Phenotype and genotype in 17 patients with Goltz–Gorlin syndrome

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ABSTRACT

Background: Goltz–Gorlin syndrome or focal dermal hypoplasia is a highly variable, X-linked dominant syndrome with abnormalities of ectodermal and mesodermal origin. In 2007, mutations in the *PORCN* gene were found to be causative in Goltz–Gorlin syndrome.

Method: A series of 17 patients with Goltz–Gorlin syndrome is reported on, and their phenotype and genotype are described.

Results: In 14 patients (13 females and one male), a *PORCN* mutation was found. Mutations included nonsense (n = 5), frameshift (n = 2), aberrant splicing (n = 2) and missense (n = 5) mutations. No genotype–phenotype correlation was found. All patients with the classical features of the syndrome had a detectable mutation. In three females with atypical signs, no mutation was found. The male patient had classical features and showed mosaicism for a *PORCN* nonsense mutation in fibroblasts. Two affected sisters had a mutation not detectable in their parents, supporting germline mosaicism. Their father had undergone radiation for testicular cancer in the past. Two classically affected females had three severely affected female fetuses which all had midline thoracic and abdominal wall defects, resembling the pentalogy of Cantrell and the limb–body wall complex. Thoracic and abdominal wall defects were also present in two surviving patients. *PORCN* mutations can possibly cause pentalogy of Cantrell and limb–body wall complexes as well. Therefore, particularly in cases with limb defects, it seems useful to search for these.

Conclusions: *PORCN* mutations can be found in all classically affected cases of Goltz–Gorlin syndrome, including males. Somatic and germline mosaicism occur. There is no evident genotype–phenotype correlation.

Goltz *et al*[1] in 1962 and Gorlin *et al*[2] in 1963 defined a syndrome with widespread features including: asymmetry of the face, trunk and extremities; focal dermal hypoplasia and hyperpigmentations, often following Blaschko lines; localised subepidermal deposits of subcutaneous fat; multiple papillomas of mucous membranes and skin surrounding body orifices; and extremely variable skeletal anomalies which particularly involve the extremities. Additional features include short stature, sparse hair, coloboma of the iris and retina, prominent and thin ears, cleft lip and palate, hypodontia and abnormally shaped teeth, occasionally internal anomalies of heart and kidneys, and developmental delay in 15% of affected people.[3] Approximately 95% of cases have been sporadic. From pedigree analyses, it was thought likely to be an X-linked dominant disorder, and ~90% of affected individuals were female. The few familial examples have been mostly mother–daughter reports.[4] Transmission from mildly affected fathers to severely affected daughters was postulated to be explainable by somatic mosaicism for the putative mutation.[5–9]

In 2007, two groups independently reported mutations in the *PORCN* gene at Xp11.23 in patients with Goltz–Gorlin syndrome.[10 11] The gene encodes an *O*-acyltransferase that catalyses cysteine *N*-palmitoylation and serine *O*-acylation in the endoplasmic reticulum, which allows membrane targeting and secretion of several Wnt proteins that have key roles in embryonic tissue development.[12] To date, 71 disease-causing mutations in *PORCN* associated with Goltz–Gorlin syndrome have been identified.[10 11 13–18] Here we report on our joint experience of a series of 17 Goltz–Gorlin patients, including an affected male, two vertical transmissions, and one pair of affected siblings without affected parents, and describe both their phenotype and genotype.

MATERIALS AND METHODS

Patients

The laboratory in the Academic Medical Center in Amsterdam started to offer *PORCN* mutation analysis on 1 January 2008. Physicians who forwarded samples between that date and 1 July 2008 of patients for whom the diagnosis was felt to be firmly established were contacted to ask them to participate, and all agreed.

Mutation analysis

Genomic DNA was extracted from peripheral leucocytes according to standard protocols. In one patient (see table 2, patient 3), DNA was also obtained from cultured fibroblasts. All coding exons (2–15) of the *PORCN* gene were amplified using intronic primers, as described.[10] PCRs were carried out in a 25 µl volume containing 50–100 ng genomic DNA, 2.5 µl 10×PCR buffer (Solis Biodyne Tartu, Estonia), 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 10 pmol each primer and 0.2 U HotFire Polymerase (Solis Biodyne). The PCR products were analysed by direct sequencing of both strands on an automated ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, California, USA). All sequence files were compared with the reference genomic sequence (NM_203475.1, variant D) and analysed with the aid of the Codon Code Aligner software (CodonCode Corporation, Dedham, Massachusetts, USA). The presence of all identified variants was confirmed by resequencing of an independent sample. Analysis and interpretation

of the mutations was conducted with the Alamut mutation interpretation software (V1.4).

