Focal segmental glomerulosclerosis and mild intellectual disability in a patient with a novel *de novo* truncating *TRIM8* mutation.

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Abstract

Mutations in the *TRIM8* gene have been described in patients with severe developmental delay, intellectual disability and epilepsy. Only six patients have been described to date. All the previous mutations were truncating variants clustered in the C-terminus of the protein. A previous patient with *TRIM8*-related epileptic encephalopathy was reported to have nephrotic syndrome. Here we describe the clinical, radiological and histological features of an 8-year-old male patient with a *TRIM8* mutation who, in contrast to previous patients, had only mild intellectual disability and well-controlled epilepsy. The patient was found to have proteinuria at 2 years of age. Renal biopsy findings were suggestive of focal segmental glomerulosclerosis. His kidney function declined and peritoneal dialysis was started at 5 years of age. He underwent renal transplant at 7 years of age. Trio-based whole genome sequencing identified a novel *de novo* heterozygous frameshift mutation in *TRIM8* (NM_030912.2) c.1198_1220del, p.(Tyr400ArgfsTer2). This patient is further evidence that *TRIM8* mutations cause a syndrome with both neurological and renal features. Our findings suggest the spectrum of *TRIM8*-related disease may be wider than previously thought with the possibility of milder neurodevelopmental problems and/or a more severe, progressive renal phenotype. We highlight the need for proteinuria screening in patients with *TRIM8* mutations.

Keywords: TRIM8; intellectual disability; epilepsy; nephrotic syndrome; focal segmental glomerulosclerosis

Introduction

Tripartite Motif (TRIM)-containing proteins are a family of proteins which regulate a broad range of biological processes including cell signalling, proliferation, differentiation, protein quality control, autophagy, immune responses, and carcinogenesis (Caratozzolo et al., 2017; Hatakeyama, 2017). Mutations in TRIM proteins are associated with several rare monogenic disorders. Examples include Charcot-Marie-Tooth disease type 2R (*TRIM2*) (Ylikallio et al., 2013), Opitz G/BBB syndrome (*MID1/TRIM18*) (Quaderi et al., 1997), autosomal recessive limb-girdle muscular dystrophy type 8 (*TRIM32*) (Frosk et al., 2002) and Mulibrey nanism (*TRIM37*) (Avela et al., 2000).

TRIM8 mutations have recently been described in patients with epilepsy, developmental delay and learning disability (Assoum et al., 2018; Sakai et al., 2016). To date, only six previous patients have been reported. All of them had heterozygous truncating mutations clustering around the C-terminal region of the TRIM8 protein. These patients had severe developmental delay, normal or mildly distinctive facial features, and mild white matter abnormalities on brain MRI. Three patients were noted to have proteinuria with one being diagnosed with nephrotic syndrome (Assoum et al., 2018). Here, we describe a patient with a C-terminal truncating *TRIM8* mutation who presented with severe renal disease but only mild neurodevelopmental problems.

Clinical report

We report an 8-year-old boy, born to unrelated white British parents. He has one older brother who is well. There was no family history of kidney disease or epilepsy. His mother had diet-controlled gestational diabetes in pregnancy. She had multiple scans during the pregnancy. There were no concerns about intra-uterine growth until late pregnancy. Some

growth restriction was suspected on the last ultrasound at 38 weeks gestation. Labour was induced a week later. The patient was delivered by vaginal delivery at 39+2 weeks gestation. His biometry at birth was weight 2942 gm (-0.9 SD), length 49 cm (-0.6 SD) and head circumference 32 cm (-2.0 SD). There were no developmental or health concerns in the neonatal period.

He was noted to have a right-sided squint at 8 weeks of age and had chronic constipation from 5 months of age. The patient was noted to have mild developmental delay. He crawled at 7 months, sat at 8 months and walked at 18 months. At the age of 2 years he used several simple words but was not forming sentences. His speech delay improved with speech and language therapy. Between 1 and 2 years of age he had recurrent episodes of fever. These were thought to be due to viral infections.

