**A recurrent mosaic mutation of *SMO*, encoding the hedgehog signal transducer Smoothened, is the major cause of Curry-Jones syndrome**

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Curry-Jones syndrome (CJS) is a multisystem disorder characterized by patchy skin lesions, polysyndactyly, diverse cerebral malformations, unicoronal craniosynostosis, iris colobomas, microphthalmia, and intestinal malrotation with myofibromas or hamartomas. Cerebellar medulloblastoma has been described in a single affected individual; in another, biopsy of skin lesions showed features of trichoblastoma. The combination of asymmetric clinical features, patchy skin manifestations and neoplastic association previously led to the suggestion that this could be a mosaic condition, possibly involving Hedgehog (Hh) signaling. Here we show that CJS is caused by recurrent somatic mosaicism for a nonsynonymous variant in *SMO* (c.1234C>T; p.Leu412Phe), encoding Smoothened (SMO), a G protein-coupled receptor that transduces Hh signaling. We identified seven mutation-proven individuals (including one previously unreported), with highly similar phenotypes, in whom we demonstrate varying amounts of the mutant allele in different tissues. We present detailed findings from magnetic resonance brain imaging in three mutation-positive cases. Somatic mutations of *SMO* that result in constitutive activation have been described in several tumors, including medulloblastoma, ameloblastoma and basal cell carcinoma. Strikingly, the most common of these mutations is the identical nonsynonymous variant encoding p.Leu412Phe. Furthermore, this substitution has been shown to activate SMO in the absence of Hh signaling, providing an explanation for tumor development in CJS. This raises therapeutic possibilities using recently generated Hh pathway inhibitors. In summary, our work uncovers the major genetic cause of CJS and illustrates strategies for gene discovery in the context of low-level tissue-specific somatic mosaicism.

The multiple congenital anomalies disorder Curry-Jones syndrome (MIM: 601707) was first presented (in abstract form) by Cynthia Curry and Marilyn Jones at the David Smith workshop on Malformations and Morphogenesis in 1987. These authors described two unrelated individuals with the shared features of unilateral coronal cranisynostosis, cutaneous syndactyly, bilateral preaxial polydactyly of the feet, and unusual streaky skin lesions. Subsequently, the term Curry-Jones syndrome (CJS) was applied to this condition.[1](#_ENREF_1); [2](#_ENREF_2)

The first formal publication on CJS was by Temple et al.[3](#_ENREF_3), which included detailed clinical descriptions of the two original subjects as well as three further unrelated individuals. Four further simplex cases have since been added to the literature, all with abnormal skin patches and preaxial polydactyly of the feet.[4-6](#_ENREF_4) Additional features found in most individuals have included ectopic hair growth, abnormalities of brain development, coloboma and/or microphthalmia, coronal suture synostosis, cutaneous syndactyly, and intestinal malrotation and/or obstruction (reviewed by Grange et al.[6](#_ENREF_6)). Figure 1A-D shows the major craniofacial, limb and dermatological features of CJS in a previously unreported individual (Subject 8 in our series); the diversity of cerebral malformations (described in more detail below) is illustrated by magnetic resonance imaging (MRI) scans of three individuals in Figure 1E-G. Mild intellectual disability has been present in some cases. In a single individual, a desmoplastic medulloblastoma (WHO grade IV/IV) of the right cerebellum, found incidentally on a followup MRI brain scan at 17 months, was treated successfully by surgical resection, chemotherapy and radiotherapy.[6](#_ENREF_6) In two individuals, biopsies of active skin lesions were reported as showing features of either trichoblastoma[6](#_ENREF_6) or nevus sebaceous.[3](#_ENREF_3) In addition, odontogenic keratocysts have been found in one individual, and lesions in the bowel, identified histologically as hamartomas or myofibromas, were reported in three instances.[3](#_ENREF_3); [6](#_ENREF_6)

The etiology of CJS was previously unknown, but three clinical observations are relevant to hypotheses for causation. First, the nine previously reported cases comprised seven males and two females with similar severity in the two sexes, making an X-linked mutation unlikely. Second, the sporadic origin of all cases, associated with patchy skin lesions and asymmetric cranial findings led Temple et al.[3](#_ENREF_3) to propose an underlying mosaic mutation; however Grange et al.[6](#_ENREF_6) favoured germline constitutional mutation in view of the consistent presentation and bilaterality of some of the other features. Third, the occurrence of medulloblastoma in basal cell nevus syndrome (BCNS; MIM: 109400) caused by mutations of *PTCH1* (MIM: 601309), which encodes the Hedgehog (Hh) receptor, as well as the overlapping cranial and limb abnormalities found in disorders caused by mutation of *GLI3* (MIM: 165240), a downstream effector of the Hh pathway, led Grange et al.[6](#_ENREF_6) to propose that perturbation of Hh signaling could underlie CJS. However, DNA sequencing of *PTCH1* and *GLI3* in two individuals with CJS was normal.[6](#_ENREF_6) The objective of the present work was to identify causative disease genes in CJS through whole-exome sequencing (WES), initially using an overlap strategy in four individuals to pinpoint disease-causing variants in the same gene.