Copy number of coding *PORCN* exons in mutation-negative patients was determined by multiplex ligation-dependent probe amplification (MLPA). Synthetic probes (Biolegio, Nijmegen, The Netherlands) targeting all coding exons of *PORCN* were designed as described previously.[19] MLPA reactions were performed essentially as previously described using MLPA kit reagents (MRC-Holland, Amsterdam The Netherlands).[20] Probe sequences are available on request. Quantitative analysis of the methylation pattern at the *AR* locus was performed as described previously.[21]

RESULTS

Phenotype

We gathered data on 17 patients with Goltz–Gorlin syndrome (age 0–64 years; mean age 29 years). Mean age of parents was not increased (mean age of fathers 31.0 years; mean age of mothers 28.6 years). There were seven familial and 10 sporadic patients. One was an adult male; the other 16 were females, of whom 13 survived infancy. One prematurely born girl was severely retarded; all other surviving patients had normal cognitive development. In five patients, however, the parents considered the level of cognitive functioning of the child to be slightly lower than was normal in the family. The cognition of the patients has not been tested formally. The major clinical features of Goltz–Gorlin syndrome in the present patients are shown in table 1 and illustrated in fig 1. Only unusual additional features and familial variability are described in more detail here.

Two unrelated female fetuses born to classically affected mothers had ectopia cordis, diaphragmatic hernia and abdominal wall defect. Another female fetus had an abdominal body wall defect (fig 2). An adult female had an inward displacement of a lateral part of her thorax, with normal skin covering of the lesion. The adult male had several areas of aplasia cutis at birth (fig 3). These were all located in the midline of the occipital region of the scalp, the anterior thoracic wall or overlying the spine in the lower thoracic region. Some had to be closed surgically in the first year of life as they did not heal spontaneously.

Table 1 Major physical features in 17 patients with Goltz–Gorlin syndrome

Physical feature	Number
Height <P3–P10	11/17
OFC <P3–P10	9/17
Thin, sparse scalp hair	13/17
Thin, protruding ears	13/16
Microphthalmia	7/17 unilateral, 3/17 bilateral
Coloboma	6/17 unilateral, 5/17 bilateral
Significantly decreased vision	5/17 unilateral, 5/17 bilateral
Tear duct obstruction	3/10 unilateral, 1/10 bilateral
Cleft lip/palate	2/17 lip–palate, 2/17 palate
Oligodontia	4/14
Enamel hypoplasia	6/12
Mammary hypoplasia in adults	4/10
Nipple hypoplasia in adults	3/10
Caudal appendage	2/17
Acral abnormalities	4/17 one limb, 5/17 two limbs, 5/17 three limbs, 1/17 four limbs
Oligodactyly	13/17
Polydactyly	1/17
Syndactyly	11/17
Nail hypoplasia	14/17
Hypohidrosis	3/9
Skin hypoplasia	17/17
Papilloma periorally	6/17
Papilloma elsewhere	3/16

OFC, occipitofrontal circumference.

In one adolescent girl, papillomas were present on the palate and vocal cords. She also had papillomas around the orifices. Another female patient had unilateral polythelia, and yet another adult female developed lymphoedema of the lower legs at the end of puberty, which did not react well to adjunctive therapy but remained static. Internal organ abnormalities included: mild hydronephrosis in two females; bilateral renal agenesis in one of the affected female fetuses; anal atresia in one and an anteriorly placed anus in another female; and a ventricular septal defect in two and bicuspid aortic valve in one female. Unusual skeletal manifestations were scoliosis in two females, unilateral symphalangism of the thumb, unilateral hypermobility of the thumb, unilateral complete lower limb aplasia below the knee, unilateral and bilateral fibular aplasia, unilateral clavicular agenesis, and a seemingly spontaneous fracture of the lower leg, each in one female. Other findings were preauricular tags in two females, dislocated lenses in two females, and, in single affected females, a preauricular pit, unilateral and bilateral moderate hearing loss, and a supracerebellar cyst.

One patient developed unilateral breast cancer at 50 years of age, and another female patient developed a large tumour of the skin of her scalp at 64 years of age (possibly a trichilemmal tumour). No histological results are available at present.

