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Novel clinical and molecular findings in Spanish patients with naevoid basal cell carcinoma syndrome*

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Summary

Background Naevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterized by developmental alterations and multiple basal cell carcinomas. Mutations in *PTCH1*, which encodes a membrane receptor for Sonic Hedgehog, are associated with the development of the disease. Most of them produce a truncated protein, which is unable to suppress Smoothened protein and continuously activates the downstream pathway.

Objectives We aimed to characterize 22 unrelated Spanish patients with NBCCS, the largest cohort with Gorlin syndrome reported to date in Spain.

Methods Genomic analysis of *PTCH1* was performed in patients with NBCCS and controls, and mutations were analysed using bioinformatics tools.

Results We report for the first time two young patients, one each with uterus didelphys and ganglioneuroma, within the context of NBCCS. One patient showing a severe phenotype of the disease had developed basal cell carcinomas since childhood. Sanger sequencing of *PTCH1* in this cohort identified 17 novel truncating mutations (11 frameshift, five nonsense and one mutation affecting an exon–intron splice site) and two novel missense mutations that were predicted to be pathogenic. The patients showed great clinical variability and inconsistent genotype–phenotype correlation, as seen in relatives carrying similar mutations.

Conclusions This study contributes to increase the pool of clinical manifestations of NBCCS, as well as increasing the number of pathogenic mutations identified in *PTCH1* predisposing to the condition. The inconsistencies found between

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Conflicts of interest

None declared.

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phenotype and genotype suggest the involvement of other modifying factors, genetic, epigenetic or environmental.

What's already known about this topic?

- Naevoid basal cell carcinoma syndrome (NBCCS) is a rare autosomal dominant disorder characterized by developmental alterations and multiple basal cell carcinomas.
- Mutations in the *PTCH1* gene are associated with the disease.
- Clinical manifestations are variable and inconsistent with the genotype.

What does this study add?

- This is the largest cohort of patients with NBCCS in the Spanish population to date.
- We have identified 17 novel truncating mutations in *PTCH1* and two novel missense mutations in these patients.
- We describe for the first time two patients with NBCCS with uterus didelphys and ganglioneuroma, respectively.

What is the translational message?

- The novel clinical manifestations and *PTCH1* mutations associated with NBCCS in this large series could contribute to the diagnosis of patients with unclear phenotypes.
- The novel *PTCH1* mutations reported in this study could also contribute to improve genetic counselling for the individuals and families affected.

Gorlin syndrome, also known as naevoid basal cell carcinoma syndrome (NBCCS, OMIM 109400), is a rare autosomal dominantly inherited condition with complete penetrance and variable clinical manifestations.[1] Features include developmental defects (i.e. bifid ribs, lamellar calcification of the falx and polydactyly) and tumorigenesis, such as basal cell carcinomas (BCCs), medulloblastomas and keratocystic odontogenic tumours.[2–4] Palmar and plantar pits also appear frequently in these patients.[4,5] Kimonis *et al.* classified these clinical findings into major and minor criteria, and patients are diagnosed when presenting one major criterion and genetic confirmation, two major criteria, or one major and two minor features.[3,6] The prevalence of this syndrome has been estimated as 1 in 56 000,[7] with both sexes equally affected.[8]

Pathogenesis of Gorlin syndrome has been associated with constitutional hemizygous inactivation of *PTCH1*, the human homologue of the *Drosophila* patched gene.[1,9] The gene maps to 9q22·3 and contains 23 exons spanning approximately 65 kb. It encodes a 1450-amino acid glycoprotein, which has 12 transmembrane-spanning domains, intracellular amino- and carboxy-terminal regions and two large hydrophilic extracellular loops.[10,11] The PTCH1 protein acts as a transmembrane receptor for Sonic Hedgehog (SHH).[12] Upon binding of SHH to PTCH1, Smoothened (SMO) protein is activated,[13] and the transduced signal leads to activation of the *GLI* family genes.[14] Mutations in *PTCH1* have been found in 40–80% of patients with NBCCS.[15] The majority of the described mutations result in premature protein truncation. These mutations concentrate mainly in the large extracellular loops, the intracellular loop and the N-terminal region of PTCH1.[16]