The study was approved by the Oxfordshire Research Ethics Committee B (reference C02.143) and the Riverside Research Ethics Committee (reference 09/H0706/20). Participants or their parents provided informed, written consent for genetic studies. Four of five samples initially chosen for analysis were from individuals with CJS reported by Temple et al.;[3](#_ENREF_3) these comprised both eyelid tissue (sample 1-2) and fibroblasts from an affected skin biopsy (sample 1-4) from Subject 1 (the individual originally described by Jones), and fibroblasts from affected skin of Subjects 2 (sample 2-3) and 3 (sample 3-2), the latter being the individual originally described by Curry. An additional fifth sample (4-1) was from blood of Subject 4, an unpublished individual with features overlapping CJS. The clinical features of these four individuals are summarized in Table 1.

Following DNA extraction we performed WES using the SeqCap EZ Human Exome Library v2.0 (NimbleGen) on the HiSeq2000 (Illumina). Paired-end reads (100 bp) were mapped to hs37d5 using Stampy version 1.0.22,[7](#_ENREF_7) and after removal of artifacts, average coverage was >25x. We called variants using Platypus[8](#_ENREF_8) and, assuming that CJS is caused by very rare autosomally located variant(s), we prioritized the data by excluding variants present in either (1) our in-house database of solved cases or (2) the Exome Variant Server (later revised to the Exome Aggregation Consortium (ExAC) dataset for reanalysis). We compared across CJS individuals for hits in the same gene. This analysis did not yield any genes with rare coding variants shared by three or four individuals, and only 3 genes with rare coding variants shared by two cases, none of which were strong candidates (Table S1).

As an alternative approach, we compared the data from the two tissues separately sequenced from Subject 1, as mosaicism for a pathogenic variant could be detectable by finding a difference in allele frequencies between the datasets. Variants were ranked according to predicted pathogenicity (annotation from ANNOVAR[9](#_ENREF_9)) and scrutinised manually for differences between the samples. Within the variant list for the fibroblast sample 1-4 we observed a nonsynonymous substitution in *SMO* (MIM: 601500; NM\_005631.4), c.1234C>T encoding p.Leu412Phe, present in 44 of 83 sequence reads. Notably, this variant was not called in the eyelid sample 1-2, although a low level of the same variant (2 of 40 sequence reads) was apparent on manual examination of the reads (Figure 2A). The fortuitous presence of two heterozygous flanking SNPs (dbSNP137, rs2228617 and rs2735842) in this individual enabled the two *SMO* alleles to be easily distinguished. We noted that when sequence reads included both the c.1234 position and one of the flanking SNPs, the mutant c.1234T reads were always in *cis* with the variant allele of the SNP in both samples. However, for the eyelid sample 1-2, the converse was not true, supporting the conclusion that the c.1234C>T mutation was mosaic in this sample (see Figure 2). The *SMO* gene encodes Smoothened (SMO), a frizzled class G-protein-coupled receptor that plays a key role in transduction of Hh signaling. Hh binding relieves Patched-mediated suppression of SMO to allow transduction of the signal, probably mediated by conformational changes within the 7-transmembrane bundle.[10](#_ENREF_10) Hence the variant matched all three criteria (autosomal, mosaic, and affecting Hh signalling) for the characteristics of a candidate gene for CJS based on clinical deduction.[3](#_ENREF_3); [6](#_ENREF_6) Adding further weight to the conclusion that this was the pathogenic variant, the Catalogue of Somatic Mutations in Cancer (COSMIC) database revealed that the *SMO* c.1234C>T was a previously known mutation hotspot in multiple tumor types (discussed further below). Dideoxy-sequencing of *SMO* exon 6 in sample 1-4 confirmed the presence of the C>T variant in apparently heterozygous state, but the mutant peak was barely visible in sample 1-2 (Figure 2B).

In the light of this finding we scrutinized the exome sequence data for *SMO* from the other three CJS individuals in greater detail. The identical c.1234C>T variant was present in 2.7% of reads (6 of 219) from sample 2-3 (Figure S1), but was absent in the other two samples (0 of 57 reads from sample 3-2 and 0 of 179 reads from sample 4-1); no other suspicious *SMO* variant was detected in the exome sequences of Subjects 3 or 4.

To gather further evidence that mosaic *SMO* mutations cause CJS, we collected and analyzed additional tissue samples from Subjects 1-4 together with a further five individuals with CJS (Subjects 5 and 6 reported by Grange et al.,[6](#_ENREF_6) Subject 7 reported by Thomas et al.,[5](#_ENREF_5) and two unpublished cases; clinical features are summarized in Table 1). Material from affected regions was prioritized and included archival formalin-fixed paraffin-embedded (FFPE) sections (Table S2). Altogether we undertook deep sequencing of *SMO* exon 6 in thirty-five different tissue samples from the nine CJS subjects (including the previously sequenced samples) and three control samples, using the Ion Torrent PGM. Table S3 lists the primer sequences and conditions used for *SMO* (NG\_023340.1) amplification and sequencing. Deep sequencing of Subject 1 DNA confirmed a ~50% frequency for c.1234C>T in the fibroblast sample 1-4 and showed that the variant was present at 11% in the eyelid sample 1-2 (Figure 3 and Table S2). Surprisingly, we did not identify any mutant c.1234T alleles in the affected skin sample (1-3) from which the fibroblasts constituting sample 1-4 were derived, perhaps indicating positive selection of mutant cells during growth in culture. The exome finding in Subject 2 that suggested the tested sample (2-3) was *SMO* mutation-positive was validated and variant frequencies of c.1234C>T ranging from 4-25% were detected in 3 of 5 samples, confirming mosaicism for the identical *SMO* mutation in this individual.

