Crucial Role Reported for TSPO in Viability and Steroidogenesis is a Misconception. Commentary: Conditional Steroidogenic Cell-Targeted Deletion of TSPO Unveils a Crucial Role in Viability and Hormone-Dependent Steroid Formation

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A commentary on Conditional Steroidogenic Cell-Targeted Deletion of TSPO Unveils a Crucial Role in Viability and Hormone-Dependent Steroid Formation by Fan J, Campioli E, Midzak A, Culty M, Papadopoulos V. Proc. Natl. Acad. Sci. U S A (2015) 112:7261–7266. doi: 10.1073/pnas.1502670112

Recent reports on Leydig cell-specific Tspo conditional knockout TspoΔ/Δ mice (1), viable global Tspo knockout (Tspo−/−) mice from two independent laboratories (2, 3), and clones of CRISPR/Cas9-mediated Tspo-deleted MA-10 Leydig cells (MA-10 TspoΔ/Δ) (4) established that TSPO is not essential for steroid hormone biosynthesis or viability [reviewed in Ref. (5, 6)]. These reports refuted 25 years of dogma that described TSPO as a mitochondrial cholesterol transport protein, indispensable for steroidogenesis. In response, the research group involved in most of the early studies linking TSPO and steroidogenesis investigated Leydig cell-specific and adrenocortical cell-specific TspoΔ/Δ mice (7) and presented results that seem to repudiate the recent findings and revive the old model. In this commentary, we would like to point out that interpretations made in the manuscript by Fan et al. (7) are seriously flawed.

**TSPO DELETION DOES NOT AFFECT VIABILITY

In Fan et al. (7), it was observed that use of Amhr2cre/+ knock-in mice (8) to generate Leydig cell-specific TspoΔ/Δ mice resulted in low Mendelian ratios for homozygous cre positive mice (HO: Amhr2cre/+ TspoΔ/Δ). This was interpreted as partial preimplantation embryo loss, and the authors concluded that TSPO is crucial for viability. This is a fundamental mistake because both Amhr2 and Tspo are in the same chromosome 15 and, therefore, cannot assort independently. The Tspo and Amhr2 genetic positions are just 18.18 cM apart, and the probability for chromosomal crossover between the two loci to get HO mice is calculated as 7.6% [based on Haldane (9), other estimates are similar]. Therefore, the low rate of 4.4% HO mice observed by Fan et al. (7) is anticipated and represents the precise biological value due to linkage of the two loci and is certainly not an indication of embryonic lethality (Figure 1) [Note: Actual values are not identical to calculated numbers...
TSPO Knockout Mice are Viable

Selvaraj et al.

TSPO Knockout Mice are Viable

Frontiers in Endocrinology | www.frontiersin.org 2 July 2016 | Volume 7 | Article 91

FIGURE 1 | Continued

F2: HE mice are backcrossed with TspoΔ/ΔAmhr2Δ/Δ mice in order to generate TspoΔ/Δ, Amhr2Δ/Δ (HO) mice. The TspoΔ/ΔAmhr2Δ/Δ mice produce only one gamete genotype, whereas HE mice produce four gamete genotypes, the ratio of which depends on the frequency of odd number of crossovers that occur between the Tspo and Amhr2 loci, and not through independent assortment, because they are in the same chromosome. Consequently, the gamete genotype necessary for generating HO offspring occurs at a calculated frequency of 7.6%. This is much lower than the classic Mendelian ratio of 25% because Tspo and Amhr2 gene loci are closely spaced in chromosome 15, and are, therefore, linked and inherited together at a high frequency. The experimental value of 4.4% observed by Fan et al. (7), is anticipated and indicates the biological value for that particular mouse strain. Therefore, interpretation of embryonic lethality in HO mice based on an incorrect expectation of 25% by Fan et al. (7) is seriously flawed.

because of differences in mouse strain, secondary regulation, specific chromosome structures, and interference at crossover sites. Expectation of 25% HO mice based on classical Mendelian principles is not applicable in this context, and the interpretation made on this basis is highly inaccurate.

To explain their proposed case of embryonic lethality, Fan et al. (7) proposed that the Amhr2Δ/Δ knock-in mice used to generate gonadal cell type-specific conditional deletions in more than 90 publications (MGI ID: 3042214), in their particular case, could induce global Tspo deletions. The justification provided was published microarray datasets that seemed to detect (with inconsistencies) an increase in Amhr2 transcription in 2-cell embryos. There was no primary data in the manuscript validating this assertion that appears highly unlikely. Even if it were to occur, global Tspo deletions would not affect the linkage and rate of HO mice as described above. In previous studies that used cre on different chromosomes, Mendelian ratios were observed during generation of viable global TspoΔ/Δ mice with similar mouse backgrounds (2, 3).

TSPO IS NOT INVOLVED IN STEROIDOGENESIS

In Fan et al. (7), the Nr5a1Δ/nTspoΔ/Δ mice showed expected Tspo deletions in Leydig cells and the adrenal cortex. Testosterone production (both baseline and after induction using hCG) was not affected, consistent with the previous report (1). Although the authors note this as “surprising,” the significance of this observation as indication that TSPO was not involved in mitochondrial cholesterol import in Leydig cells was disregarded. It is not our intention to criticize, but we are indeed under obligation to point out that the proclaimed landmark in vitro studies of Tspo disruption (10) and Tspo knockdown (11) used as foundations for asserting TSPO link to steroidogenesis were performed only using Leydig cells by this same research group. These in vitro results have since not been reproducible both in vitro (2, 4) and in vivo (1, 2) and is now also invalidated by Fan et al. (7) without an explanation.

In Nr5a1Δ/nTspoΔ/Δ mice, baseline corticosterone levels were also unaffected, consistent with the previous report (2). However, when induced with ACTH, circulating corticosterone did not increase in both heterozygous (TspoΔ/+Δ) and homozygous (TspoΔ/ΔΔ) deletions of Tspo compared to TspoΔ/+ controls, an...
Observation that had no correlation to TSPO expression levels and was in contrast to the previous report (2). This inconsistency and differences observed with regard to lipid accumulation and changes to Lhgr and Scarb1 expression in Nr5a1−/−Tspo+/− adrenals (7) may be linked to parallel findings showing that TSPO can affect lipid metabolism in cells (12). Although additional work is necessary, by providing explanations based on the unsubstantiated conjecture that TSPO is an essential cholesterol transport protein for steroidogenesis, the authors have missed an excellent opportunity to advance understanding of TSPO function.

**CONCLUDING REMARKS**

Global Tspo−/− mice are viable (2, 3) and are an excellent tool for investigating TSPO function in health and disease.

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