To date, mutation screening of *PTCH1* in the Spanish population has been performed mostly in individual cases or families,[17–20] and very rarely in cohorts.[21,22] In this study, we aimed to characterize clinically and to genotype the *PTCH1* gene in 22 unrelated individuals, the largest cohort of Spanish patients with NBCCS reported to date. We have identified 19 novel pathogenic mutations, including two novel missense mutations predicted as pathogenic. We also report novel clinical findings in two young patients.

Patients and methods

Patients

Twenty-two unrelated patients referred from different clinical centres in Spain were recruited for this study. They were diagnosed according to the Kimonis clinical criteria (Table S1; see Supporting Information).[3] In five cases, relatives were also included in the study. A list with all clinical features is presented in Table 1 and Table S2 (see Supporting Information). Information about the characteristics of BCCs was obtained for 14 patients and two relatives, as detailed in Table 2 and Table S3 (see Supporting Information). All enrolled individuals were previously informed about the nature and objectives of the study and they gave written informed consent for the genetic study. Two hundred controls were selected from

Table 1 Clinical findings and associated mutations in *PTCH1* in Spanish patients with naevoid basal cell carcinoma syndrome

ID	Sex	Age (years)	Clinical characteristics	Mutation	Exon; domain	Protein change	Reported
IC1	Female	77	BCCs, palmoplantar pits, family history, hand abnormalities	c.976_977delT	7; ECL1	p.L323X	-
IC2	Male	51	BCCs, odontogenic keratocysts, falx cerebri calcification, rib abnormalities, macrocephaly, frontal bossing, hypertelorism, Sprengel deformity, pectus deformity, vertebral deformities, hand abnormalities, mental retardation, flaccid eyelid, transmission hypoacusis	c.395–2_408delAGTTGGAGGACGA	2–3; ECL1	p.V192fs	-
IC3	Male	37	BCCs, odontogenic keratocysts, palmoplantar pits, falx cerebri calcification, rib abnormalities, family history, hypertelorism, vertebral deformities, hand deformities, occipital lobe asymmetry, left occipital lobe groove enlargement	c.1404dupC	10; SSD	p.A468fs	-
IC4	Female	15	BCCs, odontogenic keratocysts, palmoplantar pits, falx cerebri calcification, rib abnormalities, macrocephaly, hypertelorism, vertebral deformities, uterus didelphys	c.3039C>A	18; ECL4	p.Y1013X	-
IC5	Male	31	BCCs, odontogenic keratocysts, macrocephaly, congenital hydrocephaly	c.697_707delACTGCTTCTG	5; ECL1	p.D233fs	-
IC6	Male	62	BCCs, odontogenic keratocysts, palmoplantar pits, falx cerebri calcification, rib abnormalities, macrocephaly, frontal bossing, hypertelorism, Sprengel deformity, pectus deformity, mental retardation, kyphoscoliosis, turricephaly, septum pellucidum cyst, cortical and central atrophy, daltonism, nose ossification abnormalities	c.417_418insA	3; ECL1	p.E139fs	-
IC7	Male	80	BCCs, odontogenic keratocysts, palmoplantar pits, rib abnormalities, macrocephaly, hand abnormalities, kyphoscoliosis, hypoacusis, enlarged hands, colon polyps	c.724_725delCA	5; ECL1	p.Q242fs	COSMIC database (medulloblastoma)
IC8	Male	7	BCCs, macrocephaly, medulloblastoma, flat feet	c.403C>T	3; ECL1	p.R135X	COSMIC database (odontogenic keratocysts); Wicking 1997[38]
IC9	Male	47	BCCs, odontogenic keratocysts, palmoplantar pits, falx cerebri calcification, rib abnormalities, macrocephaly, hypertelorism, frontal bossing, arachnoid cyst, fused toes	c.2204_2205delTT	14; ICL3	p.F735fs	-
IC10	Female	59	BCCs, odontogenic keratocysts, falx cerebri calcification, rib abnormalities, hypertelorism	c.945+1G>A	6–7; ECL1		-
IC11	Male	65	BCCs, cleft palate, frontal bossing, hypertelorism, mental retardation, melanoma	rs149258400C>T	14; ICL3	p.P725S	1000 Genomes (uncertain significance); COSMIC database (medulloblastoma)
IC12	Male	45	BCCs, odontogenic keratocysts, falx cerebri calcification, rib abnormalities	c.1231_1232delGT	9; ECL1	p.V411fs	-
IC13	Male	43	BCCs, odontogenic keratocysts, palmar hyperkeratosis	rs140417636C>T	23; ICL6	p.R1350W	1000 Genomes (uncertain significance)
IC14	Male	37	BCCs, odontogenic keratocysts, palmoplantar pits, family history, vertebral deformities	c.1011G>A	7; ECL1	p.W337X	-