Deep sequencing of *SMO* in the samples 3-2 and 4-1 used for exome sequencing confirmed that the c.1234C>T mutation was essentially undetectable as the data were indistinguishable from the control samples (Table S2). However, a new skin biopsy sample from Subject 3 was positive (14%), with a low level (1.6%) detected in saliva from this individual. Furthermore, four of the five additional CJS cases (Subjects 5-8) were also mosaic for c.1234C>T (Figure 3 and Table S2). FFPE material from an occipital meningocele and cerebellar medulloblastoma was available from Subject 5 (corresponding to Patient 2 in Grange et al.[6](#_ENREF_6)), and analysis showed relatively high levels of mosaicism at 20% and 43% c.1234T allele, respectively. Similarly, the abdominal smooth muscle tumor sections from Subject 6 (Patient 1 in Grange et al.;[6](#_ENREF_6) samples 6-3 and 6-4) contained 35-37% c.1234T allele. In Subject 7, a mildly affected individual,[5](#_ENREF_5) we detected 3-4% c.1234T in skin (sample 7-1, taken from a hairy area of the inner leg) and associated fibroblasts (7-2), as well as a femoral bone marrow sample (7-5, 1%). Finally, we identified the c.1234T variant in samples from the large bowel of Subject 8, a previously unpublished case (Figure 1A-D and G, Table 1 and Supplementary Clinical Report). FFPE samples from the colon (8-2) and cecum (8-3) collected during investigation of intestinal malrotation and mesenteric masses (which contained hamartomatous nodules consisting of disorganized bundles of mature smooth muscle intermixed with nerve fibers and ganglion cells), had 8-9% levels of the c.1234T allele (Figure 3).

In total we identified the identical *SMO* c.1234C>T mutation in tissues from seven unrelated individuals with CJS, including the two originally described by Curry and Jones. In all but one sample (1-4, the sample in which the mutation was originally identified by exome sequencing), the c.1234T allele was present at a level substantially below 50%, indicating tissue mosaicism. Importantly for diagnosis, we had greatest success in detecting the mutation in affected tissues obtained by invasive procedures. The mutation was not reliably detected in blood samples from three mutation-positive individuals (levels of c.1234T ranged from 0.03-0.5%, Figure 3 and Table S2). We had greater success using saliva samples (mean 3.6%, range 0.11- 7.1% in four mutation-positive individuals), but in every case a different tissue sample showed a higher mutation level (Figure 3 and Table S2).

The finding of widespread mosaicism in CJS suggests that it arises post-zygotically early during embryonic development. A somewhat later acquisition of the mutation is predicted to cause isolated pathology of individual organs, so we explored this possibility in two tissue contexts, brain and skin. In the case of the brain, we sought to define further the neuroanatomical features of CJS by reviewing recent MRI scans of three mutation-positive individuals (Subjects 5, 6 and 8). A diverse range of phenotypes was present in this small sample; prominent features are illustrated in Figure 1E-G, with the complete phenotype summarized in Table S4 and presented in Figure S2. Abnormal findings included hemimegalencephaly (HMEG) with cortical dysplasia, white matter abnormalities and polymicrogyria, abnormalities of the corpus callosum, ventriculomegaly, occipital meningocele and Chiari type I malformation. Given the phenotypic overlap we tested five samples from individuals with isolated megalencephaly/HMEG, but did not identify any *SMO* mutations (Table S5). For skin we performed deep sequencing of *SMO* in 14 isolated trichoblastoma samples and a single nevus sebaceous sample, as analysis of skin biopsies from Subjects 1 and 6 had previously demonstrated nevus sebaceous[3](#_ENREF_3) and trichoblastoma,[6](#_ENREF_6) respectively. Although we detected rare SNPs, potentially pathological variants were not found (Table S5).

Although no constitutional mutations of *SMO* have previously been described, several hotspots of somatic mutation are evident in COSMIC, and it is striking that the most frequent of these is the identical c.1234C>T transition (note, this does not occur in the context of a CpG dinucleotide, so intrinsic hypermutability[11](#_ENREF_11) is not predicted). Notably, the *SMO* c.1234C>T mutation has been identified in ameloblastoma,[12](#_ENREF_12); [13](#_ENREF_13) medulloblastoma,[14](#_ENREF_14); [15](#_ENREF_15) meningioma,[16](#_ENREF_16); [17](#_ENREF_17) basal cell carcinoma (BCC)[18](#_ENREF_18); [19](#_ENREF_19) and moreover, has been reported as the oncogenic driver in some of these tumours.[12](#_ENREF_12); [19](#_ENREF_19) The mutated amino acid Leu412 locates to transmembrane helix 5 of SMO within one of three pivot regions that, by analogy with the ß2 adrenergic receptor,[20](#_ENREF_20) are likely to have a key role in the conformational changes required for receptor activation. Supporting this, expression of mutant p.Leu412Phe leads to constitutive activation in the absence of Hh ligand in *Smo*-/- mouse embryonic fibroblasts,[12](#_ENREF_12); [18](#_ENREF_18) and increased cell proliferation in ameloblast-lineage cells.[12](#_ENREF_12)