At 2 years and 2 months of age, he experienced an episode of diarrhoea, fever and abdominal pain. There was no history of rash, joint pain, hearing impairment or frank haematuria. Physical examination was normal apart from mild eczema. He was normotensive with no oedema or facial puffiness. He was noted to have significant proteinuria (2 + protein and 1+ glucose on dipstick) with a urinary protein/creatinine ratio of 207 mg/mmol (normal range < 50 mg/mmol). Serum creatinine, albumin and glucose values were normal. At 2 years and 4 months of age he was treated for an episode of coliform urinary tract infection. Urinalysis (2-3 + protein, 1-2+ blood and 1+ glucose on dipstick) confirmed persistent nephrotic range proteinuria with a protein/creatinine ratio of 519 mg/mmol. He was not oedematous and had normal serum creatinine. Several urine protein/creatinine measurements over the next 6 months ranged from 102 to 306 mg/mmol. There was no evidence of tubular proteinuria, hypercalciuria or nephrocalcinosis.

Renal ultrasound, full blood count, bone profile, liver function, plasma glucose, vitamin D, immunoglobulins, complement, anti-nuclear antibodies and anti-double stranded DNA antibodies were all within normal limits. His TSH was slightly raised (8.21 mU/L, reference range 0.30-4.40 mU/L) probably related to proteinuria, and serum ferritin was mildly low (23 µg/L, reference range 15-300 µg/L) for which he was on iron supplementation.

Renal biopsy at 3 years of age revealed global sclerosis of 11.5% (13/113) of the biopsied glomeruli (Fig. 1a). Three further glomeruli showed marked ischaemic shrinkage, one with apparent segmental sclerosis. The remainder were of normal size for age (up to 230 microns diameter), and showed no mesangial thickening or hypercellularity. Glomerular basement membrane (GBM) appeared normal on silver stain. There was a mild focal atrophy of the tubules. No glomerular deposits were seen on immunocytochemistry. On electron microscopy (Fig. 1b), glomeruli had a normal architecture with thin GBM consistent with age. Foot processes appeared mostly intact although there were a few lengths of fusion. One single focus of subendothelial deposit was seen without significant neomembrane or endothelial reaction. These changes suggested focal segmental glomerulosclerosis (FSGS), however the overall appearance was not typical of classical FSGS. A secondary cause for the nephrotic syndrome was considered unlikely given the relatively early age of onset and the normal clinical, renal and serological parameters.

Over the next 2-3 years the patient's proteinuria gradually worsened (protein/creatinine ratio 700 – 1000 mg/mmol) and he developed hypoalbuminaemia. He was never clinically nephrotic except on a couple of occasions when he had intravenous fluid therapy to treat dehydration. The proteinuria did not respond to steroids, calcineurin inhibitors and rituximab

infusion; he was maintained on regular therapy with enalapril. His renal function declined and peritoneal dialysis was commenced at 5 years of age.

In the months before peritoneal dialysis was initiated the patient experienced recurrent paroxysmal episodes of headache and vomiting. Upper gastrointestinal endoscopy and contrast studies were normal. Biopsies were taken from the oesophagus (mild chronic inflammation), stomach and duodenum (both had normal morphology). Urease test was negative. The episodes improved after optimisation of his dialysis and diet. In the months after peritoneal dialysis began the patient experienced three generalised seizures (being unresponsive and vacant, with bilateral eye twitching and profuse salivation). An interictal electroencephalogram was normal. MRI brain (Fig. 2) identified a well-defined, 7 mm T2-hyperintense/T1 hypointense focus in the superior aspect of the left cerebellar hemisphere, which was thought to be an old infarct. The cerebral white matter and other brain structures appeared normal. Sodium valproate was initiated and the peritoneal dialysis was optimised. The patient has subsequently been seizure free.

Psychometric testing at 7 years of age revealed the patient's abilities were around the 1st centile in most areas (consistent with mild intellectual disability). The patient underwent living unrelated renal transplant at the age of 7 years and 5 months. The patient was last assessed at 8 years and 3 month of age. He was generally healthy. The sodium valproate was stopped before the transplant. No seizures or paroxysmal episodes have happened since then. The transplant was functioning well. Absence of proteinuria indicated no recurrence of disease in the donor kidney. His family reported some challenging behaviour. He attended a mainstream school where he received 1:1 support 25 hours per week. On examination he had a small right-sided divergent squint, mild hypermetropia and a small umbilical hernia. He

was not dysmorphic. His height was 124 cm (-0.95 SD), weight 25 kg (-0.35 SD) and head circumference was 51 cm (-1.9 SD).