(continued)

Table 1 (continued)

ID	Sex	Age (years)	Clinical characteristics	Mutation	Exon; domain	Protein change	Reported
IC15	Male	57	BCCs, odontogenic keratocysts, palmoplantar pits, falx cerebri calcification, family history, hypertelorism, brachydactyly, broad nasal bridge, caries, scoliosis	c.2552G>A	15; ECL4	p.W851X	COSMIC database (ovarian carcinoma)
IC16	Female	32	BCCs, family history, frontal bossing, vertebral deformities, infundibular inclusion cyst, bifid spine	c.2454_2457delACTT	15; ECL4	p.L818fs	-
IC17	Male	25	BCCs, odontogenic keratocysts, palmoplantar pits, rib abnormalities, hypertelorism, fibroepithelial polyp, depression	c.258_259delCT	2; N-terminus	p.L86fs	-
IC18	Male	30	BCCs, odontogenic keratocysts, macrocephaly, frontal bossing, hypertelorism, mental retardation	c.284_285insA	2; N-terminus	p.Q95fs	Pietsch 1997[37]
IC19	Male	65	BCCs, odontogenic keratocysts, cleft palate	c.1410_1411insG	10; SSD	p.G470fs	-
IC20	Female	49	BCCs, odontogenic keratocysts, falx cerebri calcification, family history, macrocephaly, cleft palate, hypertelorism, vertebral deformities	c.1138C>T	8; ECL1	p.E380X	Pastorino 2005[39]
IC21	Male	6	Macrocephaly, frontal bossing, medulloblastoma	c.3531_3534delCTTT	21; ICL6	p.F1177fs	-
IC22	Female	–	BCCs, odontogenic keratocysts, macrocephaly, cleft palate, frontal bossing, hypertelorism, Sprengel deformity, mental retardation	c.3140_3152delTTCTGAACCCCTG	18; TM helix 9	p.V1057X	-

IC, index case; BCC, basal cell carcinoma; ECL, extracellular loop; ICL, intracellular loop; TM, transmembrane; SSD, sterol-sensing domain.

individuals over 65 years of age who were not diagnosed with cancer. Samples were obtained according to the law for clinical research in Spain and following approval from the ethics committee of each local centre.

PTCH1 sequencing

Genomic DNA was isolated from blood using standard protocols. Exons 2–23 of *PTCH1* were amplified by polymerase chain reaction (PCR) (RefSeq NM_000264), along with the relevant exon–intron boundaries. Exon 1b was excluded due to its extreme GC-rich sequence. Primers for PCR and sequencing were designed using Primer3 v4·0·0 software (Table S4; see Supporting Information).[23,24] Patients were screened by Sanger sequencing using an ABI377 sequencer (Applied Biosystems, Foster City, CA, U.S.A.) at the Central Sequencing Service of the University of Salamanca, Spain. Mutations found were confirmed on two independent PCR products. Two databases were used to find previously reported variants: dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php).

