The prostate-specific protein, transglutaminase 4 (TG4), is an autoantigen associated with male subfertility

Siiri E. Iismaa[1,2]

[1]Division of Molecular Cardiology and Biophysics, Victor Chang Cardiac Research Institute, Sydney, NSW 2010, Australia, [2]St. Vincent's Clinical School, University of New South Wales, Sydney, NSW 2052, Australia

Correspondence to: Siiri E. Iismaa. Division of Molecular Cardiology and Biophysics, Victor Chang Cardiac Research Institute, 405 Liverpool St., Darlinghurst, Sydney, NSW 2010, Australia. Email: s.iismaa@victorchang.edu.au.

Submitted Aug 15, 2016. Accepted for publication Aug 22, 2016.
doi: 10.21037/atm.2016.10.02
View this article at: http://dx.doi.org/10.21037/atm.2016.10.02

Autoimmune polyglandular syndrome type 1 (APS1) is a rare autoimmune disease that affects multiple endocrine glands. It is a monogenic autosomal recessive disorder caused by loss-of-function mutations in the autoimmune regulator (*AIRE*) gene located on human chromosome 21q22.3. AIRE is a transcription factor that is expressed in medullary thymic epithelial cells (mTECs) (1) and extrathymic Aire-expressing cells (eTACs) that are resident in the secondary lymphoid organs (2). AIRE initiates the transcription of thousands of tissue-specific antigens that are usually only expressed in peripheral tissues. Autoreactive T cells that bind strongly to these self-antigens are thereby eliminated in the thymus and peripheral tissues through apoptosis. This prevents the immune system from attacking the body itself. When AIRE is defective, autoreactive T cells survive to bind their corresponding self-proteins, thereby creating an autoimmune reaction (1). APS1 is thus a major disease model for autoimmunity (3) and the Aire-deficient mouse serves as a good animal model for studying autoimmune organ destruction (1,4).

Infertility is a common manifestation of APS1 in both male and female patients (5). Most female APS1 patient infertility can be explained by autoimmune ovarian failure, which often commences at an early age, e.g., in the teens or early adult years. Gonadal failure in male APS1 patients is rare and occurs mostly at an adult age. The male Aire-deficient mouse, which is subfertile, exhibits normal testis anatomy and histology, yet the prostate gland is a major target of autoimmune destruction (6). The causes of male infertility have remained unclear until the recent work of Landegren *et al.* published in *Science Translational Medicine* (7), which reports that the prostate-specific secretory protein transglutaminase 4 (TG4), is a male-specific autoantigen in APS1 patients that could contribute to subfertility.

To identify male-specific autoantibodies against antigens specific to male reproductive organs, Landegren *et al.* (7) screened human protein arrays with sera from male and female APS1 patients from Finland, Norway and Sweden, and sex-matched healthy control subjects. Their screen identified one significant target, TG4, which is expressed specifically in the prostate epithelium. Using a radioligand binding assay to screen for TG4 autoantibodies in an extended cohort of APS1 patients as well as healthy controls and various disease cohorts, Landegren *et al.* (7) validated TG4 as a highly specific autoantigen for adult male APS1 patients. TG4 autoantibodies were not detected in male patients of pre-pubertal age, but were prevalent in male patients of postpubertal age. Male patient serum samples collected consecutively before and after puberty revealed that TG4 autoantibodies first arose at puberty and were sustained thereafter. This parallels TG4 expression, which begins during early puberty (8).

To further investigate the involvement of TG4 as a prostate autoantigen in male subfertility, Landegren *et al.* (7) used Aire-deficient mice. Radioligand binding assays detected autoantibodies against murine TG4 in the serum of all male Aire-deficient mice but not in the serum of female Aire-deficient mice or wild-type mice. Male Aire-deficient mice displayed prostatitis characterized by increased mRNA expression of markers of T helper 1 (Th1) cells (7), which is consistent with Th1 cells being the major contributors to autoimmune

pathology in these mice (9). Landegren *et al*. (7) detected *Tgm4* mRNA expression in pooled mTECs from wild-type but not Aire-deficient mice, indicating Aire-dependent thymic expression of TG4. This links the lack of tolerance for TG4 in the prostate gland of Aire-deficient mice to its consequent autoimmune destruction. Although chronic prostatitis has not been described as a typical clinical manifestation in men with APS1, prostatitis is frequently asymptomatic and its clinical manifestation may not yet be appreciated (6). *Tgm4* mRNA was also absent from the prostate tissue of Aire-deficient but not wild-type mice, thereby connecting destructive prostatitis with compromised TG4 secretion. The role of TG4 in male reproductive physiology is not clearly defined, however, male TG4 knockout mice do not form a copulatory plug and are severely reduced in fertility despite normal sperm count, motility and reproductive morphology (10).