The familial cases were: an affected mother and female fetus; an affected mother and two affected female fetuses; and two affected sisters. In the latter family, both parents were clinically unaffected on careful evaluation, and the *PORCN* mutation, present in the sisters, was not found in either parent. It may be of importance that the father had testicular cancer in early adulthood, which had been treated by irradiation. In the two other familial cases, the mothers were classically affected. All three fetuses were more severely affected than the mothers. These two mothers had experienced no miscarriages, and together they had two healthy daughters and one healthy son.

Genotype

Seventeen patients were analysed for point mutations by direct sequence analysis of the coding exons of the *PORCN* gene. In 14 patients (13 females, one male), sequence analysis revealed mutations, heterozygous and hemizygous, respectively. No DNA was available in the three affected fetuses, but, as their mothers had a detectable mutation, it seems very likely that they had the same mutation. No mutation was found in three females. Protein alteration and location throughout PORCN are summarised in table 2 and fig 4.

Mutations were premature nonsense (n = 5), frameshifts (n = 2), aberrant splicing (n = 2) and missense (n = 5) mutations. All sequence alterations detected are likely to be pathogenic, as the transcription products are predicted to result in prematurely truncated proteins, potentially targeted for nonsense-mediated decay, or in dysfunctional proteins. All changes are predicted to result in prematurely truncated or dysfunctional proteins. Three changes (p.Trp444X, p.Trp448X and p.Gly452TrpfsX19) occur in exon 15 and affect the C-terminus of the PORCN protein, a region that is not included in the MBOAT (membrane-bound *O*-acyltransferase) functional

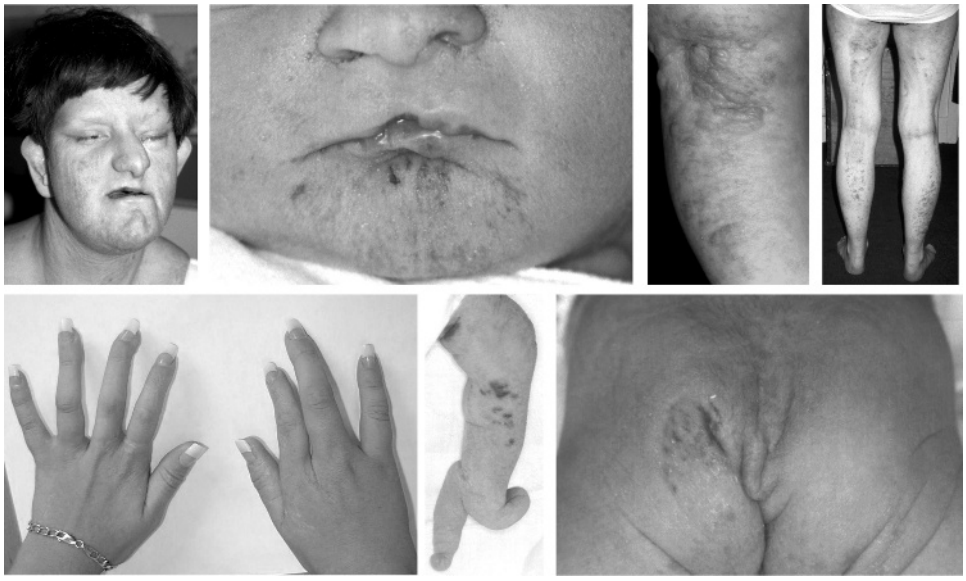

Figure 1 Selection of characteristic findings in females with Goltz–Gorlin syndrome including the general facial phenotype, irregular vermillion of the upper and lower lip, fat herniations in the elbow, streaky pigmentations following Blaschko lines, asymmetrical mildly affected hands, severely affected lower limb and caudal appendage.

domain, spanning amino acids 115–402. However, exon 15 is present in all *PORCN* alternatively spliced transcript variants, and its amino acid sequence is strongly conserved throughout species, suggesting that integrity of the C-terminal part of the protein is essential for proper functional activity. Furthermore, four different missense mutations were identified in five patients, including the same mutation in female siblings. The sisters had the p.Ser297Leu substitution, where the hydrophobic amino acid leucine replaces the hydrophilic serine. This variant was not found in the parents, which suggests a germline mutation, possibly related to testicular irradiation in the father. Serine at 297 is a fully conserved residue, located within the MBOAT domain of PORCN. The other three missense mutations (fig 4) also affect highly conserved residues within the MBOAT domain. The p.Gly168Arg and p.Arg365Gln substitutions have been previously reported.[13 18] None of the missense mutations was detected in 180 control X chromosomes, and no other variants were found in the coding region of *PORCN*.

