

Fig S1

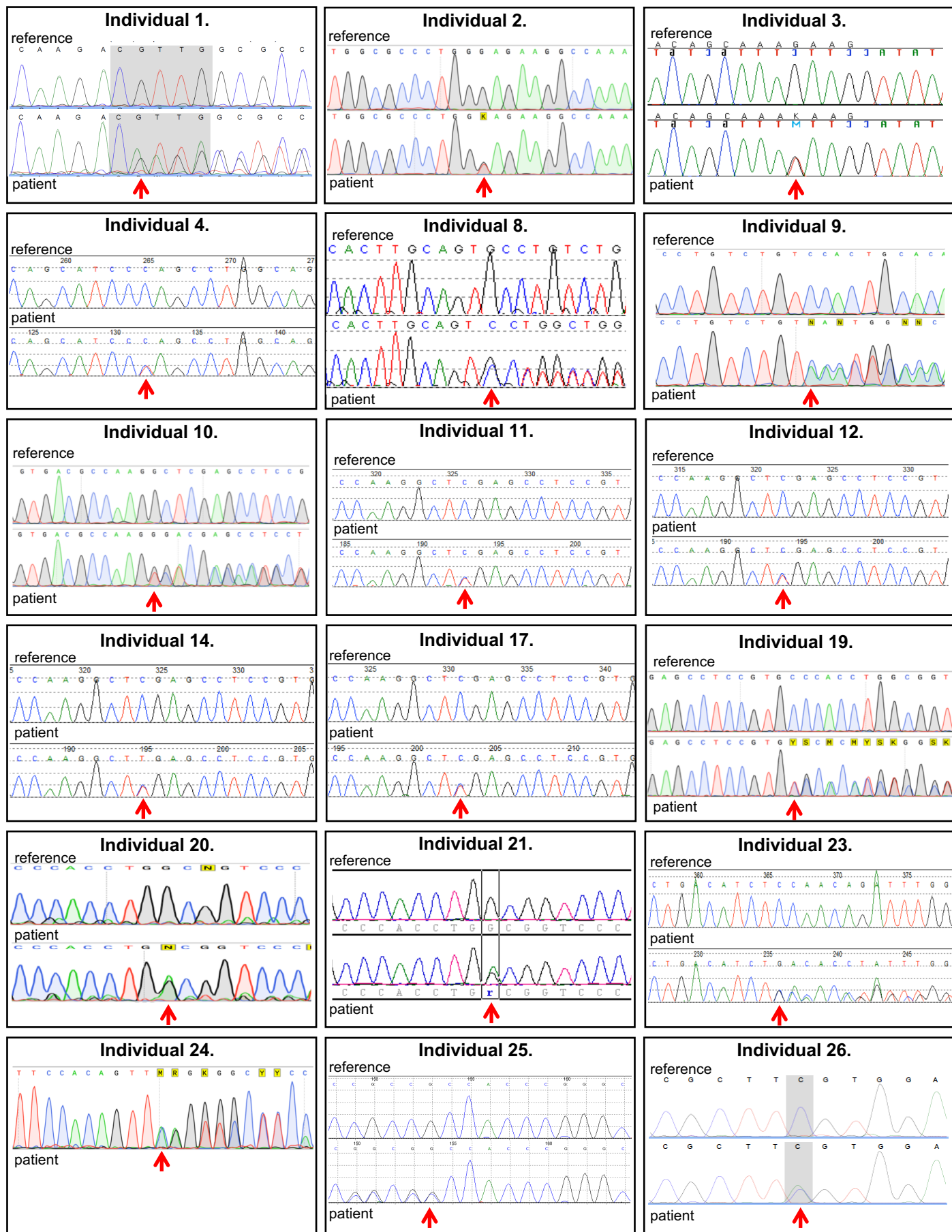


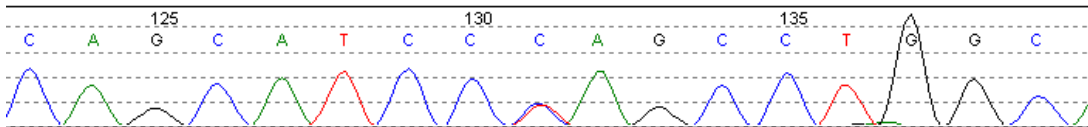
Figure S1. Chromatograms of *MNI* variants.

