Fact Sheet: Naturally-occurring spontaneous mutation impacting thyroid anatomy identified in C57BL/6NTac strain

Summary

Taconic has identified a naturally-occurring spontaneous mutation in C57BL/6NTac mice (model #B6, hereafter called B6NTac) that is strongly associated with early-onset thyroid dysplasia. This variant arose before 2011 and became widespread in global B6NTac colonies. **This allele did not become fixed, and Taconic has eliminated it from all B6NTac Production Colonies**. It will be eliminated over time from aged holding and associated lines on the B6NTac background. We expect this may be of concern primarily to customers studying thyroid biology and/or using aged animals. The B6NTac strain and related hybrid, diet-induced and GEM models on this strain background will remain available for sale.

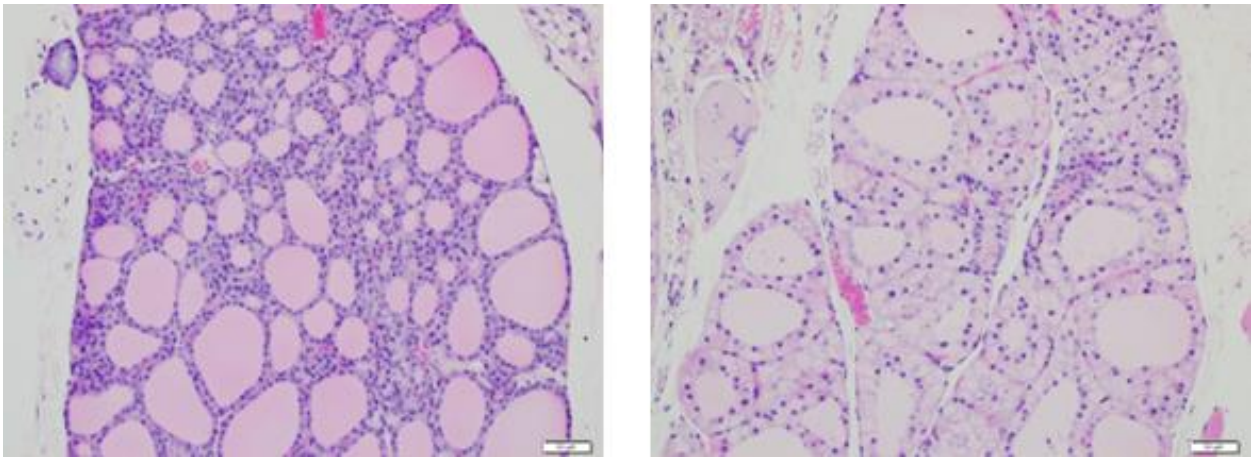
- While this spontaneous mutation is associated with progressive anatomical anomalies in the thyroid, affected mice have normal thyroid function as measured by T3 and T4 hormone levels in serum at younger ages (10-16 weeks) as compared to other B6 substrains. This dysplasia is progressive and may lead to thyroid tumors in aged homozygous mice (6-12 mo).
- This phenotype is autosomal dominant, meaning heterozygotes and homozygotes both display abnormal thyroid anatomy.
- This spontaneous mutation is specific to B6NTac mice and is not present in B6JBOM or B6 substrains from other vendors.
- This allele arose before 2011 and became widespread in global B6NTac colonies.
- Taconic has actively selected this variant out all B6NTac colonies. Saleable B6NTac animals from all US and DK colonies starting with week of birth May 22, 2023, are wild type for the mutation.
- This variant may be present commercial GEMs on the C57BL/6NTac background.
    - *The rasH2™ mouse is not affected as a unique B6 substrain is used to produce it.*
    - Testing and selection activities for ~30 commercial GEM strains are in process.
- All new Custom Model Generation Solutions (CMGS) projects on the B6NTac background started after February 20, 2023, use B6NTac ES cells and/or donor animals that do not carry the spontaneous mutation. Prior to this date, CMGS project that use B6NTac donor animals (not ES cells) may have been impacted.
- Colony Management Solutions (CMS) projects that have used Taconic B6NTac mice as donors for embryology services or breeders may be affected.

