Letter.

Transplantation.

To the editor:

Stem cell transplantation for tetratricopeptide repeat domain 7A deficiency: long-term follow up.

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Running title: HSCT in TTC7A deficiency: long-term follow up

Submitted (01/28/2016)
The online version of the article contains a data supplement.

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Key Words

Haematopoietic Stem Cell Transplantation, TTC7A, very early onset inflammatory bowel disease

Word Count: 1190

Figures:1

Tables:1

References count:18

Mutations in the tetratricopeptide repeat domain 7A (*TTC7A)* gene cause a severe form of very early-onset inflammatory bowel disease (VEOIBD)[1]. TTC7A has a crucial role in chaperoning the enzyme phosphatidylinositol-4-Kinase-3-alpha from the Trans-Golgi apparatus to the plasma membrane to facilitate phosphorylation of phosphatidylinositol (PI). The composition of the plasma membrane in particular levels of phosphorylated PI (PI-4P) are crucial for preserving epithelial cell polarity and survival[1-3]. The clinical spectrum of the disease varies from multiple intestinal atresias (MIA) to severe autoimmune enterocolitis clinically evident by infantile-onset intestinal obstruction/failure, bleeding and diarrhoea[1,4-6]. Furthermore, the disease can be associated with severe immunodeficiency or autoimmune phenomena owing to the central role of TTC7A in thymic architecture[7]. Limited published data on TTC7A deficient patients suggests a median survival <12 months of age[1,4-9]. Allogeneic hematopoietic stem cell transplantation (HSCT) represents a possible treatment option for therapy-refractory VEOIBD with or without genetic diagnosis, especially when associated with immunological impairment[10-13]. Moreover, evidence on the interaction between haematopoietic donor and host epithelial cells in stem cell transplantation might lead to potential benefit of HSCT in certain epithelial disorder[14-16]. TTC7A deficient patients with associated immunodeficiency may therefore benefit from HSCT, however the effect of engrafted donor cells on chronic intestinal inflammation and gut epithelial tissue regeneration is unknown[14-15]. Follow-up data on HSCT in TTC7A-deficiency is required to understand the impact of this treatment on the disease phenotype and to guide the management of future cases. In this study, we report the clinical and histological evolution of four TTC7A-deficient patients who are alive 19 to 114 months post HSCT.

Anthropometric data, clinical features and laboratory results from affected patients were extracted from clinical notes and prospective databases. One of the four patients included in this study was previously reported with shorter post-HSCT follow up[6]. We recruited candidates for molecular evaluation, including NGS, as part of the PETIT Study (Patients with Early-onseT Intestinal inflammaTion Study). The molecular diagnosis was established through whole exome sequencing in three patients (in one case as previously reported[6], for the other two Beckman Coulter Genomics on Illumina HiSeq2000, SureSelect Human All Exon Kit Version 4 Agilent Technologies; Alignment: Burrows-Wheeler Aligner software; Refinement: Genome Analysis Tool Kit) and targeted sequencing in one patient (PCR according to standardised diagnostic laboratory criteria, protocol on request). Intestinal biopsies were fixed, paraffin embedded and stained (haematoxylin and eosin) according to

standardised diagnostic laboratory criteria (protocol on request). Patients were informed and consented for functional studies and genetic sequencing as part of the PETIT Study. Ethical approval was obtained from the National Research Ethics Service Committee London, Bloomsbury. Between 2005 and 2013, three patients with TTC7A deficiency were treated at Great Ormond Street Hospital, London, UK and one at Boston Children's Hospital in Boston, MA, USA (extended medical histories in supplemental material). In one case, a family history of early infantile deaths due to obstructive intestinal disease in the patients' siblings was established. All children were diagnosed antenatally with bowel obstruction, required multiple surgeries in the neonatal period to address gut strictures and were parenteral nutrition (PN) dependent within the first six months of life. In three patients attempts to control intestinal inflammation by deploying multiple immunosuppressant agents were undertaken without consistent improvement. There was evidence of immune dysfunction/dysregulation in all patients varying from severe T- and NK-cell deficiency with impaired mitogen response and low T-cell receptor excision circles (TRECs) to mild TRECs impairment (Table 1, detailed in Table S3,S4). Patients received HSCT after reduced toxicity conditioning or serotherapy alone at 3, 9, 14 and 17 months of life (Table 1). At the time of writing, all children were alive at 19, 50, 66 and 114 months post-HSCT. Donor engraftment was complete in one and mixed in three patients with normal immune reconstitution in all children (Table 1, detailed in Table S3). Three patients required major surgical revisions for on-going gut strictures post-HSCT, and two are still on immunosuppression. None of the patients could be weaned off PN, and two are currently listed for multivisceral transplantation due to additional liver dysfunction. Intestinal inflammation and abnormal epithelial features persisted beyond HSCT in all children (supplemental material, table S1) consistent with ongoing primary disease as opposed to a possible allogeneic phenomenon (i.e. gut graft versus host disease). Post HSCT, one patient developed severe sensorineural deafness and immune mediated hypothyroidism, one developed "flaky skin" phenotype and lung dysfunction of unclear origin. In three children, advanced liver disease with bridging fibrosis possibly due to long-term PN was established.

Published data on TTC7A deficiency is scarce. Overall 52 cases with genetically confirmed TTC7A deficiency were reported in the literature [3, 6-9,15-16]. Clinical outcomes are available for 45 patients, 30 of whom have died (median age at death eight months). Fifteen reported pts are alive at a median age of 27 months. All of them have a severe gastro-intestinal disease (severe diarrhoea, gut failure, MIA), 6 are PN dependent, 11 show signs of immunodeficiency and/or autoimmune disorders[1,6-8]. Most of the reported patients were treated symptomatically and managed with supportive medical and surgical care. One reported case was referred for combined small intestine and HSCT but was lost to follow-up

[6]. Six published patients underwent HSCT of whom five died within the first year post procedure (3 from infections, 1 from disease progression, 1 from unknown causes)[6,8,9,15]. The only survivor is reported here as P4.