In silico analysis

Theoretical behaviour of the novel missense mutations was analysed using eight different bioinformatics algorithms: PolyPhen-2,[25] SIFT,[26] Pmut,[27] PANTHER,[28] Align-GVGD,[29] MutationTaster,[30] SNPS3D[31] and PSIPRED.[32] Multiple sequence alignment among 17 related PTCH1 protein sequences was performed using Clustal Omega.[33,34]

Results

The study cohort of patients with NBCCS comprised 22 individuals, 73% of whom were male. The median and interquartile range of the age was 46 (30·5–60·5) years. BCCs (95% of patients) and odontogenic keratocysts (77% of patients) were the most common major criteria found, followed by palmoplantar pits (41%), falx calcification (41%), rib abnormalities (36%) and a family history of Gorlin syndrome (27%). As minor clinical characteristics, patients showed congenital malformations in 86% of the cases, of which hypertelorism (54%), frontal bossing (36%) and cleft palate (18%) were the most common features. Fifty percent of the analysed patients presented macrocephaly. Radiological and skeletal abnormalities detected in the series of patients comprised vertebral abnormalities (23%), radiological defects in the hands or feet (14%), Sprengel malformation (14%) and pectoral deformities (9%). Either mental retardation or low intellectual index was described in 18% of the cases. Only one patient developed medulloblastoma. Here we report a uterus didelphys in a 15-year-old girl with NBCCS. Table 1 shows the details of the clinical features described in the cohort.

Table 2 Clinical characteristics of basal cell carcinomas identified in the patients with naevoid basal cell carcinoma syndrome

ID	Number of BCCs	Age at onset of first BCC (years)	Histopathological subtype	Location	Chronic sun exposure	Clinical evolution	Mutation
IC1	> 50	38	Nodular, superficial, sclerodermiform	Head and neck, trunk	No	Good[a]	c.976_977delT
IC2	109	21	Nodular, superficial	Head and neck, trunk	Yes	Good[a]	c.395–2_408delAGTTGGAGGACGA
IC5	> 20	–	–	–	–	–	c.697_707delACTGCTTCTG
IC6	> 20	41	Nodular, superficial	Head and neck, trunk	Yes	Good	c.417_418insA
IC7	> 20	51	Nodular, superficial	Head and neck, trunk	Yes	Good	c.724_725delCA
IC8	> 100	8	Nodular, two of them infiltrative on the scalp	Head and neck (predominantly scalp), back, major folds and periumbilical region	No (radiotherapy for medulloblastoma)	Good[a]	c.403C>T
IC9	> 100	26	Nodular (naevoid)	Head and neck, trunk (mainly in back)	Yes	Good	c.2204_2205delTT
IC12	> 10	17	–	Head and neck, trunk	No	Good	c.1231_1232delGT
IC13	> 20	26	Nodular	Head and neck (scalp), limbs and trunk	No (exposure on holidays)	Good	rs140417636C>T
IC14	> 10	34	Superficial	Head and neck, trunk, legs	No	Good	c.1011G>A
IC16	55	24	Nodular, superficial	Head, trunk and limbs	–	Good[a]	c.2454_2457delACTT
IC17	18	25	Nodular, superficial	Head, trunk and hands	–	Good	c.258_259delCT
IC18	> 50	22	Nodular, superficial	Head and neck, trunk and legs	–	Participating in MIKIE trial (vismodegib vs. placebo)[35]	c.284_285insA
IC22	> 30	–	–	–	–	–	c.3140_3152delTTCTGAACCCCTG

IC, index case; BCC, basal cell carcinoma. [a]Relapse of some BCCs due to the surgical technique used to remove them.

Details about the characteristics of the BCCs were obtained from 14 index cases (Table 2) and two relatives (Table S3; see Supporting Information). In total 43% of the cases showed more than 50 BCCs, with a maximum of 109 BCCs identified on the same patient. The majority were nodular and superficial, with the sclerodermiform and infiltrative types reported in two patients. The average age at appearance of the first BCC was 28 years, ranging from childhood (8 years) to 51 years. All cases presented carcinomas in the head, neck and trunk, and only three of them reported BCCs spread along the limbs. Prognosis was good in half of the patients; four cases showed a relapse in some BCCs, due to the use of electrocoagulation to treat the superficial types, instead of excision. One of the patients was included in the MIKIE clinical trial – a randomized, double-blind, regimen-controlled, phase II, multicentre study to assess the efficacy and safety of two different vismodegib regimens in patients with multiple BCCs[35] – and therefore he could not be followed up.