TG4 belongs to the transglutaminase family of proteins, which includes nine human members: a structural protein, protein 4.2, that lacks catalytic activity and eight zymogens/enzymes designated factor XIII-A and TG1-TG7, that catalyze a variety of calcium- and thiol-dependent posttranslational protein-modifying reactions (11). All reactions involve a glutamine-containing protein or peptide as the first substrate followed by a direct attack of the glutamine by either water (deamidation reaction) or a second substrate such as an amine (transamidation reaction) or an alcohol (esterification reaction), to generate a deamidated, cross-linked or esterified product, respectively (11). The different TG family members are restricted in their substrate specificity and tissue distribution, and have distinct biological roles (11). Protein 4.2 functions in ion transport across the red blood cell membrane; factor XIII-A has an extracellular cross-linking role in blood coagulation and also plays a role in inflammation and bone synthesis; TG1, TG3 and TG5 have intracellular cross-linking roles in skin barrier development; TG2 has a pleiotropic role in pathophysiological conditions (11); TG4 enhances fertility in males (10) and has been implicated in prostate cancer progression (12); TG6 has a role in cerebellar functioning (13) and the function of TG7 is not known.

TG4 is not the first TG family member to be implicated in autoimmune disease. Several other members have been identified as major autoantigens in distinct autoimmune diseases. Autoantibodies against factor XIII-A result in acquired FXIII deficiency, a rare and severe bleeding disorder associated with a significant mortality rate [reviewed in (14)]. Gluten-sensitivity diseases triggered by gluten present in wheat, barley and rye commonly manifest as an enteropathy (celiac disease, characterized by chronic inflammation of the small intestinal mucosa, malabsorption and diarrhea), and/or dermatopathy (dermatitis herpetiformis, a blistering skin disease) or neuropathy (gluten ataxia involving cerebellar dysfunction due to loss of Purkinje cells). Symptoms resolve with a gluten-free diet and reoccur upon resumption of gluten ingestion. Patients develop antibodies against TG-deamidated gluten epitopes and autoantibodies against TG2 in celiac disease (15), against TG3 in dermatitis herpetiformis (16) or against TG6 in gluten ataxia (17). TG-mediated deamidation of gluten peptides (15,18) improves the binding of the peptides to HLA-DQ2 and HLA-DQ8 molecules present on the surface of antigen-presenting cells, which initiates a T-cell-mediated immune response and mediates the humoral adaptive response that stimulates B-cells to produce antibodies specific to deamidated gluten (19). The production of anti-transglutaminase autoantibodies in gluten-sensitivity disorders is likely mediated by the formation of covalent enzyme-peptide complexes, which act as hapten-carrier complexes (15,18,19).

In addition to TG4 being identified as a major Aire-dependent autoantigen in APS1 patients and in Aire-deficient mice (7), previous work has associated another Aire-dependent, prostate-specific protein, human semenogelin (seminal vesicle protein 2 in the mouse), as an autoantigen in chronic prostatitis patients and Aire-deficient mice (6). Semenogelins are major seminal vesicle secreted proteins in human semen that form the gelatinous fluid that immobilises sperm upon ejaculation. Semenogelins have also been reported to be substrates for TG (20). Thus, although the mechanism by which the T-cell-mediated immune response is initiated differs between the chronic prostatitis of the Aire-deficient mouse and gluten sensitivity diseases, there are parallels in terms of autoantibody production against the TG enzyme and its substrate(s).

The discovery by Landegren *et al*. (7) of TG4 as a major autoantigen in APS1 is not only an important step forward in understanding the infertility associated with this disorder but may lead to therapeutic strategies to treat young male APS1 patients to prevent infertility. This study also highlights a number of fundamental unanswered questions for biochemists and biologists in relation to male fertility. For example, does the interaction of TG4 with its substrates

result in deamidation, transamidation or esterification of the substrate? What is the effect of TG4 and/or the TG4 enzyme-substrate reaction on male fertility in terms of effects on sperm activity or antigenicity in the female reproductive tract, oocyte fertilization, or post-fertilization events such as implantation? Clearly, there is much yet to be learned about the functional role of TG4 and its substrates in male reproductive physiology and the study by Landegren *et al.* (7) has pointed us in fruitful directions for future research.