No mutation was found in three females. A skin biopsy specimen taken from an affected area in one of them failed to show a mutation. No consent was obtained to study other tissues in the other females without mutation. In these mutation-negative females, MLPA analysis was applied to search for small deletions or duplications within the *PORCN* gene: no variations in copy number of *PORCN* exons was found (data not shown). Analysis of exons 2, 5 and 12 was not informative, however, due to signal intensity below detection level.

In the two classically affected females who had three severely affected female fetuses (table 2; patients 5 and 8), an extremely skewed X-inactivation pattern was observed (98/2 and 95/5, respectively).

No mutation was initially detected in lymphocytes in the adult male, who was classically affected, except for relatively mild limb anomalies. Sequence analysis on DNA isolated from fibroblasts cultured from two skin biopsy samples taken from affected and unaffected areas revealed a pathogenic mutation (p.Gln191X) in both samples. The mutant sequence was found superimposed on the wild-type sequence, with a lower signal

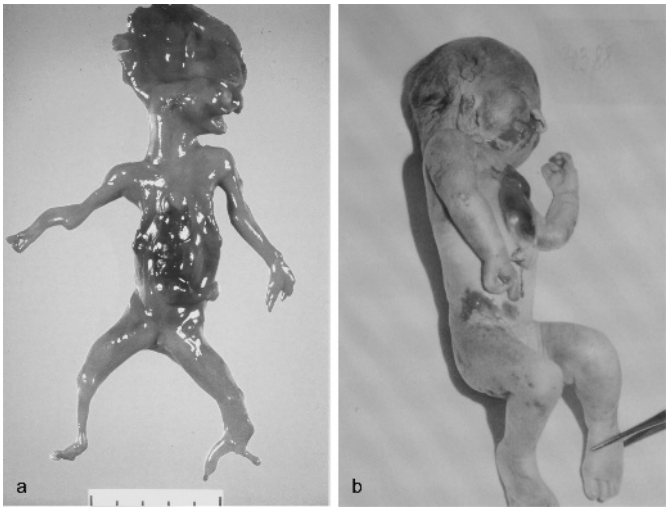

Figure 2 Two severely affected female fetuses born to women with classical Goltz–Gorlin syndrome. Note the thoracic–abdominal body wall defect (a) and ectopia cordis (b).

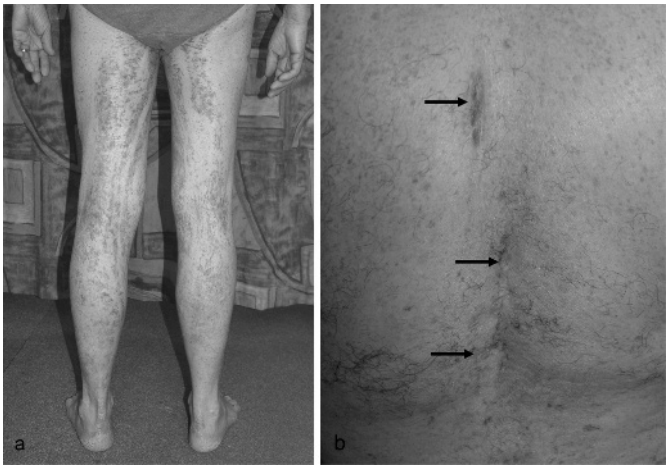

Figure 3 Adult male with molecularly confirmed Goltz–Gorlin syndrome. Note skin lesions following Blaschko lines (a) and areas of aplasia cutis overlying the mid-thoracic spine (arrows) (b).

