ORIGINAL ARTICLE

Two novel CCDC88C mutations confirm the role of DAPLE in autosomal recessive congenital hydrocephalus

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Received 23 August 2012
Revised 23 August 2012
Accepted 7 September 2012
Published Online First
5 October 2012

ABSTRACT

Background Human congenital non-syndromic hydrocephalus is a vastly heterogeneous condition. A subgroup of cases are not secondary to a specific cause (eg, a neural tube defect), and within this subgroup, autosomal recessive inheritance has been described. One homozygous mutation in the DAPLE (Dvl-associating protein with a high frequency of leucine residues) protein-encoding gene CCDC88C (coiled-coil domain containing 88C) has recently been reported in a single family. The role of this gene has not been validated in another family, and no other autosomal recessive gene has been reported.

Methods We used homozygosity mapping and whole exome sequencing in two families with primary, non-syndromic congenital hydrocephalus from two different ethnic backgrounds.

Results In each family, we identified a novel homozygous mutation of CCDC88C. One mutation produced a premature stop codon at position 312 of the protein, while the second mutation induced a frameshift in the last exon, producing a stop codon that truncated the extreme C-terminus of DAPLE, including the 2026-2028 Gly-Cys-Val motif known to bind the post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1) (PDZ) domain of Dishevelled.

Conclusions Our data validate CCDC88C as causing autosomal recessive, primary non-syndromic congenital hydrocephalus, suggesting this gene may be an important cause of congenital hydrocephalus, and underscore the important role of the C-terminal PDZ domain-binding motif in the DAPLE protein.

INTRODUCTION

Congenital hydrocephalus is a common and potentially devastating condition whose molecular mechanisms are still poorly understood, affecting 0.6 per 1000 live births in Western countries.[1] Hydrocephalus is defined as a disturbance of cerebrospinal fluid circulation causing accumulation of ventricular cerebrospinal fluid, which results in progressive ventricular dilatation. A dilated third ventricle associated with a normal fourth ventricle is usually associated with an obstruction of the aqueduct of Sylvius, and hence referred to as non-communicating hydrocephalus.[2] The causes of congenital hydrocephalus are vastly heterogeneous. The majority of cases are secondary to neural tube defects, intracranial haemorrhages, trauma, tumours, teratogens or primitive brain malformations. The remaining cases of congenital hydrocephalus can be divided into syndromic (two-thirds of cases) and non-syndromic (a third). Syndromic causes of congenital hydrocephalus include cytogenetic anomalies and many rare syndromes such as the L1-syndrome, skeletal dysplasias, metabolic disorders, dystroglycanopathies, Meckel syndrome, as well as a large subset of unknown causes. Non-syndromic congenital hydrocephalus is familial in only a minority of cases, 11% in one clinical series, and the recurrence risk for sporadic cases is small, 2%–4%.[3] An X-linked form is associated with mutations in L1CAM.[4] After excluding those cases, the male to female ratio remains >2,[3] suggesting a higher sensibility in the male or additional X-linked loci. It has been estimated that approximately 40% of hydrocephali with aqueductal stenosis result from a Mendelian cause.[5] There are furthermore many reports of idiopathic hydrocephalus in siblings of both sexes and/or in consanguineous children from unaffected parents, strongly suggesting autosomal recessive cause(s).[3]

Recently, a first autosomal recessive cause of non-syndromic congenital hydrocephalus was described by Ekici *et al*[6] in a family where two female patients had non-syndromic hydrocephali, associated with a splice site mutation of the *CCDC88C* (coiled-coil domain containing 88C) gene resulting in a premature stop codon (p.S1591HfsX7). The patients had ventricular dilatation with an interhemispheric cyst, a small vermis and an enlarged posterior fossa. The only living patient had no psychomotor delay at the age of 3 years and 3 months. *CCDC88C* encodes DAPLE (Dishevelled-associating protein with a high frequency of leucine residues), a dishevelled-associated protein that is a negative regulator of Wnt signalling.[7]

Here, we describe two previously unreported multiplex families with novel mutations of the CCDC88C gene, confirming its role in idiopathic autosomal recessive congenital hydrocephalus.

PATIENTS AND METHODS

The study and all experiments were approved by the Université Libre de Bruxelles Erasme Hospital and the Hadassah medical centre ethical review boards. Written informed consent was obtained from participants or guardians.