Fig S2

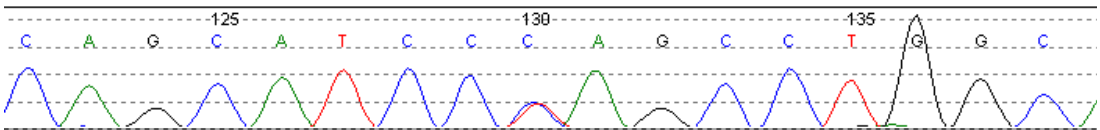
MN1, c.3817C>T, p.(Gln1273*)



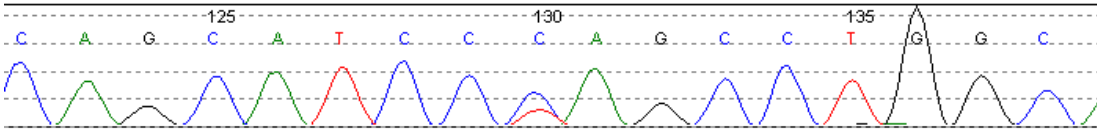
Proband
(individual 5)



Brother
(individual 6)



Father
(individual 7)



Mother

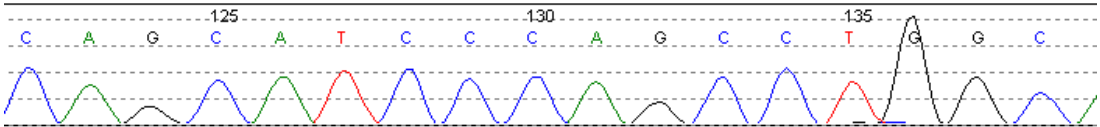


Figure S2. Evidence for somatic mosaicism of the *MNI* variant c.3817C>T, p.(Gln1273*) in individual 7. A red arrow indicates the variant in chromatograms of the proband and her brother (individuals 5 and 6) and their father (individual 7; note reduced peak size for the mutant allele).

