by whole exome sequencing in a patient with an unknown overgrowth syndrome, retrospectively fully compatible with SGBS (Das Bhowmik & Dalal, 2015). The last variant, a frameshift mutation c.1243del, p.(Val415Trpfs*27), leading to a stop premature codon, was highlighted by next-generation sequencing targeting a panel of genes associated with intellectual disability in a cohort of 996 patients with moderate-to-severe intellectual disability (Grozeva et al., 2015). However, owing to the lack of additional clinical data we were not able to interpret this variant regarding SGBS phenotype, and it was not included in the present review. This illustrates that, with the advent of such large-scale approaches, new

*GPC3* rare variants may be revealed incidentally. An accurate evaluation of these variants coupled with a thorough phenotyping will be needed in order to assess their potential involvement in SGBS. This report by describing for the first time the wide mutational spectrum of *GPC3* could help clinicians and geneticists in confirming a clinical diagnosis and, more importantly, in interpreting incidental variants of *GPC3* which will be found with next-generation sequencing.

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## DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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