Patients and families

Family I

A non-consanguineous Jewish Ashkenazi family was referred for evaluation of familial congenital

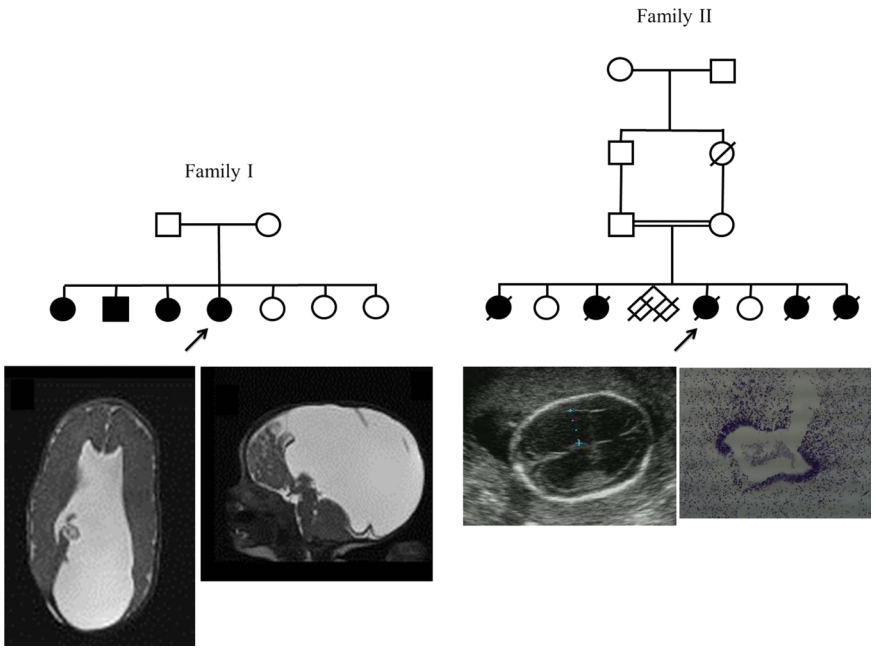

Figure 1 Phenotypic data. Brain imaging of a proband (arrow) is shown beneath each respective family tree. In family I, axial (left panel) and midline sagittal (right panel) T2-weighted MRI images of the proband's brain demonstrate a large midline cystic structure and dilated lateral ventricles. In family II, prenatal ultrasound at 20 weeks of gestational age revealed severe dilatation of lateral ventricles. Neuropathological microscopy showed an open aqueduct of Sylvius in fetus III.6. This figure is only reproduced in colour in the online version.

hydrocephalus. Four children, aged from 1 to 19 years at the time of evaluation, were affected whereas the parents and three other siblings were healthy (figure 1) and no further cases were known in the extended family except a male maternal second cousin who suffered from hydrocephalus and was not evaluated during the present investigations. The affected children shared a phenotype of congenital hydrocephalus and seizures (table 1). The head circumference at birth ranged from 39.5 to 49 cm and thus necessitated delivery by caesarean section. Perinatal course was otherwise uneventful. Initial MRIs were available for three of the patients, demonstrating changes consistent with hydrocephalus or hydrocephalus drained by shunt. In two of them midline cystic structure was present, and in one a big extra-axial parietal cyst was seen (figure 1). No cross-sectional study was available for the remaining patient. The fourth ventricle was not enlarged in any of the patients. Child I.3 presented the additional feature of biparietal polymicrogyria. Shunting procedures were performed within the first week of life. Motor development has been retarded. Focal seizures have been noted in all affected patients from a few weeks of age up to 3 years. The seizures are presently easily controlled with medications. The motor and cognitive difficulties are variable but within the range of moderate to severe mental retardation and may relate to complications of shunt placement or to an associated malformation of the brain.