Table 2 Genotype in 14 patients with Goltz–Gorlin syndrome

Patient	Sex	Nucleotide change	Protein alteration	de novo	Reference
1	F	c.283C>T	p.Arg95X	+	Bornholdt *et al*[13]
2	F	c.509G>A	p.Trp170X	na	Harmsen *et al*[14]
3	M	c.571C>T	p.Gln191X	na	Novel
4	F	c.1331G>A	p.Trp444X	na	Novel
5	F	c.1344G>A	p.Trp448X	na	Novel
6	F	c.637delT	p.Tyr213ThrfsX27	+	Novel
7	F	c.1353dup	p.Gly452TrpfsX19	na	Novel
8	F	c.947-2A>C		na	Novel
9	F	c.1284+1G>A		+	Novel
10	F	c.502G>A	p.Gly168Arg	+	Bornholdt *et al*[13]
11+12	F+F	c.890C>T	p.Ser297Leu	+	Novel
13	F	c.1094G>A	p.Arg365Gln	na	Leoyklang *et al*[18] Bornholdt *et al*[13]
14	F	c.1120G>C	p.Ala374Pro	na	Novel

F, female; M, male; na, parental DNA not available.

intensity at the mutation site in non-affected fibroblasts (fig 5), suggesting somatic mosaicism. Chromosome analysis showed the patient to have a normal male karyotype (46, XY).

DISCUSSION

In this article, we describe the clinical and molecular features of 17 patients with Goltz–Gorlin syndrome. In 14 (13 females, one male) of the 17 patients, a mutation in the *PORCN* gene was found. All 13 females are classically affected, with typical features of Goltz–Gorlin syndrome.[4] Statistical analysis failed to show a significant correlation between the nature and localisation of the mutation and the phenotype (eye, skin, limbs) (data not shown). This is in agreement with previous reports.[13 14]

In three mildly affected females, no point mutations, small deletions or duplications were found. As MLPA analysis for three non-contiguous exons of the *PORCN* gene failed, we cannot rule out the possibility that deletions or duplications of one of these exons might have occurred in these patients. Until now, only larger microdeletions, encompassing the entire *PORCN* gene and sometimes also neighbouring genes, have been reported, however.[10 11 13] These patients were not classically affected: features were limited to focal dermal hypoplasia and hyperpigmentation following Blaschko lines; no papillomas, eye, limb or other abnormalities were present. These cases were each evaluated by the late Professor Robert J Gorlin in 1995, who confirmed the diagnosis. One may speculate whether the Goltz–Gorlin syndrome diagnosis remains applicable in them. We conclude that a mutation has been found in all the classically affected patients, but in none of the atypical patients.

The mother of the two girls with abdominal wall defects (one with ectopia cordis with diaphragmatic hernia and abdominal wall defect, the other with only an abdominal body wall defect) had a premature nonsense mutation. She has classical features of the syndrome. The mother of the other severely affected fetus has classical features of the disorder too, and a missense mutation. In neither of the mothers was there evidence of mosaicism based on sequencing of lymphocyte DNA (no other tissues were available). Both showed extreme skewing by X-chromosome inactivation analysis, making a difference in X-inactivation a less likely explanation for the difference in severity between mothers and fetuses. The data suggest that other factors (environmental, epigenetic, modifying genes) are of importance in determining the phenotype in individuals with *PORCN* mutations. Others were of the same opinion.[10 14 15]

The affected fetuses showed a phenotype that resembles either the limb–body wall complex (including ectopia cordis) or the pentalogy of Cantrell (midline abdominal wall defects, sternum defects, anterior diaphragmatic defects, pericardial defects, congenital heart defects).[22–25] In the limb–body wall complex in particular, there have been case reports of limb defects, including ectrodactyly.[26–28] These have been explained as resulting from amniotic bands. The thoracic wall defect in one of the present affected females and the areas of aplasia cutis in the midline of the anterior and posterior thorax of the present affected male may be related defects. We suggest that some cases with a diagnosis of limb–body wall complex or pentalogy of Cantrell may in fact be severely affected fetuses with Goltz–Gorlin syndrome, and *PORCN* mutation analysis may be indicated in such patients, particularly if limb defects are present.

Although rare, male cases have occurred and some father-to-daughter transmissions have been reported. Fathers exhibit mild signs of the disease such as skin lesions on only one part of the body: the left thigh in one case and involving only one arm and one knee in another. These cases are thought to be mosaic.[5–9] In the presently reported affected male, the mutation was detectable only in fibroblasts and not in lymphocytes. The imbalance in signal intensities at the mutation site in fibroblasts from affected skin and normal skin indicated under-representation of the mutant allele and suggests mosaicism for this mutation. This finding is similar to the results in recently reported affected males in whom signal intensity was also reported to be weaker.[13] The finding of a larger percentage of mutated cells in the biopsy specimen from affected skin compared with unaffected skin in our patient is in agreement with this. If present in previously described affected fathers of