Over 20 private mutations in the TTC7A gene have been described ranging from homozygous deletions as demonstrated in the first publication associating the TTC7A gene with MIA[4] to compound heterozygous missense mutations[1,6,8,9]. There is insufficient evidence to suggest a correlation between type or position of the mutation within the TTC7A gene and clinical severity. This is confirmed by our findings. Survival, clinical and histological evolution of the disease appeared to be similar in our patients despite markedly different genotypes. P2 harboured a large homozygous deletion in exon 1 (Table 1, Figure 1) leading to a premature stop codon at the start of exon two (amino acid position 67) rendering the presence of a functional TTC7A protein highly unlikely. Considering that 3 patients in our cohort underwent HSCT at a higher median age than the reported median age of death in the literature, we suggest that disease severity plays a central role in early patient survival and eligibility to HSCT. All our patients are alive post HSCT, suggesting that reduced or minimal toxicity conditioning is a critical point to avoid transplantation-related mortality and still obtain T-cell engraftment. Our findings suggest that while HSCT was feasible and safe, correcting the immune dysfunction through HSCT in TT7A patients did not influence the epithelial phenotype nor promoted enteral tolerance. Restoring immunocompetence in patients with concomitant immunodeficiency may nevertheless increase the chance for long-term survival limiting infection-related comorbidities across multiple surgeries and long-term PN. The role of HSCT in preventing immune mediated phenomena which appear later in life of TTC7A deficient patients has yet to be established, and is therefore difficult to include in the decision-making process[7]. Small bowel transplantation in genetically confirmed TTC7A deficiency has not yet been reported but given the overall improving results of intestinal transplantation in children, this might be an option in selected cases[17,18].

Authorship:

Contribution: JK, DE, and HU were in charge of the genetic diagnosis and analysis for the reported patients; JK, GL and PV wrote the paper; SYP, AW, PA, JS, RC, KR, GNJ, MG, ME , NShah, LN and PV were in charge of the clinical management of the patients pre, during an post transplant, they collected and made the follow up data available for the study, DR and NSebire analysed the longitudinal histopathology data from the patients.

Conflict of interest disclosure: the authors declare no competing financial interests.

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Patient	Genotype	Immunology	Gut Phenotype	IS pre HSCT	Donor	Stem-Cell Source	Conditioning Regimen	GvHD	Chimerism
P1	p.Glu191fs p.I854Phe	Low IgG levels Impaired mitogen stimulation	Pyloric stenosis Microcolon Autoimmune enterocolitis (PE)	Steroids Azathioprine Infliximab Daclizumab	Unrelated HLA 8/10	CB	TBI 2 Gy Cyclophosphamide 50 mg/kg Fludarabine 200 mg/m^2 ATG 7.5 mg/kg	None	CD3 = 51% donor CD15 = 15% donor
P2	p.Gly45_Ala55del p.Gly45_Ala55del	Low IgG levels Reduced TRECs	D/AC/TC Atresias Microcolon Autoimmune enterocolitis (PE)	Steroids Infliximab Basiliximab Cyclosporine	Sibling HLA 10/10	BM	Treosulfan 42 gr/m^2 Cyclophosphamide 120 mg/kg	Acute skin Grade I	CD3 = 100% donor CD15 = 100% donor
P3	p.Glu71Lys p.Glu96*	Low IgG levels Low T/NK-Cells Reduced TRECs Abnormal mitogen stimulation	Exomphalos Pyloric/Ileal atresias Microcolon Autoimmune enterocolitis (PE)	Steroids	Unrelated HLA 12/12	PBSC	Treosulfan 36 gr/m^2 Fludarabine 150 mg/m^2 Alemtuzumab 1 mg/kg	None	CD3 = 96% donor CD15 = 7% donor
P4	p.K606R p.S672P	Low IgG levels Low T cells Absent TRECs Abnormal mitogen stimulation	Pyloric stenosis Jejunal and colonic atresias Microcolon	None	Sibling HLA 9/10	BM	Equine ATG 30 mg/kg	None	CD3 = 100% donor CD15 = 6% donor

Table 1. Characteristics of patients with TTC7A deficiency undergoing HSCT (TRECs = T-Cell Receptor Excision Circles, PE = panenteric (small and large bowel), D/AC/TC = Duodenum/Ascending Colon/Transverse Colon, IS = Immunesupression, CB = cord blood, BM = bone marrow, PBSC = peripheral blood stem cells, TBI = total body irradiation , ATG = anti-thymocyte globulin). Additional data on histological evolution post HSCT in supplemental material.

Figure 1. Pedigree for patient 2 with TTC7A deficiency (A). Integrative Genomics Viewer of whole exome sequencing data from patient 2 (B): Broad grey bars represent sequenced reads aligned to the reference genome. Thin grey bars represent missing/deleted segments (large 34 base pair deletion demonstrated in exon one, confirmed on Sanger sequencing). **H&E stained gut biopsies taken pre HSCT and 6 months and 18 months post HSCT (C, from left to right) from patient 2.** Unfilled white arrows: apoptotic debris. Filled white arrows: inilftration of the lamina propria with lymphocytes, eosinophils, plasma cells and neutrophils. **Position of all causative mutations within *TTC7A* gene** (D, top line: Exon 1-20) and position within TTC7A protein (lower line: Tetratricopeptide repeat (TPR) domains 1-9) for all four patients.

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