Family II

A first cousin couple of Palestinian origin underwent five terminations of pregnancies following the diagnosis of marked ventricular dilatation at mid-gestation. This couple also had a twin miscarriage at 10 weeks gestation. The parents and two daughters aged 13 and 11 years were healthy (figure 1). The standard karyotypes were normal in both parents. All fetuses from terminated pregnancies were females (table 2). The first was terminated at 21 weeks of gestational age (WGA) because of right ventricle dilation. The fetus was female, eumorphic and eutrophic. Brain and cerebellum were small relative to the skull. Pathology studies did not show visceral anomalies but unfortunately the brain could not be analysed. A second fetus was interrupted at 24 WGA and had severe dilatation of the lateral ventricles. No additional study was performed. A third fetus was interrupted at 20 WGA. The lateral ventricles were measured at 18 mm (marked ventriculomegaly, normal: <10 mm[8]). The third and fourth ventricles were normal. A standard karyotype was normal (46, XX). A fetogram revealed craniofacial disproportion with large cranial sutures and 'butterfly' vertebral anlage. No autopsy was performed. A fourth pregnancy was terminated at 21 WGA. The lateral ventricles were measured at 15 and 14 mm. The axial transcerebellar diameter was 16 mm (normal: 21.4 ± 1.3 mm[9]). The fetus was eutrophic and hyperteloric. The lungs showed lymphangiectasias and were thickened. Brain pathology showed lateral ventricular dilatation with a thin 6-layered cortex. The aqueduct of Sylvius was permeable. A standard karyotype was normal (46, XX). The last pregnancy was interrupted at 20 WGA. Lateral ventricles were measured at 15 and 17 mm. The third ventricle was also dilated. The axial transcerebellar diameter was 17 mm (normal: 20.3 ± 1.2 mm[9]). A standard karyotype was normal

Table 1 Clinical data from family patient number/sex/head circumference at birth/current age

	Age at first VP-shunt	Number of revisions of VP-shunt	Age at first seizure	Current cognitive function	Current motor function
I-1/Female/46 cm/19 years	5 days	2	3 years	Mild–moderate mental retardation	Unsteady gait
I-2/Male/46 cm/18 years	Within first week of life	7	6–8 weeks	No verbal communication. Severe mental retardation	Able to sit
I-3/Female/39.5 cm/16 years	1 day	1	3 years	Age-appropriate. Learning disability	Age-appropriate
I-4/Female/ 49 cm/2 years	1 day	5	7 months	Developmental quotient approximately 75	Crawls. No independent walking yet

VP; ventriculo-peritoneal.

Table 2 Anatomical data from family II

Foetus	Age at termination of pregnancy (gestational weeks)	Ventriculomegaly	Other brain malformations
II.1	21	Right lateral ventricle	Small cerebellum
II.3	24	Bilateral	
II.6	20	Bilateral: 18 mm	
II.8	21	Bilateral: 14 and 15 mm	Small cerebellum
II.9	20	Lateral: 15 and 17 mm and 3th ventricle	Small cerebellum, choroid plexus anomalies

(46, XX), and multiplex ligation-dependent probe amplification (MLPA) analysis of chromosomal subtelomeric regions detected no rearrangement.

Homozygosity mapping and Sanger sequencing

DNA of all members of family I and of all affected fetuses in family II except the first one (no DNA available) and DNA of the mother were genotyped using GeneChip Human 250K Nsp Arrays (Affymetrix, Inc.) following the manufacturer's instructions. Homozygous stretches were delineated using the Homozygosity Mapper software[10] (Berlin, Germany) and the Affymetrix Genotyping Console Software V.4.0 (Santa Clara, CA, USA).

Sanger sequencing

PCR primers for all candidate genes were designed using the Exonprimer software (http://ihg.helmholtz-muenchen.de/ihg/ExonPrimer.html). The coding regions and exon–intron junctions of the candidate genes were sequenced by the Sanger method using the Big Dye Terminator cycle sequencing kit v2 (Applied Biosystems, Foster City, California, USA), and analysed on a 3130 Genetic Analyser sequencing machine (Applied Biosystems). Sequences were analysed in silico for mutations using the SeqScape software V.2.0. (Applied Biosystems).

Exome sequencing

The exome was captured using the TruSeq capture kit (Illumina (San Diego, CA, USA)) and paired-end sequenced over 100 bp on a Illumina HiSeq2000 sequencer (AROS (Aarhus, Denmark) applied biotechnology (Carlsbad, CA, USA)). A total of 68 million reads were processed using the Genome Analysis ToolKit best practice recommendations.[11] Precisely, reads were aligned to the human genome GRCh37 with Burrows–Wheeler Alignment tool,[12] duplicate reads were removed using Picard MarkDuplicates (http://picard.sourceforge.net), local realignment around indels, base quality score recalibration, SNPS/indels calling and quality score recalibration were performed using Genome Analysis ToolKit, and variant annotation was done with SNPeff (Cingolani, P, 'snpEff: Variant effect prediction', http://snpeff.sourceforge.net, 2012). Depth of coverage was computed using BEDtools coveragebed[13] over all coding exons from Ensembl release 66, giving a mean depth of 88X. Overall, 91.6% of Ensembl exonic sequences were covered ≥10X depth.