Fig S3

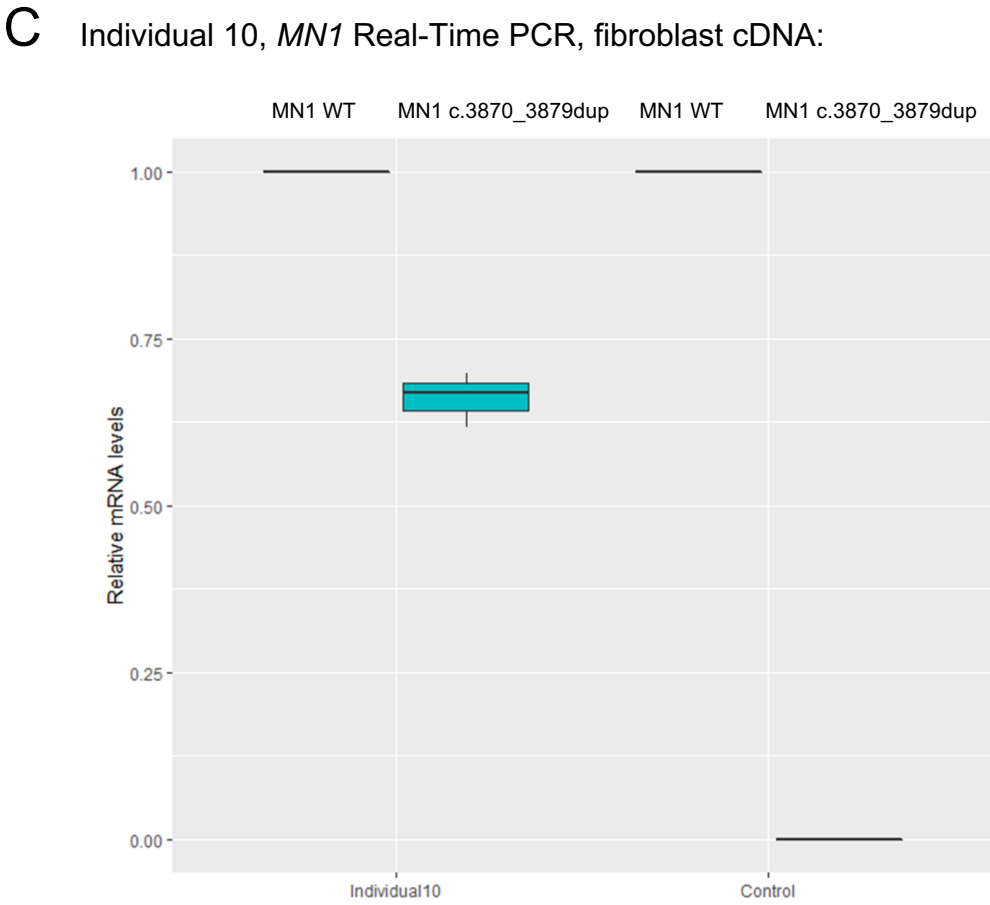
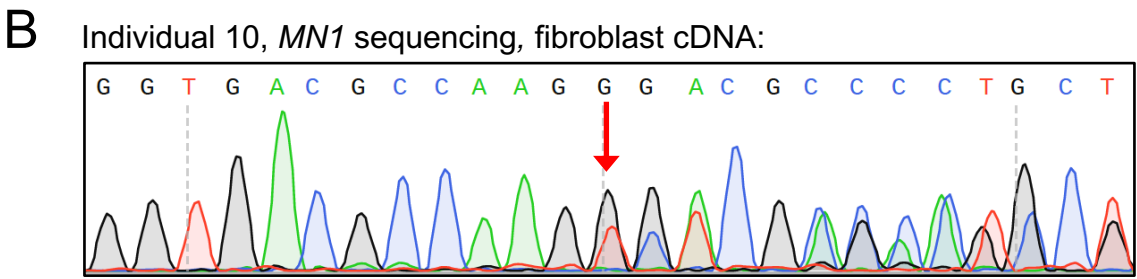
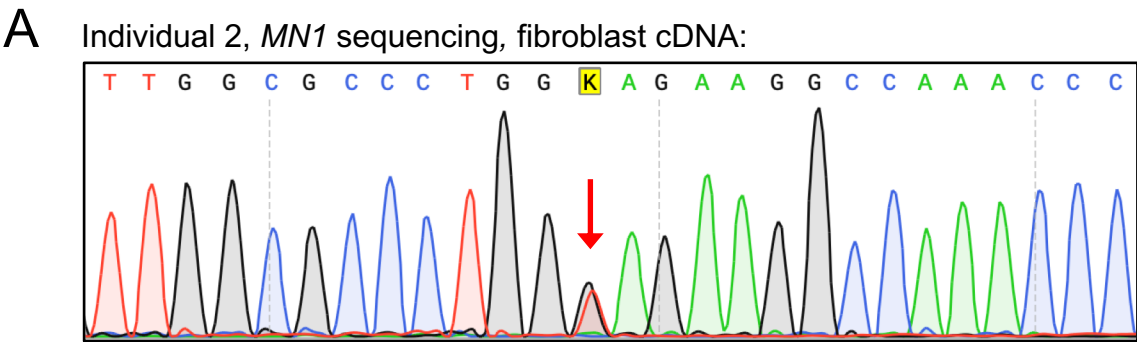
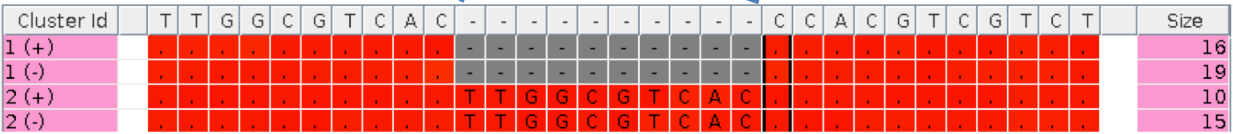