In vertebrates there are 3 Hh ligands, Sonic Hedgehog (SHH; MIM: 600725), Desert Hedgehog (DHH; MIM: 605423) and Indian Hedgehog (IHH; MIM: 600726), which by binding to the receptor Patched (encoded by *PTCH1* or *PTCH2* [MIM:603673]) remove inhibition of SMO. Downstream effectors of Hh signal transduction, notably the transcription factors GLI2 (MIM: 165230) and GLI3, are normally tethered by SUFU (Suppressor of fused homolog; MIM: 607035) at the base of the primary cilium where they are proteolytically processed to repressor forms (GLI2-R and GLI3-R, respectively). SMO activation leads to KIF7-dependent translocation towards the tip of the primary cilium and transport of full-length activated GLI proteins (GLI2-A and GLI3-A) into the nucleus, enabling transcriptional activation (reviewed by Briscoe and Therond,[21](#_ENREF_21) McCabe and Leahy,[22](#_ENREF_22) Arensdorf et al.[23](#_ENREF_23)). Activation of the PI3K/AKT/mTOR and/or PKA-pathways can independently lead to GLI activation, indicating the potential for significant cross-talk with the Hh pathway (Wang 2012).[24](#_ENREF_24) Constitutional mutations that mimic the consequences of Hh signal activation include loss-of-function mutations in negative regulators acting at multiple stages of the Hh pathway, such as *RAB23* (Carpenter syndrome [MIM: 606144][25](#_ENREF_25)); *PTCH1* (BCNS[26-28](#_ENREF_26)), *SUFU* (BCNS[29](#_ENREF_29); [30](#_ENREF_30)), *KIF7* (acrocallosal syndrome[31](#_ENREF_31)) and *GLI3* (Greig cephalopolysyndactyly[32](#_ENREF_32); [33](#_ENREF_33)). In addition, regulatory mutations of *IHH* cause Philadelphia craniosynostosis.[34](#_ENREF_34) Reflecting the net consequence of excessive Hh signal transduction, several clinical features of CJS overlap the above disorders, as previously noted.[6](#_ENREF_6) These include craniosynostosis[35](#_ENREF_35) and syn/polydactyly[36](#_ENREF_36) in Carpenter and Philadelphia craniosynostosis syndromes, cerebral malformations in acrocallosal syndrome[31](#_ENREF_31) and odontogenic keratocysts and skin involvement in BCNS.[37](#_ENREF_37) We also note overlap in the cerebral features with mosaic activation of components of the PI3K/AKT/mTOR pathway, including Proteus syndrome (MIM: 164730), fibroadipose hyperplasia and CLOVES syndrome (MIM: 612918), and HMEG (reviewed in Keppler-Noreuil et al.;[38](#_ENREF_38) Hevner,[39](#_ENREF_39) and Jansen et al.[40](#_ENREF_40)). To our knowledge, abnormalities of the bowel have not previously been highlighted as a frequent feature of disorders associated with activation of the Hh signalling, but appear common in CJS (including myofibromas or hamartomas, and malrotation). These observations are consistent with the documented role of Shh and Ihh, produced by the endodermal epithelium, as primary factors in the patterning and organogenesis of the gut,[41](#_ENREF_41) where Hh signaling from the endoderm controls growth of the adjacent mesenchyme.[42](#_ENREF_42) Furthermore, mouse embryos in which *Smo* is deleted from the gut mesenchyme have severely reduced proliferation and differentiation of the intestinal mesenchyme, with a reduction in the number of smooth muscle cells and enteric neurons.[43](#_ENREF_43)

Given the observed association of CJS with neoplastic diseases (trichoblastoma and cerebellar medulloblastoma), a particularly important consequence of identifying the activating p.Leu412Phe substitution in SMO concerns the potential therapeutic implications for management of affected individuals. Recently there has been considerable interest in the development of SMO inhibitors to reduce Hh pathway activation (reviewed by Arensdorf et al.[23](#_ENREF_23)). Importantly, however, it appears that the p.Leu412Phe substitution confers resistance to vismodegib, a clinically approved inhibitor of SMO that makes contacts with the extracellular domain and ligand-biding pocket, supporting a role for Leu412 in autoinhibition and/or structural stability.[18](#_ENREF_18); [19](#_ENREF_19) This suggests that in CJS-associated tumors, use of inhibitors, such as arsenic trioxide or other more specific GLI-inhibitors or PI3K/AKT/mTOR inhibitors, that act at later stages of the Hh signal transduction pathway should be explored,[29](#_ENREF_29) and indeed there is evidence that this approach can be effective against p.Leu412Phe mutants.[12](#_ENREF_12); [18](#_ENREF_18)

In summary, these data show that the major phenotypic features of CJS are attributable to excessive activation of Hh signalling owing to a specific c.1234C>T (p.Leu412Phe) somatic mutation. The finding that all seven mutation-positive cases are mosaic may indicate that constitutional mutations are not compatible with life. The mutation is likely to be associated with a spectrum of negative and positive selective consequences during organismal growth and homeostasis, depending on cell type, which likely explains the apparent evolution of skin lesions and low mutation levels in blood (negative selection), and predisposition to tumorigenesis (positive selection). Interestingly, a recent report of a subject with segmental BCNS associated with the identical SMO p.Leu412Phe substitution[44](#_ENREF_44) is compatible with a later occurrence of the somatic mutation during development, compared to subjects with the classical CJS phenotype. We were unable to detect c.1234C>T in Subjects 4 and 9, from whom only blood and saliva samples were available, and deep sequencing of the entire *SMO* coding region did not identify any alternative variants (data not shown). Genetic heterogeneity, and/or suboptimal tissue sampling may account for the negative results in these two cases.