Genetic testing included array comparative genomic hybridization and sequencing of a gene panel for steroid resistant nephrotic syndrome (70 genes). The results of both tests were normal. Trio-based whole genome sequencing as part of the 100,000 Genomes Project (Genomics England, 2019) identified a *de novo* heterozygous frameshift mutation in *TRIM8* (NM_030912.2) c.1198_1220del, p.(Tyr400ArgfsTer2), chr10(GRCh38): g.102656896_102656918del. The variant was validated and confirmed to be *de novo* by Sanger sequencing in the All Wales Medical Genomics Service laboratory (Fig. 3a). The variant was not present in gnomAD. No other potentially pathogenic variants in other genes associated with kidney or neurological disease were identified. The variant was interpreted to be likely pathogenic based on the Association for Clinical Genomic Science Best Practice Guidelines for Variant Classification 2019 (PVS1_strong, PM2, PS2_supporting) (Ellard et al., 2019). The variant has been submitted to the DECIPHER database (Patient 411907).

The 100,000 Genomes Project was approved by the Health Research Authority Research Ethics Committee East of England – Cambridge South (REC Ref 14/EE/1112). The parents of the patient provided written informed consent for publication of this report.

Discussion

Mutations in *TRIM8* have been reported as a rare cause of epilepsy and intellectual disability (Assoum et al., 2018; Sakai et al., 2016). In this report, we describe a seventh individual with a *de novo* heterozygous truncating mutation in the C-terminal region of TRIM8. The six previous patients with *TRIM8* mutations had intractable seizures and severe developmental

delay (absent or minimal speech, delayed walking or non-ambulant). In contrast, our patient presented with a mild neurodevelopmental phenotype (mild speech delay, borderline motor milestones, and mild intellectual disability) and a small number of seizures (possibly triggered by dialysis and/or electrolyte imbalance) that have been controlled by a single antiepileptic medication. The reason(s) for this difference in severity are unclear. One possibility is that *TRIM8* mutations cause a broad spectrum of neurodevelopmental outcomes. Individuals with mild neurological phenotypes may be less likely to have genomic testing, leading to an ascertainment bias. Alternatively, we considered whether the mutation might be mosaic. The wild type allele was seen in 14 out of 21 sequencing reads. This difference is not significantly skewed (binomial P-value = 0.09); however, mosaicism remains a possible explanation for the patient's mild neurological phenotype.

As with previous patients, our patient's mutation is in the last exon of *TRIM8* (Fig. 3b). It has been proposed that these mutations escape nonsense-mediated decay (Assoum et al., 2018). The resulting stable but truncated product may have dominant-negative effects. Two of the previously reported mutations were located more 5' in the last exon, while four were located more 3'. Therefore, a difference in the size of the truncated protein is unlikely to explain the difference in the severity of the neurological phenotype. Interestingly, the variants known to be associated with renal involvement tended to be closer to the C-terminal (Fig. 3b) apart from p.Gln423* which did not fit this pattern. Some variability in the age of onset and treatment response of seizures was noted among previous patients (Assoum et al., 2018). The patient with the earliest-onset seizures (2 months) had profound developmental delay and pharmaco-resistant epilepsy; whilst the patient with the latest-onset seizures (3 years 5 months) had earlier motor milestones, showed no regression and had seizure control on levetiracetam monotherapy. Therefore, it is possible that genetic background or

environmental factors may modulate seizure threshold in patients. A higher threshold may delay onset and reduce the severity of seizures, preserving brain development.

Proteinuria has now been documented in 4 of the 7 reported TRIM8 patients (Assoum et al., 2018). Our patient and one previous subject had evidence of a urinary tract infection at the time of testing (blood, bacteria and white cells). Another previous patient was diagnosed with nephrotic syndrome associated with steroid-resistant glomerulonephritis. None of the previous patients were reported to have impaired renal function. In contrast, our patient had a FSGS-like phenotype with progressive renal impairment leading to end stage renal failure. Once again, the reasons for this difference are unclear. We note that four of the previous patients were younger than our patient at the time of reporting (the others were still only 10 and 13 years old). The spectrum of renal outcome in patients with TRIM8-related disease remains to be established. However, based on these observations, we would recommend that all TRIM8 patients have periodic surveillance for proteinuria and that those with nephrotic-range proteinuria have their renal function closely monitored in the long term.