Figure S3. Stable expression of the mutant *MNI* transcript in skin fibroblasts of individuals 2 and 10, by cDNA sequencing and Real-Time PCR. In (A) and (B), *MNI* cDNA was amplified by PCR using a sense primer in exon 1 and an antisense primer in exon 2. A red arrow indicates the mutation in each case. In (C), Real-Time PCR was performed using wild-type (WT) and mutant c.3870_3879dup *MNI* probes specific to each sequence. For patient and control fibroblast cDNA samples, the Ct values for *MNI* WT and c.3870_3879dup probes were normalized to *ACTB*, then c.3870_3879dup values were normalized to WT (set as 1). Box-and-whisker plot indicates median, first and third quartiles with vertical line showing the maximum and minimum values from three repeat experiments.

A Individual 10, *MN1* fibroblast RNA-Seq:



B Individual 21, *MN1* fibroblast RNA-Seq:

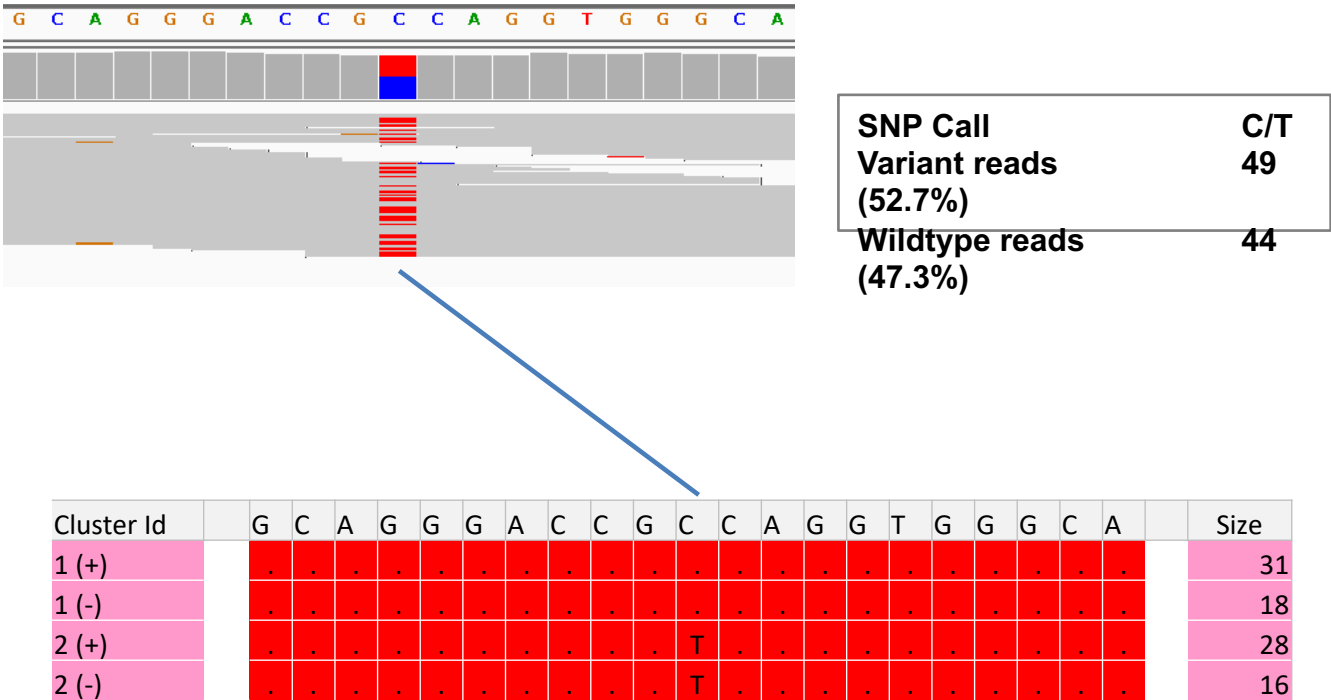


Figure S4. RNA-Seq demonstrating stable expression of the mutant *MNI* transcript in skin fibroblasts of individuals 10 (A) and 21 (B).