**Supplemental Data**

Supplemental data includes 5 tables, 2 figures and a Clinical Report for Subject 8.

Table S1. Genes for which rare coding variants were present in two of the samples analyzed by exome sequencing.

Table S2. Deep sequencing of *SMO* c.1234C>T in CJS samples – read depths and percentage mutant allele.

Table S3. Primer sequences and amplification conditions used for genetic analysis of *SMO*.

Table S4. Comparison of brain imaging in Subjects 5, 6 and 8.

Table S5. Deep sequencing of *SMO* in brain, trichoblastoma and nevus sebaceous samples.

Figure S1. Exome sequencing data from Subject 2.

Figure S2. MRI neuroimaging features from Subjects 5, 6 and 8.

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**Web Resources**

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/

Exome Variant Server, http://evs.gs.washington.edu/EVS/

Exome Aggregation Consortium, http://exac.broadinstitute.org/

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/

COSMIC, http:// cancer.sanger.ac.uk/cosmic

Leiden Open Variation Database (LOVD), http://chromium.lovd.nl/LOVD2

**Accession Numbers**

*SMO*: NM\_005631.4 (mRNA) and NG\_023340.1 (genomic)

**References**

**Figure legends**

**Figure 1.** **Clinical and radiological features, and brain imaging, of individuals with CJS caused by *SMO* mutation.** (A-D) Subject 8 showing (A) Facial features aged 2 yr, note asymmetry, frontal bossing and scarring of right eyelid. (B) Computed tomographic (CT) head scan aged 7 months showing right coronal synostosis (arrow) and bifurcated sagittal suture with fontanellar bone. (C) The right foot is medially deviated with a duplicated hallux and partial cutaneous syndactyly of digits 1-3. (D) The skin has linear streaks of hypopigmentation with atrophy, this was not visible at birth but had developed by the age of 6 months. (E-G) Magnetic resonance imaging (MRI) of brain. (E) Subject 5 aged 1 yr 5 months, note left-sided hemimegalencephaly with extensive cortical malformation (arrows) and ventriculomegaly (asterisk). (F) Subject 6 aged 3 months, there are subtle abnormalities of gyri and cortex (white arrows), note cyst in left thalamus (black arrow) that connects with 3rd ventricle-midline cyst. (G) Subject 8 aged 1 day, showing thin corpus callosum, mildly hypoplastic cerebellum and occipital cystic lesion, pathologically confirmed to represent a lymphangiomatous malformation (white arrow).

**Figure 2. Identification of a mosaic *SMO* 1234C>T mutation. (**A) GBrowse[45](#_ENREF_45) visualization of exome sequence data from two tissues of Subject 1. The upper panel shows the location of *SMO* on chromosome 7q32.1 and the middle and lower panels display a 144 bp alignment of sequencing reads aligned to exon 6 and the following intron; bases that match the reference sequence are boxed in black and variant reads are in red. The red arrows indicate the position of the c.1234C>T mutation (indicated by a ‘t’ within a red box) which is present in 53% reads in tissue 1-4 (middle panel; not all reads shown) but only in 2.7% reads in tissue 1-2 (lower panel). Flanking heterozygous SNPs are indicated by black arrows. (B) Dideoxy-sequence traces for the 1234C>T mutation. Top, in sample 1-4, the mutation appears to be present in heterozygous state, with no evidence of dilution by non-mutant cells (red arrow). Below, in samples 1-2 and 2-3, a small proportion of the mutant T allele is suspected to be present, based both on presence of a small T peak and on reduced relative height of the normal C peak (pink arrows, compare with control at bottom). By contrast, samples 3-2 and 4-1 appear negative for the mutation.

**Figure 3. Quantification of tissue mosaicism for *SMO* 1234C>T mutation.** Deep sequence data plot of proportion of mutant T allele in tissue samples collected from Subjects 1-9. Similar tissue sources are grouped by color, indicated by colored rectangle below the corresponding sample number. Individual tissue identifications along the horizontal axis correspond to the numbering listed in Table S2.