PCR amplification and sequencing of 22 exons, including exon–intron boundaries, of *PTCH1* in the NBCCS cohort detected 19 truncating mutations (Table 1). Most of the mutations found were frameshift (52%). We identified one mutation affecting the splice site for exon–intron 6 (c.945+1G>A). A deletion of 13 nucleotides in intron 2 (c.935–2408delAGTTGGAGGACGA) resulted in removal of the splice site for intron 2–exon 3, together with 11 nucleotides in exon 3, creating a premature stop codon and a truncated protein. This was therefore considered a frameshift mutation. Mutations were found along the gene, although the majority of these inactivating variants affected the SHH-binding domain of

the PTCH1 protein (65%), involving the two extracellular loops, and only 10% of them were located within the sterol-sensing domain, essential for suppressing SMO activity (Fig. 1).[36] Frameshift mutation p.Q95fs and two nonsense mutations, p.R135X and p. E380X, have been described previously.[37–39] Mutations p.Q242fs and p.W851X were detected in tumours (medulloblastoma and ovarian carcinoma, respectively) and were reported in the COSMIC database (http://cancer.sanger.ac.uk/cosmic),[40] but no association to NBCCS has yet been established. The remaining 15 truncating mutations are described for the first time in this study.

Genetic screening also detected two missense mutations, one each located in the intracellular loop 3 and C-terminus of PTCH1. Mutation p.P725S was previously detected in medulloblastoma tumours and reported in the COSMIC database. The 1000 Genomes Database (http://www.1000genomes.org)[41] also reported this variant (rs149258400), as well as p.R1350W (rs140417636), both with unclear clinical significance. All these substituted amino acids were evolutionarily strictly conserved among PTCH1 orthologues (Fig. S1; see Supporting Information). *In silico* bioinformatics analysis predicted that p.R1350W was deleterious, while p.P725S could represent a neutral change (Table S5; see Supporting Information). These variations were not found in 400 alleles from healthy individuals. We could not establish a direct correlation between the type of mutation found in *PTCH1* and the clinical characteristics of the patients, the type of BCCs identified or the severity of the disease.

In five cases (IC1, IC3, IC14 and IC15), relatives diagnosed with Gorlin syndrome were also genotyped. They carried the same mutation as the index case, showing that these variations segregate with the disease (Table S2; see Supporting Information). We report a 4-year-old child with ganglioneuroma and NBCCS. His father was also affected with NBCCS, but did not show any nervous system manifestation. Clinical manifestations were different among family members with the same *PTCH1* mutation. We collected information about clinical characteristics of BCCs from two relatives (IC1-I and IC1-II). They showed lower numbers of BBCs, appearing earlier in age than in the index case. They show a similar histopathological subtype and distribution in the body. In both cases, the prognosis was good.

Discussion

We have screened 22 Spanish patients with Gorlin syndrome and identified 19 novel mutations in the *PTCH1* gene. The male-to-female ratio was 2·7 : 1. All studied individuals were diagnosed according to the Kimonis clinical criteria for NBCCS. We did not find significant discrepancies in the frequencies of the main major criteria between the study patients and previously published series,[3,5,42,43] apart from a greater incidence of hypertelorism at 56%, compared with only 5% found in the literature.[5] Patients in our study also showed a significantly reduced falx calcification incidence among individuals > 20 years old when compared with previous studies.[42,44] Minor criteria involve less-specific features and, therefore, a larger inconsistency among them has been reported.