The TRIM8 protein is widely expressed including in the primitive glomeruli of the developing kidney (Reymond et al., 2001). The C-terminal region of TRIM8 is highly conserved across vertebrate species and has a crucial role in the subcellular localisation of the protein product (Huang et al., 2016; Reymond et al., 2001) . Expression studies in HeLa and U2OS cells showed that wild type TRIM8 protein localised intracellularly to specific nuclear bodies in a speckled distribution. Targeted deletion of the C-terminal domain led to a loss of nuclear staining in favour of a diffuse cytoplasmic staining (Reymond et al., 2001). One important pathway that may be affected by mislocalisation of TRIM8 is the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signalling pathway.

TRIM8 has been shown to negatively regulate protein inhibitor of activated STAT3 (PIAS3) and suppressor of cytokine signalling 1 (SOCS1), two inhibitors of the JAK/STAT pathway (Hatakeyama, 2017; Okumura et al., 2010; Toniato et al., 2002). Inappropriate activation of JAK-STAT signalling has been linked to a range of glomerular renal disease, including HIV-induced nephropathy (Feng et al., 2009; Gu et al., 2013), diabetic kidney disease (Berthier et al., 2009; Zhang et al., 2017) and FSGS (Chuang and He, 2010; Pace et al., 2019).

In summary, the findings in this patient provide further evidence that truncating mutations in the C-terminal region of TRIM8 cause a syndrome with both neurological and renal features. These findings also suggest that the spectrum of TRIM8-related disease may be broader than previously thought, with the potential for less severe neurodevelopmental problems but a more severe renal phenotype. Given the progression to end stage renal failure in our patient, we would recommend periodic proteinuria screening in patients with C-terminal truncating mutations in TRIM8.