Fig S5



Figure S5. Spectrum of ear morphologies in MCTT syndrome patients. Note the upper portion of the pinna is frequently over-folded or dysplastic. Patient numbers are indicated.

Fig S6



Figure S6. A facial composite image was generated at Face2Gene

(<https://www.face2gene.com>) using one photo from each of 10 MCTT syndrome patients between the ages of 4 and 10 years.

Supplementary Table legends

Table S1. Medical details of 23 individuals harbouring C-terminal *MNI* truncating mutations and five individuals harbouring N-terminal *MNI* truncating mutations or whole *MNI* deletions. #; three individuals (13, 16 and 19) have been previously published in the context of large-scale sequencing studies of patients with developmental disorders. A brief clinical summary was published for individual 13 (Rossi *et al.*, 2017), while no clinical information was previously provided for individuals 16 and 19 (Deciphering Developmental Disorders Study, 2017); in each case the significance of the *MNI* variant was not established. Brief clinical information was published for individual 21 in (Ishak *et al.*, 2012; Tully *et al.*, 2012) without identification of the *MNI* variant. For individual 28 harboring a large deletion that includes *MNI*, brief clinical information was previously published (Friedman *et al.*, 2006).

Abbreviations: ABR, auditory brainstem response; ASD, atrial septal defect; ATNR, asymmetrical tonic neck reflex; CMA, chromosomal microarray; CNV, copy number variant; CPAP, continuous positive airway pressure; CSF, cerebrospinal fluid; CT, computed tomography; DHPLC, denaturing high-performance liquid chromatography; DQ, developmental quotient; EEG, electroencephalogram; GAG, glycosaminoglycan; IAM, internal auditory meatus; ICD, inter-canthal distance; IPD, inter-pupillary distance; IUGR, intrauterine growth restriction; LSCS, lower segment Caesarean section; MRI, magnetic resonance imaging; NA, not available; ND, not done; NICU, neonatal intensive care unit; NSVD, normal spontaneous vaginal delivery; OCD, outer-canthal distance; OM, otitis media; PDA, patent ductus arteriosus; PET, pressure equalization tube; PFO, patent foramen ovale; SAB, spontaneous abortion; SCBU, Special Care Baby Unit; SFU, Society for Fetal Urology; SNHL, sensorineural hearing loss; T and A, tonsillectomy and adenoidectomy; T-L, thoracolumbar; UGI, upper gastrointestinal series; US, ultrasound; VE, vacuum extraction; VSD, ventricular septal defect; VUS, variant of unknown significance; WES, whole exome

sequencing; WGS, whole genome sequencing; WISC, Wechsler Intelligence Scale for Children; WPPSI, Wechsler Preschool and Primary Scale of Intelligence; XR, X-ray.

Table S2. Sequences of primers and probes.

References

Rossi M, El-Khechen D, Black MH, Farwell Hagman KD, Tang S, Powis Z. Outcomes of Diagnostic Exome Sequencing in Patients With Diagnosed or Suspected Autism Spectrum Disorders. *Pediatr Neurol* 2017; 70: 34-43.e2.

Ishak GE, Dempsey JC, Shaw DWW, Tully H, Adam MP, Sanchez-Lara PA, et al. Rhombencephalosynapsis: a hindbrain malformation associated with incomplete separation of midbrain and forebrain, hydrocephalus and a broad spectrum of severity. *Brain* 2012; 135: 1370–1386.

Tully HM, Dempsey JC, Ishak GE, Adam MP, Curry CJR, Sanchez-Lara P, et al. Beyond Gómez-López-Hernández syndrome: recurring phenotypic themes in rhombencephalosynapsis. *Am J Med Genet A* 2012; 158A: 2393–2406.