Beyond diagnostic criteria, we report some low-frequency clinical findings in patients with NBCCS, namely uterine myoma and arachnoid cyst. Furthermore, we report a patient with NBCCS presenting uterus didelphys, a malformation produced by the complete failure of fusion in the paired Mullerian ducts, resulting in duplication of the uterine corpus and cervix.[45] This condition has been associated with other genitourinary malformations, such as renal agenesis,[46] also present in patients with NBCCS. In our series, we also identified a young patient with NBCCS with ganglioneuroma, a benign neurogenic tumour, originating from the neuroepithelium along with sympathetic ganglia.[47] To date, none of the above-mentioned conditions has previously been reported in patients with NBCCS. Therefore, the findings presented here enlarge the spectrum of uterine and central nervous system abnormalities, which are common features of Gorlin syndrome.

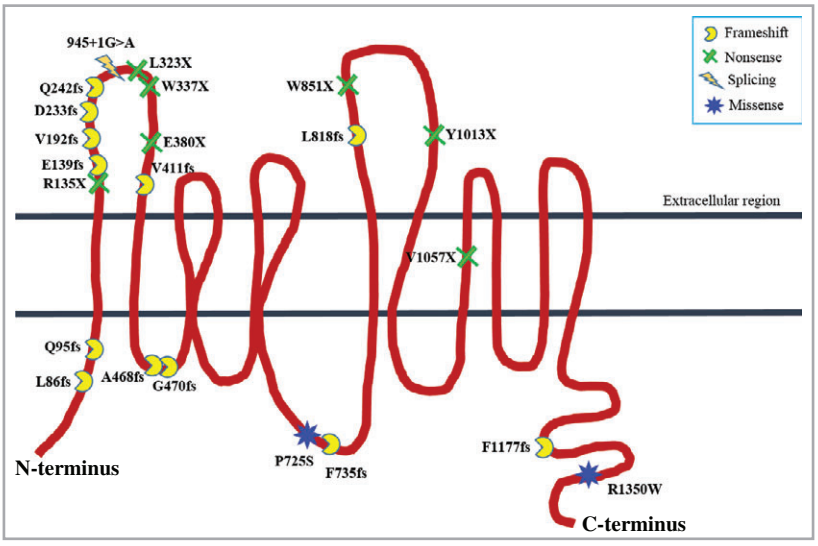

Fig 1. Graphical representation of the mutations found in the *PTCH1* gene in the screening cohort with naevoid basal cell carcinoma syndrome.

BCCs identified on the patients showed a similar trend on histopathological subtype, location in the body and progression in all patients; however, age of appearance of the first BCC was broad, from childhood (8 years old) to 51 years old. Although BBCs occur only rarely in childhood and youth, we identified two patients from our cohort who started to develop BCCs at a very young age. The case first identified at 8 years of age showed a severe phenotype, including infiltrative BCCs on the scalp, larger body surface area covered with BCCs, relapse of some of them and medulloblastoma.

To our knowledge, this is the largest study performed to date in patients with Gorlin syndrome in the Spanish population, and one of the largest cohorts of patients with NBCCS reported worldwide. Furthermore, we have identified 19 novel mutations in *PTCH1* associated with the disease, including two missense variants. Genetic screening of 22 exons and intron–exon boundaries of the *PTCH1* gene revealed truncating mutations in 87% of the patients, mainly frameshift alteration, in agreement with the literature, and missense variations in the remaining individuals. Exon 1 was not included in the study due to its diversity at the 5′-extreme end. Only exon 1b is able to inhibit SMO activity fully, but this exon has not yet been investigated due to its extremely GC-rich sequence.[48–50]

In this study, unrelated patients showed different mutations in *PTCH1*, without any recurrent findings, which is consistent with the documented high frequency of spontaneous mutations in this gene.[51–55] Although no hotspots for mutations were found, most of the truncating mutations were located within the first and fourth extracellular loops, both involved in the binding of SHH. Alterations in this region prevent ligand binding and, therefore, pathway activation. Furthermore, two missense mutations were identified, previously reported in the 1000 Genomes Database, with an unknown clinical meaning. Missense mutation p.P725S was reported in medulloblastoma tumours;[56] however, the patient with NBCCS carrying this mutation did not present this tumour. Instead, he was diagnosed with melanoma. This is the first time these missense mutations have been found in patients with Gorlin syndrome.