Declaration of interests

Acknowledgments

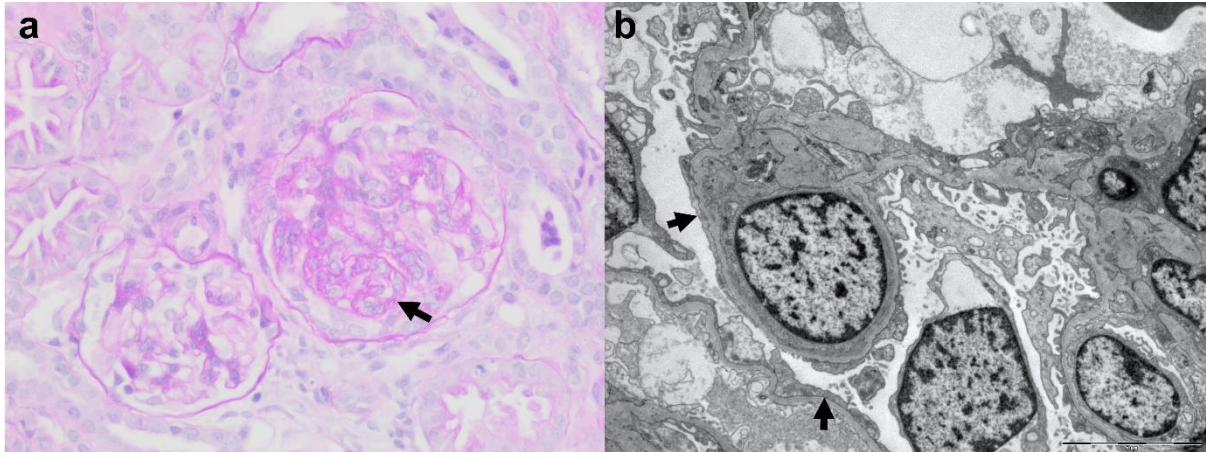## Funding

Figure Titles and Legends

Figure 1. Images of the renal histopathology. a) Light microscopy, Periodic Acid Schiff stain, x200 magnification. Two glomeruli are seen. The smaller left one appears normal, while the larger right one shows segmental collapse and sclerosis (arrow). There is no evidence of any crescents or necrosis. b) Electron microscopy, x3000 magnification. The image displays glomerular basement membrane, podocytes and the edge of the mesangium area. There are areas of foot process effacement identified in the lower left part of the image (arrows).
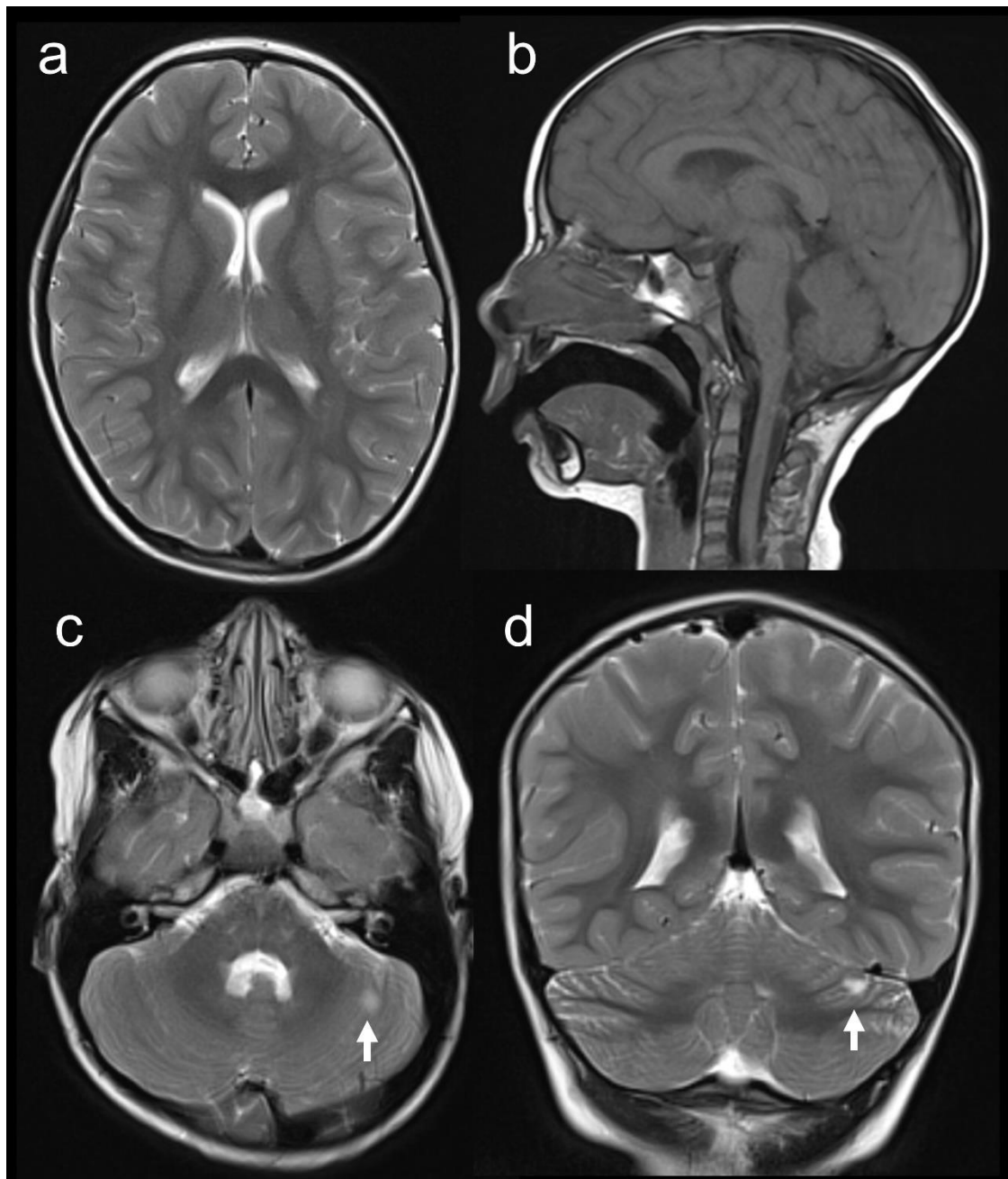
Figure 2. MRI brain scan images from the patient at 5 years of age. a) T2-weighted axial image showing normal gyration and white matter. No white matter abnormalities are seen in the cerebral hemispheres. b) T1-weighted sagittal