 **Table 1 Clinical features of subjects diagnosed with Curry-Jones syndrome**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Subject # | Gendera | Cranio-synostosis | Brain | Developmental attainment | Eyes | Skin | Hair | GI tract | Cutaneous syndactyly  | Polydactyly | Tumors | Other | Previously reported? |
| 1 SMO + | F | RC | Possible cyst in trigone of right ventricle | Normal | R amblyopia, skin overgrowth | Waxy, streaky lesions; Sebaceus nevus | Ectopic patch of hair above R eye | GI bleeding; chronic constipation | R 2/3, L 1/2/3 (H) | R trifid hallux |  | Asymmetric face | Temple et al. (1995)Case 4 |
| 2 SMO + | M | LC | ACC; R HMEG; R VMEG | Mild delay | Normal | Raised, linear white streaks; biopsy normal | Ectopic hair patch near eyes | Intestinal obstruction | R 2/3/4, L 2/3 (H); R 2/3, L 3/4 (F) | Broad thumbs; R,L preaxial polydactyly (F) | Myofibromas of large bowel | Lip pits; Seizures | Temple et al. (1995)Case 1 |
| 3 SMO + | M | LC | Partial ACC; asymmetric dilated ventricles | Mild ID; IQ <70 | R,L microphthalmia; R iris coloboma | Raised, scar-like pale lesions; biopsy non-specific | Ectopic patch of hair above R eye | Esophageal dysmotility | R,L 1/2/3/4 (H) | R,L bifid halluces |  | Asymmetric face; fused central incisor; oligodontia; freckled areas on soles of feet; Oesophageal problem | Temple et al. (1995)Case 3 |
| 4  | M | Bicoronal | Dilated ventricles; choroid plexus cyst | Mild delay | Congenital glaucoma; secondary cataract | Pigmentary anomalies in Blashko’s lines most notable on limbs | Chaotic hair patterning of lateral portions of eyebrows | Diarrhoeal episodes | Both feet, 2/3/4 | Broad halluces |  No | Cleft palate  |  No |
| 5SMO + | M | None | Mild ACC; L HMEG; R VMEG and PMG; Occipital meningocele; Chiari I malformation | Mild to moderate developmental delay | L mild colobomatous microphthalmia with unusually shaped pupil | Raised, linear streaks (L arm and leg, chin, few other areas) | Ectopic hair on cheek | Malrotation; intestinal pseudo-obstruction; chronic constipation; subtotal colectomy for volvulus and obstipation | R 2/3, L 1/2/3/4 (H); R,L 3/4 (F) | R thumb nubbin; R,L preaxial polydactyly (F) | Desmoplastic medulloblastoma of cerebellum; odontogenic keratocysts; benign colonic polyps and smooth muscle hamartoma | Large anterior fontanelle; lip pits | Grange et al. (2008) Patient 2 |
| 6 SMO + | M | None | Partial ACC; MEG; VMEG; Chiari I malformation | Mild delay (1-2 yrs behind academically) | R,L iris colobomas | Hypopigmented streaky lesions | Ectopic hair patch near eyes | Malrotation | R 2/3, L 1/2/3 (H); L1/2 (F) | R,L preaxial polydactyly (H); L preaxial polydactyly (F) | Trichoblastoma; smooth muscle hamartomas of GI tract | Mildly symmetric face, lip pit | Grange et al. (2008) Patient 1 |
| 7 SMO + | M | None | Normal | Normal | Normal | White, patchy skin behind knees | Abnormal hair growth on shoulders and limbs | Normal | R 1/2/3/4 (H) | Thumb broad; L,R preaxial polydactyly |  | Plagiocephaly | Thomas et al. (2006) |
| 8 SMO + | F | RC | Partial ACC; Moderate cerebral asymmetry consistent with L HMEG; PMG | Mild delay | Normal; dysmorphic R eyelid | Linear areas of hypopigmented skin atrophy (extremities and trunk) |  | Malrotation; intermittent pseudo-obstruction; serosal nodules in the appendix, mesentery, and duodenum | Variable R,L 1/2/3 (H,F) | R,L duplicated thumbs and halluces | Scalp lymphangiomas: benign hamartomatous lesion with features of lymphangioma and nevus sebaceous; serosal hamartomas in duodenum, appendix, mesentery | Accessory bone at anterior fontanelle; wormian bones of L posterior skull; L leg longer than R; lumbar scoliosis | No |
| 9  | F | None | ACC, dilated ventricles, macrocephaly | Psychomotor delay | Bilateral colobomas of iris, retina and choroid, strabismus, nystagmus | Hypopigmented patch on her trunk | High frontal hairline | Chronic constipation | R, L 3/4 (H) | R, L duplicated thumbs and halluces | Lipomyelomeningocele | Rudimentary sacral vertebrae | No |

 aAbbreviations: gender, F, female, M, male; craniosynostosis, LC, left coronal, RC, right coronal; brain, ACC, agenesis of the corpus callosum, MEG, megalencephaly, HMEG, hemimegalencephaly; VMEG, ventriculomegaly, PMG, polymicrogyria; developmental attainment, ID, intellectual development; limbs, H, hands, F, feet.

1. Cohen, M.M., Jr. (1988). Craniosynostosis update 1987. Am J Med Genet Suppl 4, 99-148.

2. Gorlin, R.J., Cohen, M.M., Jr., and Levin, L.S. (1990). Syndromes with craniosynostosis: general aspects and well-known syndromes. In Syndromes of the Head and Neck. (New York, Oxford University Press), pp 519-539.

3. Temple, I.K., Eccles, D.M., Winter, R.M., Baraitser, M., Carr, S.B., Shortland, D., Jones, M.C., and Curry, C. (1995). Craniofacial abnormalities, agenesis of the corpus callosum, polysyndactyly and abnormal skin and gut development--the Curry Jones syndrome. Clin Dysmorphol 4, 116-129.

4. Mingarelli, R., Mokini, V., Castriota Scanderbeg, A., and Dallapiccola, B. (1999). Brachycephalosyndactyly with ptosis, cataract, colobomas, and linear areas of skin depigmentation. Clin Dysmorphol 8, 73-75.