RESULTS

In view of the common ancestry of the parents in family I and consanguinity in family II, we presumed that homozygous, identical-by-descent mutations caused autosomal recessive hydrocephalus in both families, respectively. We thus searched, in each family separately, for homozygous genomic region(s) in the DNA samples of the affected individuals, not shared by the unaffected siblings. To this end, we used GeneChip Human 250K Nsp Arrays (Affymetrix, Inc.).

In family I, we identified a single homozygous region larger than 2 Mb, which localised to chromosome 14 at 89.6–94.9 Mb (numbering according to hg18) (rs2401868-rs1211780) and contained 430 SNP markers (figure 2B). Within this region, there were 60 protein-encoding genes, and CCDC88C appeared as an obvious candidate.[6] Sequencing of the 30 coding exons of CCDC88C and their flanking intronic sequences revealed a single homozygous mutation, c.934C>T (p.R312X). The mutation was present in a homozygous state in all four patients, whereas the parents and two of the unaffected siblings were heterozygous and one sibling was homozygous for the wild-type allele. Only one of 721 unrelated Ashkenazi controls carried the mutation.

In family II, the 250K Nsp array showed a 27.6 Mb concordant homozygous region on chromosome 10q21.3-q23.3 between 65.8 and 93.4 Mb (rs2660121-rs1341992, hg18) (figure 2A). This region contained a heterozygous deletion in the *CTNNA3* gene. This homozygous stretch was also present in the unaffected mother, who was heterozygous for the same *CTNNA3* deletion. We ruled out a mutation of the other allele of this gene and excluded this region. A second concordant homozygous region was present on chromosome 14q while not homozygous in the mother. This region spanned 6.5 Mb between 90.9 and 96.6 MB (rs8003716-rs4905446, hg18) (figure 2B). The whole exome was sequenced and data were filtered for homozygous null alleles in this 14q region. We found a homozygous two base-pair deletion in the last exon of the *CCDC88C* gene. This frameshift mutation p.E1949GfsX26 results in a premature stop codon. The mutation was homozygous in the affected fetuses and heterozygous in the parents and in both healthy sisters.

DISCUSSION

Human congenital non-syndromic hydrocephalus is a vastly heterogeneous condition, where several bona fide autosomal recessive cases have been reported.[14–25] Recently, one homozygous mutation of *CCDC88C* was associated with this disorder in one consanguineous family.[6] Here we report two novel homozygous mutations of *CCDC88C* in two independently ascertained families with multiplex cases, providing genetic validation of *CCDC88C* as a cause of non-syndromic hydrocephalus in humans.

In family II we used a combination of whole exome sequencing and homozygosity mapping to identify a homozygous-by-descent truncating mutation of *CCDC88C*. In consanguineous families, this strategy is currently rapid and efficient for identifying mutations of novel genes or mutations of known genes in very heterogeneous disorders. Rapid progress in exome data curation is likely to make the homozygosity mapping part of this strategy unnecessary in a near future.

Both mutations were truncating and consistent with null alleles. *CCDC88C* encodes a 2028 amino acid protein, named DAPLE or HkRP2 (Hook-related protein 2). DAPLE belongs to the same protein family as GIV/Girdin/HkRP1 recently characterised as an actin-binding protein that promotes cell migration.[26] Both proteins share a conserved N-terminal domain and a central coiled-coil domain with 48% identity, but diverge at the C-terminus.[27] In its N-terminus, DAPLE contains a Hook domain which has been proposed to link various organelles to microtubules in the HOOK protein family.[28 29] The central