image showing normal midline structures. c) T2-weighted axial and d) coronal images showing the T2-hyperintense/T1-hypointense focus in the superior aspect of the left cerebellar hemisphere (white arrows).
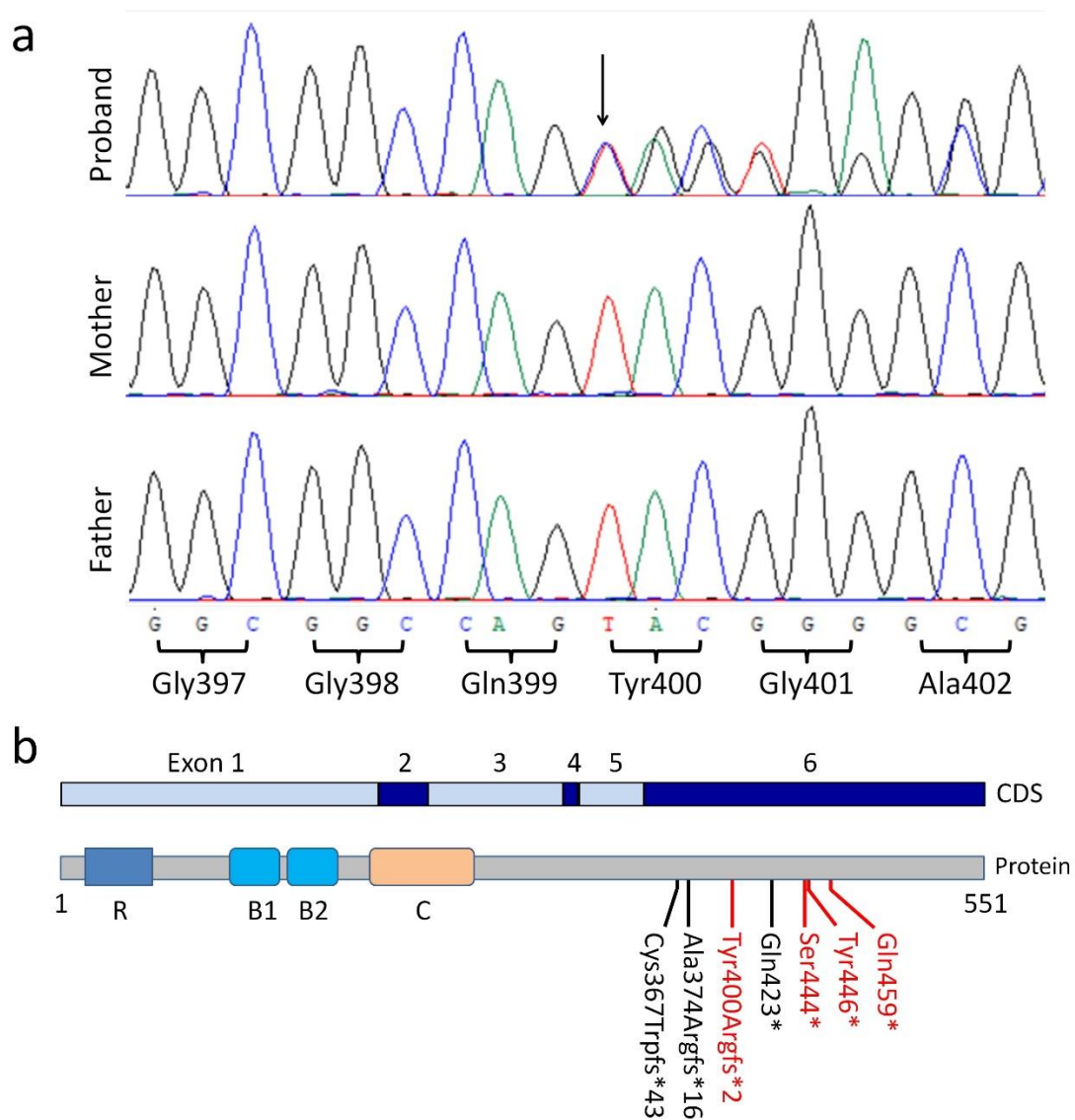
Figure 3. TRIM8 mutations. a) Chromatograms of the Sanger sequencing from the patient and both his parents. The position of the *de novo* frameshift mutation is marked by the arrow. b) Location of the TRIM8 mutations relative to the coding sequence (CDS) or protein. Location of the R(ing-type zinc finger) motif, B1 and B2 (B- Box) domains and C(oiled- coil) motif are shown. Mutations are localized to the C- terminal region of the protein. The mutations reported by Sakai et al. and Assoum et al. are indicated in the figure along with our patient. Mutations associated with known renal involvement are highlighted in red.

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