5. Thomas, E.R., Wakeling, E.L., Goodman, F.R., Dickinson, J.C., Hall, C.M., and Brady, A.F. (2006). Mild case of Curry-Jones syndrome. Clin Dysmorphol 15, 115-117.

6. Grange, D.K., Clericuzio, C.L., Bayliss, S.J., Berk, D.R., Heideman, R.L., Higginson, J.K., Julian, S., and Lind, A. (2008). Two new patients with Curry-Jones syndrome with trichoblastoma and medulloblastoma suggest an etiologic role of the sonic hedgehog-patched-GLI pathway. Am J Med Genet A 146A, 2589-2597.

7. Lunter, G., and Goodson, M. (2011). Stampy: a statistical algorithm for sensitive and fast mapping of Illumina sequence reads. Genome Res 21, 936-939.

8. Rimmer, A., Phan, H., Mathieson, I., Iqbal, Z., Twigg, S.R., Wilkie, A.O., McVean, G., and Lunter, G. (2014). Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. Nat Genet 46, 912-918.

9. Yang, H., and Wang, K. (2015). Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 10, 1556-1566.

10. Wang, C., Wu, H., Katritch, V., Han, G.W., Huang, X.P., Liu, W., Siu, F.Y., Roth, B.L., Cherezov, V., and Stevens, R.C. (2013). Structure of the human smoothened receptor bound to an antitumour agent. Nature 497, 338-343.

11. Rahbari, R., Wuster, A., Lindsay, S.J., Hardwick, R.J., Alexandrov, L.B., Al Turki, S., Dominiczak, A., Morris, A., Porteous, D., Smith, B., et al. (2016). Timing, rates and spectra of human germline mutation. Nat Genet 48, 126-133.

12. Sweeney, R.T., McClary, A.C., Myers, B.R., Biscocho, J., Neahring, L., Kwei, K.A., Qu, K., Gong, X., Ng, T., Jones, C.D., et al. (2014). Identification of recurrent SMO and BRAF mutations in ameloblastomas. Nat Genet 46, 722-725.

13. Brown, N.A., Rolland, D., McHugh, J.B., Weigelin, H.C., Zhao, L., Lim, M.S., Elenitoba-Johnson, K.S., and Betz, B.L. (2014). Activating FGFR2-RAS-BRAF mutations in ameloblastoma. Clin Cancer Res 20, 5517-5526.

14. Jones, D.T., Jager, N., Kool, M., Zichner, T., Hutter, B., Sultan, M., Cho, Y.J., Pugh, T.J., Hovestadt, V., Stutz, A.M., et al. (2012). Dissecting the genomic complexity underlying medulloblastoma. Nature 488, 100-105.

15. Pugh, T.J., Weeraratne, S.D., Archer, T.C., Pomeranz Krummel, D.A., Auclair, D., Bochicchio, J., Carneiro, M.O., Carter, S.L., Cibulskis, K., Erlich, R.L., et al. (2012). Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature 488, 106-110.

16. Brastianos, P.K., Horowitz, P.M., Santagata, S., Jones, R.T., McKenna, A., Getz, G., Ligon, K.L., Palescandolo, E., Van Hummelen, P., Ducar, M.D., et al. (2013). Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat Genet 45, 285-289.

17. Clark, V.E., Erson-Omay, E.Z., Serin, A., Yin, J., Cotney, J., Ozduman, K., Avsar, T., Li, J., Murray, P.B., Henegariu, O., et al. (2013). Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science 339, 1077-1080.

18. Atwood, S.X., Sarin, K.Y., Whitson, R.J., Li, J.R., Kim, G., Rezaee, M., Ally, M.S., Kim, J., Yao, C., Chang, A.L., et al. (2015). Smoothened variants explain the majority of drug resistance in basal cell carcinoma. Cancer Cell 27, 342-353.

19. Sharpe, H.J., Pau, G., Dijkgraaf, G.J., Basset-Seguin, N., Modrusan, Z., Januario, T., Tsui, V., Durham, A.B., Dlugosz, A.A., Haverty, P.M., et al. (2015). Genomic analysis of smoothened inhibitor resistance in basal cell carcinoma. Cancer Cell 27, 327-341.

20. Katritch, V., Cherezov, V., and Stevens, R.C. (2013). Structure-function of the G protein-coupled receptor superfamily. Annu Rev Pharmacol Toxicol 53, 531-556.

21. Briscoe, J., and Therond, P.P. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol 14, 416-429.

22. McCabe, J.M., and Leahy, D.J. (2015). Smoothened goes molecular: new pieces in the hedgehog signaling puzzle. J Biol Chem 290, 3500-3507.

23. Arensdorf, A.M., Marada, S., and Ogden, S.K. (2016). Smoothened Regulation: A Tale of Two Signals. Trends Pharmacol Sci 37, 62-72.

24. Wang, Y., Ding, Q., Yen, C.J., Xia, W., Izzo, J.G., Lang, J.Y., Li, C.W., Hsu, J.L., Miller, S.A., Wang, X., et al. (2012). The crosstalk of mTOR/S6K1 and Hedgehog pathways. Cancer Cell 21, 374-387.

25. Jenkins, D., Seelow, D., Jehee, F.S., Perlyn, C.A., Alonso, L.G., Bueno, D.F., Donnai, D., Josifova, D., Mathijssen, I.M., Morton, J.E., et al. (2007). RAB23 mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. Am J Hum Genet 80, 1162-1170.