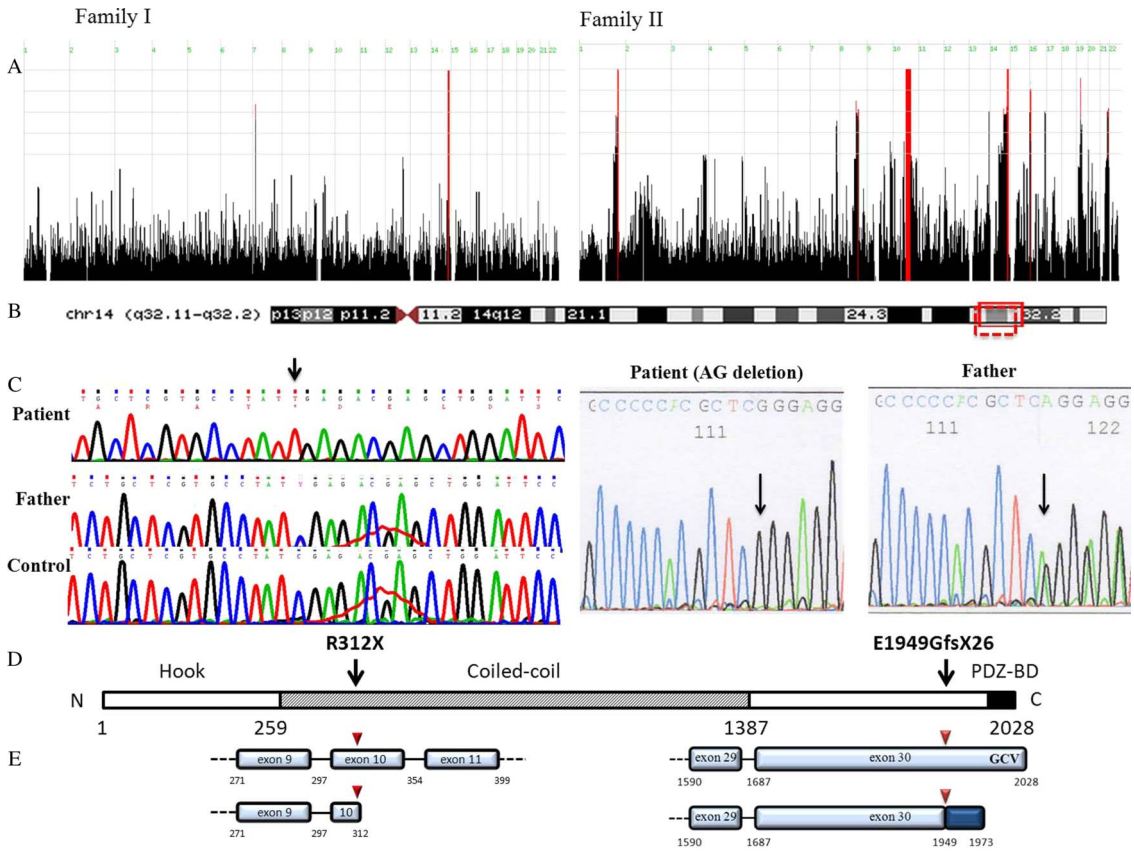

Figure 2 (A) Homozygosity mapping results using the homozygosity Mapper software. (B) Homozygous region at chromosome 14q32 in family I (dotted box) and II (full box). (C) Sanger sequencing electrophoregram of both mutations (vertical arrows). In family II, the patients were homozygous for a two base pair Adenine-Guanine (AG) deletion. The parents and healthy sisters were heterozygous. (D) A representation of the DAPLE protein, showing the N-terminal Hook motif, the central coiled-coil region and the C-terminus consisting of motif Gly-Cys-Val (GCV) that binds the PDZ domain of Dishevelled. The E1949GfsX26 mutation is located in the last exon. (E) Nonsense mutation R312X at codon 312 truncates more that 80% of the protein in family I. The frameshift mutation E1949GfsX26 at codon 1949 (AG deletion) in family II produces a stop at codon 1973. This mutation truncates a C-terminal segment of the protein that contains the distal GCV tripeptide motif that binds the PDZ domains of Dvl. This figure is only reproduced in colour in the online version.