In silico prediction of clinical significance and analysis of 400 alleles from healthy individuals suggested that p.R1350W was pathogenic. The amino acid affected was strictly conserved in a large number of PTCH1 orthologues, and located within a very well-conserved region of the protein. Missense mutation p.P725S was predicted to be a neutral change; however, it has already been reported as pathogenic in medulloblastoma tumours.[56] It substitutes a strictly conserved amino acid, located within a well-conserved area of the protein, which suggests a functional significance. Absence of this variant in 400 alleles from healthy individuals used as controls also supports its pathogenicity. Further studies need to be performed in order to address fully its role at the protein level.

Only one splicing mutation was detected, changing G to A at the donor site, affecting the exon 6–exon 7 junction. A different variant in this position has been previously reported in an Italian patient with Gorlin syndrome (c.945+1G>C).[53] The Italian patient presented BCCs, odontogenic keratocysts, eye abnormalities and ovarian fibroma, while patient IC10 studied here showed a more severe phenotype (BCCs, odontogenic keratocysts, calcification of the falx cerebri, rib abnormalities and hypertelorism), confirming the extreme variability of the clinical features present in patients with NBCCS. In general, we found great heterogeneity in the mutations described in the *PTCH1* gene (missense, nonsense, frameshift) associated with the appearance of the disease, in line with previous genetic analyses performed in case reports and series of patients. The patients with Gorlin syndrome analysed here also present high clinical heterogeneity, even inside a pedigree, which complicates the genotype–phenotype correlation in this trait.

To date there are no established guidelines for the best management of BCCs in Gorlin syndrome. The National Comprehensive Cancer Network guidelines mention that radiotherapy is contraindicated in this disease.[57] Thus, surgical excision has been the most frequent method to remove tumours in these patients. Given the large number of tumours, different surgical approaches have been used, including excision followed by electrosurgery, conventional surgery and Mohs micrographic surgery. Furthermore, topical medications such as imiquimod, topical photodynamic therapy, fluorouracil, laser and oral retinoids have been implemented with variable effectiveness.[58] As the data available for the chemotherapy used in NBCCS are obtained from case reports or small case series,[58] no consensus has been obtained for the management of BCCs in Gorlin syndrome, and patients are usually treated on an individual basis. In 2012, vismodegib emerged as a safe and effective option for the management of BCCs in Gorlin syndrome in selected cases.[59]

The variability of the treatment options available for these patients, as described above, shows the need for studies in large cohorts of patients with Gorlin syndrome, which could result in common management guidelines for these patients. To date, there is no available treatment to cover the great phenotypic variability described in these patients, and, therefore, we suggest to continue treating them on an individual basis until more research and clinical trials could support a safe and efficient general practice.

In conclusion, this is the largest genetic analysis to date in Spanish patients with NBCCS. We have enlarged the range of uterine and nervous system alterations described in patients with NBCCS, and detected 19 novel mutations in the *PTCH1* gene associated with the disease, including two missense variants predicted as pathogenic. These novel clinical and molecular findings could contribute to the understanding of the disease for future treatments and genetic counselling.

Lack of correlation between genotype and phenotype, including clinical characteristics of BCCs, suggests that other biological events or genes may also be involved in the disease severity. Further investigation needs to be done to address the clinical variability.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Multiple sequence alignment of *PTCH1* to show conservation of (a) p.P725S and (b) p.R1350W.

Table S1 Kimonis criteria used in the diagnosis of naevoid basal cell carcinoma syndrome.

Table S2 Clinical features of the affected relatives.

Table S3 Clinical characteristics of basal cell carcinomas identified in the relatives.

Table S4 Sequence of oligonucleotides designed to amplify exons 2–23 of *PTCH1*.

Table S5 Results from the *in silico* analysis of the novel missense mutations. (a) c.2173C>T, p.P725S; (b) c.4048C>T, p.R1350W.