Detailed description of the spontaneous mutation and its biological impact

As a result of normal genetic processes, the C57BL/6NTac strain acquired a natural spontaneous mutation that is strongly associated with early-onset thyroid dysplasia. The genetic variant is a type of retrotransposon called long interspersed nuclear element (LINE) which inserted into an intron within the *Thyroglobulin* gene. This allele has been designated $Tg^{tdys\text{-}Tac}$. RNAseq data suggest that presence of the LINE causes exon 26 of Thyroglobulin to be excluded from the mRNA in the majority of transcripts. A genotyping assay is available to screen for the presence of this allele.

Histopathological analysis reveals that heterozygous or homozygous carriers of this variant develop mild thyroid dysplasia as early as 10 weeks of age. This dysplasia is progressive and may lead to thyroid tumors in aged mice (6-12 mo), though penetrance is unclear. A Taconic customer has reported high penetrance of thyroid tumors in older B6NTac mice (6-12 months), but in a small study performed by Taconic of 10 aged carriers from 26-38 weeks of age, histological analysis of thyroid cross-sections did confirm dysplasia, but did not reveal any tumors. Further analysis of aged animals is underway. Affected C57BL/6NTac animals at 10-16 weeks of age produce T3 and T4 thyroid hormones in serum at levels comparable to unaffected animals of the C57BL/6NTac strain, and these levels are comparable to other B6 substrains and laboratory mice in general. Taconic is in the process of assessing thyroid function in older animals.

Normal B6NTac thyroid (left), B6NTac thyroid with dysplasia (right)



This finding is most relevant to researchers studying thyroid biology and/or working with aged mice. Taconic has no information to indicate that this spontaneous mutation impacts other organ systems.

How did this spontaneous mutation occur?

This is a spontaneous genetic variant that occurred as part of natural processes in reproduction. The mouse genome contains a significant amount of retrotransposon DNA, and these genetic elements routinely change location through retrotransposition. This occurs frequently in somatic tissues. When a LINE element changes location in a germ cell, this introduces a new germ-line spontaneous mutation.

This genetic variant in the *Thyroglobulin* gene likely arose in the Taconic B6NTac foundation colony before 2011 and became distributed in various global production colonies over time.

For more information on mouse germline mutations introduced by retrotransposition, see Gagnier et al.[1]

Actions Taconic is taking to eliminate this spontaneous mutation in C57BL/6NTac mice

Although genetic variants arise as part of natural reproductive processes, Taconic strives to maintain inbred strains with stable genetics. Because this spontaneous mutation has an identified phenotypic consequence and it did not become fixed in the genome of the B6NTac

strain, Taconic will eliminate this allele through selective breeding over time. Taconic has already eliminated this allele in the B6NTac Foundation Colony as well as all global Pedigreed Expansion Colonies, first-, second and third-layer Expansion Colonies and some Production Colonies.

- All European B6NTac Production Colony breeders are now free of the *Thyroglobulin* mutation, and saleable B6NTac inventory starting with week of birth February 20, 2023, is free of the mutation.
- All US B6NTac Production Colony breeders are now free of the *Thyroglobulin* mutation.
    - All US B6NTac saleable inventory at the Opportunist Free health standard is free of the mutation starting with week of birth February 20, 2023.
    - All US B6NTac saleable inventory at the Murine Pathogen Free health standard is free of the mutation as of week of birth May 22, 2023.
    - All B6NTac saleable inventory at the Germ Free health standard is free of the mutation for all 2023 weeks of birth.

Certain commercial GEMs on the B6NTac background are affected by the mutation, and Taconic will eliminate the mutation from these lines via selection or backcrossing over time. See individual product pages for more detail.