region is rich in leucine residues (16%). It contains three leucine zipper domains and shows homology to myosin heavy chain. Through this structured domain, Daple is able to homo-oligomerise.[7] In GIV, this central coiled-coil region, which is 66% identical to DAPLE, mediates the interaction with protein Gα. The three C-terminal amino acid residues of the DAPLE protein constitute a PDZ domain-binding motif, Gly-Cys-Val (amino acids 2007–2009 in mouse mDaple, 2026–2028 in human DAPLE) (figure 2D). The consensus sequences of the PDZ domain-binding motifs are generally classified as Class I: Ser/Thr-X-Φ, where Φ is any hydrophobic amino acid and X is any amino acid, class II: Φ-X-Φ and class III: Glu/Asp-X-Φ.[30 31] Although the three C-terminal amino acids of DAPLE are not typical, it has been shown that mutation of either Gly2007 or Val2009 leads to the inability of mDaple to bind the PDZ domain of Dishevelled, an important scaffold protein of the Wnt signalling pathway. Expression of mDaple in mouse fibroblast L cells inhibited Wnt-3a-induced accumulation of β-catenin and T cell factor transcriptional activity. Expression of mDaple in the dorsal region of Xenopus embryos inhibited axis formation, which is known to be regulated by the canonical Wnt signalling pathway. The injection of mDaple or xDaple mRNA in *Xenopus* inhibits Dvl-dependent axis duplication.[7 32] Recently, the importance of Daple–Dvl interaction was demonstrated in non-canonical Wnt signalling-mediated Rac activation pathway and cell motility.[33]

Using targeted quantitative RT-PCR and expression profiling of Wnt signalling genes, Ekici *et al*[6] described specific alterations of mRNA levels in the patient but not in his healthy mother: upregulation of β-catenin (even though β-catenin is also moderately upregulated in the mother) and of its downstream target Lef1 and reduced expression of GSK3 and Frat mRNAs.

The mutated residue in family I, p.R312X, is located in the first coiled-coil domain of DAPLE and generates a premature stop codon, which would result in a severely truncated or absent protein if subject to nonsense-mediated mRNA decay. Conversely, in family II, the frameshift mutation p.E1949GfsX26 is responsible for a premature stop codon at the very end of the polypeptide, likely to generate a stable protein essentially missing the PDZ domain-binding motif but retaining intact coiled-coil domains (figure 2D,E). In the single family with autosomal recessive non-syndromic hydrocephalus previously reported by Ekici *et al*,[6] the *CCDC88C* mutation, p.S1591HfsX7, consisted of a substitution in the donor splice-site of DAPLE intron 29 resulting in the loss of the two last exons, 29 and 30. In this family, as well as in our present family II, the premature stop codon in the penultimate or last exon is not expected to cause nonsense mediated mRNA decay. The persistence of a transcript with a hook and coiled-coil domains in these families, as opposed to the absence or severe truncation of the protein in family I, could perhaps explain why additional phenotypic features were observed in family I. It is

however possible that the severe cognitive and motor deficiencies seen in patients from family I result from shunt-related complications. The interhemispheric cyst observed in some patients could result from the increased intraventricular pressure. Indeed, this kind of cyst is observed in 25% of congenital hydrocephalus cases and is consistent with the occurrence of a ventricular diverticulum during ventricular dilatation.[34] Moreover, intracranial cysts generally appear late in pregnancy (55% between 20 and 30 WGA and 45% after 30 WGA).[35]

In conclusion, we identified two novel mutations of *CCDC88C* in two independent families with autosomal recessive congenital non-syndromic hydrocephalus. Our results confirm the role of *CCDC88C* in this genetically heterogeneous condition, and underscore the role of the C-terminal tripeptide of DAPLE which binds the PDZ domain of Dishevelled in the Wnt pathway.

Acknowledgements We thank the patients' families, N Van Regemorter, C Hans, B Pichon, and the cytogenetics lab technicians for cell cultures, V Segers and H Khadim for pathological studies and obstetricians. We wish to thank Rabbi Bochner and Bonei Olam for generous support. We thank Colin Molter and in silico db (https://insilico.ulb.ac.be/) for exome data management and useful discussions.

Contributors AD performed experimental work, CJ and JD recruited patients, NiS devised and performed bioinformatics analysis of exome sequencing data, NaS did radiological evaluation, IP and OE supervised experimental work, MA recruited patients and devised the experiments, SE did clinical phenotyping.

Competing interests None.

Patient consent Obtained.

Ethics approval Approved by the Université Libre de Bruxelles Erasme Hospital and the Hadassah Medical Center ethical review boards.

Provenance and peer review Not commissioned; externally peer reviewed.

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