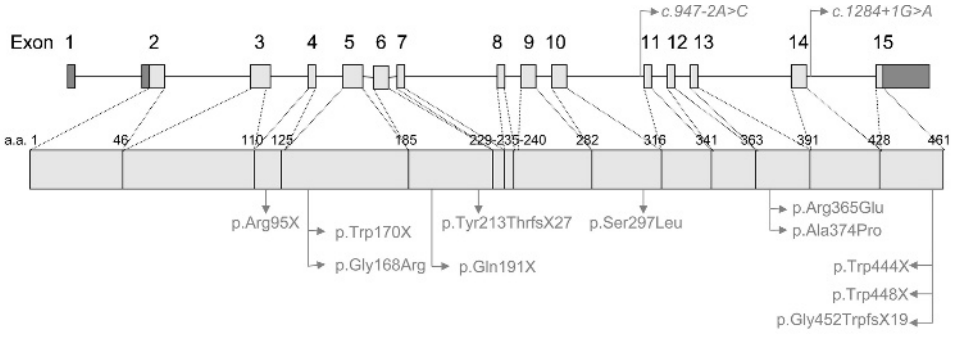

Figure 4 Overview of *PORCN* mutations identified in 17 patients with Goltz–Gorlin syndrome. Exon–intron organisation of *PORCN*: coding regions are in light blue, and non-coding regions in dark blue. PORCN protein structure and amino acid (a.a.) numbering is given according to reference sequence NM_203475.1, variant D. The arrows indicate approximate positions of the mutations. Splicing mutations are in italics.

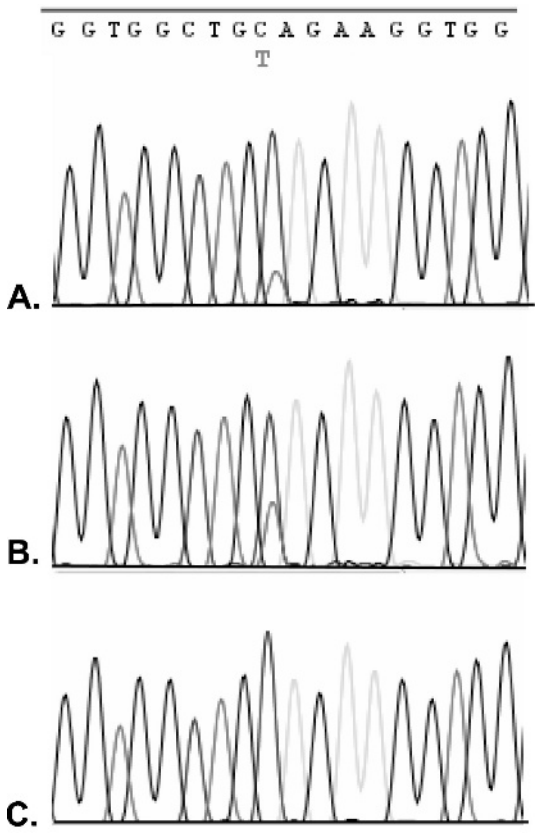

Figure 5 Mosaicism for the c.571C>T *PORCN* mutation (p.Gln191X) in a male with Goltz–Gorlin syndrome. (A) Sequence from non-affected fibroblasts of patient 3; (B) sequence from affected fibroblasts of patient 3; (C) sequence from a control individual.

classically affected daughters, it would explain why fathers are usually only mildly affected. Sequencing of genomic DNA from peripheral blood lymphocytes in females with relatively mild phenotype also show the amount of mutant DNA to be <50% of the wild-type allele, which supports somatic mosaicism.[10 14 17]

The observation of two affected sisters with a mutation without detectable mutation in the parents is suggestive of germline mosaicism. The radiation the father received in early adulthood because of testicular cancer may explain this. Exposure of mouse germ cells to radiation results in various adverse effects, including abortion, malformation and cancer in the offspring depending on the dose, although these findings have not been proven in human studies.[29]

CONCLUSIONS

In 14 of 17 patients with Goltz–Gorlin syndrome, a mutation in the *PORCN* gene was found. All of these individuals had classical features of the syndrome. All three girls born to affected mothers were severely affected. Both somatic and germline mosaicism occur. Some patients with a diagnosis of limb–body wall complex or pentalogy of Cantrell may in fact be severely affected fetuses with Goltz–Gorlin syndrome, and *PORCN* mutation analysis may be indicated.

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