Acknowledgements

Funding: SE Iismaa is funded by Australian Research Council Stem Cells Australia, Special Initiative in Stem Cell Science grant (SR110001002) and a Leducq Transatlantic Network of Excellence in Cardiovascular Research grant.

Footnote

Provenance: This is a Guest Commentary commissioned by Section Editor Ran Mo, MD (Department of Cardiothoracic Surgery, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China).
Conflicts of Interest: The author has no conflicts of interest to declare.

Comment on: Landegren N, Sharon D, Shum AK, *et al.* Transglutaminase 4 as a prostate autoantigen in male subfertility. Sci Transl Med 2015;7:292ra101.

References

1. Anderson MS, Venanzi ES, Klein L, et al. Projection of an immunological self shadow within the thymus by the aire protein. Science 2002;298:1395-401.
2. Gardner JM, Devoss JJ, Friedman RS, et al. Deletional tolerance mediated by extrathymic Aire-expressing cells. Science 2008;321:843-7.
3. Mathis D, Benoist C. A decade of AIRE. Nat Rev Immunol 2007;7:645-50.
4. Ramsey C, Winqvist O, Puhakka L, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. Hum Mol Genet 2002;11:397-409.
5. Husebye ES, Perheentupa J, Rautemaa R, et al. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. J Intern Med 2009;265:514-29.
6. Hou Y, DeVoss J, Dao V, et al. An aberrant prostate antigen-specific immune response causes prostatitis in mice and is associated with chronic prostatitis in humans. J Clin Invest 2009;119:2031-41.
7. Landegren N, Sharon D, Shum AK, et al. Transglutaminase 4 as a prostate autoantigen in male subfertility. Sci Transl Med 2015;7:292ra101.
8. Pritchard C, Mecham B, Dumpit R, et al. Conserved gene expression programs integrate mammalian prostate development and tumorigenesis. Cancer Res 2009;69:1739-47.
9. Devoss JJ, Shum AK, Johannes KP, et al. Effector mechanisms of the autoimmune syndrome in the murine model of autoimmune polyglandular syndrome type 1. J Immunol 2008;181:4072-9.
10. Dean MD. Genetic disruption of the copulatory plug in mice leads to severely reduced fertility. PLoS Genet 2013;9:e1003185.
11. Iismaa SE, Mearns BM, Lorand L, et al. Transglutaminases and disease: lessons from genetically engineered mouse models and inherited disorders. Physiol Rev 2009;89:991-1023.
12. Jiang WG, Ablin RJ. Prostate transglutaminase: a unique transglutaminase and its role in prostate cancer. Biomark Med 2011;5:285-91.
13. Thomas H, Beck K, Adamczyk M, et al. Transglutaminase 6: a protein associated with central nervous system development and motor function. Amino Acids 2013;44:161-77.
14. Franchini M, Frattini F, Crestani S, et al. Acquired FXIII inhibitors: a systematic review. J Thromb Thrombolysis 2013;36:109-14.
15. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797-801.
16. Sárdy M, Kárpáti S, Merkl B, et al. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. J Exp Med 2002;195:747-57.
17. Hadjivassiliou M, Aeschlimann P, Strigun A, et al. Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. Ann Neurol 2008;64:332-43.
18. Stamnaes J, Dorum S, Fleckenstein B, et al. Gluten T cell epitope targeting by TG3 and TG6; implications for dermatitis herpetiformis and gluten ataxia. Amino Acids

2010;39:1183-91.
19. Stamnaes J, Sollid LM. Celiac disease: Autoimmunity in response to food antigen. Semin Immunol 2015;27:343-52.
20. Peter A, Lilja H, Lundwall A, et al. Semenogelin I and semenogelin II, the major gel-forming proteins in human semen, are substrates for transglutaminase. Eur J Biochem 1998;252:216-21.

Cite this article as: Iismaa SE. The prostate-specific protein, transglutaminase 4 (TG4), is an autoantigen associated with male subfertility. Ann Transl Med 2016;4(Suppl 1):S35. doi: 10.21037/atm.2016.10.02