26. Hahn, H., Wicking, C., Zaphiropoulous, P.G., Gailani, M.R., Shanley, S., Chidambaram, A., Vorechovsky, I., Holmberg, E., Unden, A.B., Gillies, S., et al. (1996). Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 85, 841-851.

27. Johnson, R.L., Rothman, A.L., Xie, J., Goodrich, L.V., Bare, J.W., Bonifas, J.M., Quinn, A.G., Myers, R.M., Cox, D.R., Epstein, E.H., Jr., et al. (1996). Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272, 1668-1671.

28. Ming, J.E., Kaupas, M.E., Roessler, E., Brunner, H.G., Golabi, M., Tekin, M., Stratton, R.F., Sujansky, E., Bale, S.J., and Muenke, M. (2002). Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. Hum Genet 110, 297-301.

29. Kool, M., Jones, D.T., Jager, N., Northcott, P.A., Pugh, T.J., Hovestadt, V., Piro, R.M., Esparza, L.A., Markant, S.L., Remke, M., et al. (2014). Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. Cancer Cell 25, 393-405.

30. Smith, M.J., Beetz, C., Williams, S.G., Bhaskar, S.S., O'Sullivan, J., Anderson, B., Daly, S.B., Urquhart, J.E., Bholah, Z., Oudit, D., et al. (2014). Germline mutations in SUFU cause Gorlin syndrome-associated childhood medulloblastoma and redefine the risk associated with PTCH1 mutations. J Clin Oncol 32, 4155-4161.

31. Putoux, A., Thomas, S., Coene, K.L., Davis, E.E., Alanay, Y., Ogur, G., Uz, E., Buzas, D., Gomes, C., Patrier, S., et al. (2011). KIF7 mutations cause fetal hydrolethalus and acrocallosal syndromes. Nat Genet 43, 601-606.

32. Vortkamp, A., Gessler, M., and Grzeschik, K.H. (1991). GLI3 zinc-finger gene interrupted by translocations in Greig syndrome families. Nature 352, 539-540.

33. Johnston, J.J., Sapp, J.C., Turner, J.T., Amor, D., Aftimos, S., Aleck, K.A., Bocian, M., Bodurtha, J.N., Cox, G.F., Curry, C.J., et al. (2010). Molecular analysis expands the spectrum of phenotypes associated with GLI3 mutations. Hum Mutat 31, 1142-1154.

34. Klopocki, E., Lohan, S., Brancati, F., Koll, R., Brehm, A., Seemann, P., Dathe, K., Stricker, S., Hecht, J., Bosse, K., et al. (2011). Copy-number variations involving the IHH locus are associated with syndactyly and craniosynostosis. Am J Hum Genet 88, 70-75.

35. Twigg, S.R., and Wilkie, A.O. (2015). A Genetic-Pathophysiological Framework for Craniosynostosis. Am J Hum Genet 97, 359-377.

36. Anderson, E., Peluso, S., Lettice, L.A., and Hill, R.E. (2012). Human limb abnormalities caused by disruption of hedgehog signaling. Trends Genet 28, 364-373.

37. Athar, M., Li, C., Kim, A.L., Spiegelman, V.S., and Bickers, D.R. (2014). Sonic hedgehog signaling in Basal cell nevus syndrome. Cancer Res 74, 4967-4975.

38. Keppler-Noreuil, K.M., Rios, J.J., Parker, V.E., Semple, R.K., Lindhurst, M.J., Sapp, J.C., Alomari, A., Ezaki, M., Dobyns, W., and Biesecker, L.G. (2015). PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am J Med Genet A 167A, 287-295.

39. Hevner, R.F. (2015). Brain overgrowth in disorders of RTK-PI3K-AKT signaling: a mosaic of malformations. Semin Perinatol 39, 36-43.

40. Jansen, L.A., Mirzaa, G.M., Ishak, G.E., O'Roak, B.J., Hiatt, J.B., Roden, W.H., Gunter, S.A., Christian, S.L., Collins, S., Adams, C., et al. (2015). PI3K/AKT pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. Brain 138, 1613-1628.

41. Ramalho-Santos, M., Melton, D.A., and McMahon, A.P. (2000). Hedgehog signals regulate multiple aspects of gastrointestinal development. Development 127, 2763-2772.

42. Mao, J., Kim, B.M., Rajurkar, M., Shivdasani, R.A., and McMahon, A.P. (2010). Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract. Development 137, 1721-1729.

43. Huang, H., Cotton, J.L., Wang, Y., Rajurkar, M., Zhu, L.J., Lewis, B.C., and Mao, J. (2013). Specific requirement of Gli transcription factors in Hedgehog-mediated intestinal development. J Biol Chem 288, 17589-17596.

44. Khamaysi, Z., Bochner, R., Indelman, M., Magal, L., Avitan-Hersh, E., Sarig, O., Sprecher, E., and Bergman, R. (2016). Segmental Basal cell nevus syndrome caused by an activating mutation in Smoothened. Br J Dermatol.

45. Stein, L.D., Mungall, C., Shu, S., Caudy, M., Mangone, M., Day, A., Nickerson, E., Stajich, J.E., Harris, T.W., Arva, A., et al. (2002). The generic genome browser: a building block for a model organism system database. Genome Res 12, 1599-1610.