All CMGS projects utilizing B6NTac ES cells or live animals will utilize donors that are wild type (WT) for the spontaneous mutation moving forward. Likewise, Taconic will source B6NTac donors that are WT for the spontaneous mutation for all ExpressMODEL® services globally.

For all other embryology services, Taconic will source B6NTac donors that are WT for rederivations and rapid expansions as of Mar 2023 for work performed in the EU and starting in ~July 2023 for work performed in the US.

Taconic will source B6NTac donors that are WT for the spontaneous mutation for all CMS projects where B6NTac breeders are needed, with implementation immediately for projects in Denmark and Germantown and as of ~Apr 15, 2023 for projects in San Diego and Cambridge City.

FAQs

Are other commercial strains at Taconic affected?

The C57BL/6JBomTac strain does not carry this mutation. Taconic has many GEM lines on the C57BL/6NTac background, some of which are affected. The NASH and DIO diet-induced models as well as certain F1 hybrid models are generated from the B6NTac, and these models have been remediated already.

How was this spontaneous mutation identified?

As a result of a few customer complaints regarding abnormal histopathology findings in B6 mice received within the past few years, Taconic opened an investigation, with significant collaborative participation by one of the customers originally reporting an issue.

The investigation included sampling of Taconic colonies for histopathology, hormone level analysis, pedigree analysis, test matings, whole genome sequencing and RNA expression analysis and genotyping. The spontaneous mutation was identified and verified as the cause of

the abnormal thyroid anatomy. Taconic and the collaborating customer intend to publish this work as a peer-reviewed paper in the future.

Why did Taconic not detect the mutation sooner?

The Taconic quality program has several components, including major quality control programs in genetic integrity and strain harmonization. This program aims to ensure that our animals are genetically pure, and that animals obtained from one Taconic site are as genetically similar as possible to those obtained from other Taconic sites.

Additionally, Taconic maintains cryopreserved stocks of its main inbred strains in order regularly refresh colonies and avoid genetic drift. As spontaneous mutations are not preventable, best practices in strain management dictate that a strain be genetically refreshed through recovery of embryos from a set stock with some frequency. The B6NTac strain was refreshed in 2018 using embryos from a 2014 cryopreservation. Unfortunately, the refreshed B6NTac stock already possessed the variant, indicating that the mutation occurred prior to 2011. By selecting breeders with normal thyroid histology after the 2018 refresh, the allele was removed from the Foundation Colony. This was confirmed by PCR verification on genomic DNA extracted from all FC breeders.

Taconic's genetic monitoring program uses single nucleotide polymorphism (SNP) panels at varying densities. The standard panel of 2050 SNPs or custom SNP panels used by Taconic for genetic monitoring is excellent at detecting accidental mismatings between strains, but it cannot detect most individual genetic changes. Thus our routine genetic monitoring did not detect this variant.

Detection of spontaneous mutations and de-novo natural genetic variation in inbred strains is challenging. Unless there is an observable phenotype, nothing less than whole genome sequencing at high density would detect most spontaneous mutations. Sequencing of inbred strains as a genetic monitoring technique is not practical at this time, and this is not done by Taconic or any vendor.

For more information on the Taconic genetic monitoring program see http://www.taconic.com/quality/genetic-integrity.

How do I know if my line carries this genetic variant?

B6NTac mice purchased between 2012-present may carry the spontaneous mutation, and the mutant allele may have been present prior to 2012. If you used B6NTac to maintain a GEM line, the mutation may or may not be present depending on the details of your mating scheme. If you wish to analyze whether the mutation is present in your GEM line, we recommend testing your line with this genotyping assay:

Detection of *Tg*$^{tdys\text{-}Tac}$

A genotyping assay is also available at Transnetyx for customer use. Request access to B6-Thyroglobulin-TX-1.

My experiments are working fine. Do I need to do anything?

No. It is likely that the spontaneous mutation does not affect your organ system of interest unless you are studying thyroid biology. Researchers working with aged animals may also want to be aware of this variant.

Are there any other genetic variants or mutations that I should be aware of?

The development of new genetic variants by spontaneous mutational mechanisms is a natural process that cannot be prevented. Various spontaneous mutations have become fixed in commonly used inbred strains, and as whole genome sequencing becomes more common, additional mutations are likely to be discovered. For example, in B6 substrains, the following spontaneous mutations are known:

		Deletion	Deletion	Deletion	Deletion	Point mutation	Deletion	Missense mutation
Substrain	Vendor	*Nnt*$^{C57BL/6J}$	*Snca* deletion	*Crb1*^{rd8}	*Mmrn1* deletion	*Cyfip2*^{M1N}	Partial deletion Y chromosome	*Nlrp12*$^{C57BL/6J}$
C57BL/6NTac	Taconic			Yes		Yes		
C57BL/6JBomTac	Taconic						Yes	Not yet determined
C57BL/6NHsd	Envigo			Yes		Yes		
C57BL/6JOlaHsd	Envigo	Yes	Yes		Yes			Yes
C57BL/6RccHsd	Envigo							
C57BL/6J	Jax	Yes						Yes
C57BL/6NJ	Jax			Yes		Yes		
C57BL/6NCrl	CRL			Yes		Yes		
Reference	-	[2]	[3]	[4]	[5]	[6]	[7]	[8]

I have a question that isn't addressed here.

For questions regarding Colony Management or Custom Model Generation projects, please contact your Scientific Program Manager.

For questions regarding commercial animal models, please contact your Field Application Scientist or email info@taconic.com.

---

1 Gagnier, L., Belancio, V.P. & Mager, D.L. (2019) Mouse germ line mutations due to retrotransposon insertions. Mobile DNA 10, 15.

2 Freeman, H. C., Hugill, A., Dear, N. T., Ashcroft, F. M., & Cox, R. D. (2006). Deletion of nicotinamide nucleotide transhydrogenase. Diabetes, 55(7):2153-2156.

3 Specht, C. G., & Schoepfer, R. (2001). Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. BMC neuroscience, 2:11.

4 Mattapallil, M. J., Wawrousek, E. F., Chan, C. C., Zhao, H., Roychoudhury, J., Ferguson, T. A., Caspi, R. R. (2012). The Rd8 Mutation of the Crb1 Gene Is Present in Vendor Lines of C57BL/6N Mice and Embryonic Stem Cells, and Confounds Ocular Induced Mutant Phenotypes rd8 Mutation in Vendor B6 Mice and ES Cells. Investigative ophthalmology & visual science, 53(6):2921-2927.

5 Gajović, S., Mitrečić, D., Augustinčić, L., Iaconcig, A., Muro, A. F. (2006). Unexpected rescue of alpha-synuclein and multimerin1 deletion in C57BL/6JOlaHsd mice by beta-adducin knockout. Transgenic research, 15(2),:255-259.

6 Kumar, V., Kim, K., Joseph, C., Kourrich, S., Yoo, S. H., Huang, H. C., Vitaterna, M.H., Pardo-Manuel de Villena, F., Churchill, G., Bonci, A., Takahashi, J. S. (2013). C57BL/6N mutation in cytoplasmic FMRP interacting protein 2 regulates cocaine response. Science, 342(6165): 1508-1512.

7 MacBride MM, Navis A, Dasari A, Perez AV. Mild reproductive impact of a Y chromosome deletion on a C57BL/6J substrain. Mamm Genome. 2017 Jun;28(5-6):155-165.

8 Ulland TK, Jain N, Hornick EE, Elliott EI, Clay GM, Sadler JJ, Mills KA, Janowski AM, Volk AP, Wang K, Legge KL, Gakhar L, Bourdi M, Ferguson PJ, Wilson ME, Cassel SL, Sutterwala FS. (2016) Nlrp12 mutation causes C57BL/6J strain-specific defect in neutrophil recruitment. Nat Commun. 7:13180.