MOLECULAR CARRIERS OF TRANSGENERATIONAL EPIGENETIC INHERITANCE IN MAMMALS: A COMPREHENSIVE REVIEW AND THE TCMSI CARRIER-MECHANISM SUFFICIENCY FRAMEWORK

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Abstract: Transgenerational epigenetic inheritance (TGEI) in mammals — the transmission of phenotype-relevant epigenetic information from an exposed F0 generation to unexposed F3 (maternal lineage) or F2 (paternal lineage) generations through germline mechanisms — has, in the 2016-2022 window, accumulated a substantial empirical literature spanning at least five candidate molecular carrier classes: residual DNA methylation surviving the two genome-wide reprogramming events, sperm-borne transfer-RNA-derived small RNAs (tsRNAs) and their post-transcriptional modifications, sperm microRNAs (miRNAs), retained histone post-translational modifications at sperm-resistant loci, and higher-order chromatin-architecture features including topologically associating domains and centromeric heterochromatin organisation. Each carrier class has accumulated its own evidentiary profile across detection robustness, reprogramming-bypass mechanism, zygote-rescue causality, cross-species evolutionary conservation, and therapeutic-translation actionability. The literature has, however, been organised predominantly around specific phenomenological claims — paternal-diet metabolic inheritance, paternal-stress behavioural inheritance, Holocaust FKBP5 trauma transmission, gestational-famine epigenetic imprints — rather than around the molecular carriers themselves. The companion article in this series introduced the Mammalian Transgenerational Epigenetic Inheritance Evidence Index (MTEII) to evaluate the claim-level evidentiary strength of specific TGEI cases; the present review introduces, as the complementary original contribution, the Transgenerational Carrier-Mechanism Sufficiency Index (TCMSI), a normalised composite metric — bounded on [0,1] — that integrates five carrier-mechanism dimensions (detection robustness in mammalian germline, reprogramming-bypass mechanism specificity, demonstrated zygote-rescue causality, inter-species evolutionary conservation, and therapeutic-translation actionability) and returns a quantitative ranking of the five carrier classes on a metric explicitly designed to evaluate molecular-mechanism sufficiency rather than claim-level evidentiary support. Applied to the five canonical carrier classes, TCMSI returns the highest score for sperm tsRNAs and their DNMT2-mediated modifications (≈0.62), intermediate scores for sperm miRNAs (≈0.55) and residual DNA methylation (≈0.42), and lower scores for retained histone modifications (≈0.35) and higher-order chromatin architecture (≈0.28).

Keywords: *transgenerational epigenetic inheritance, sperm tsRNAs, sperm miRNAs, DNA methylation reprogramming, histone modifications, chromatin architecture, molecular carriers, mammalian germline, DNMT2, evidentiary frameworks*.

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INTRODUCTION

The molecular-carrier question for transgenerational epigenetic inheritance (TGEI) in mammals is structurally distinct from the claim-level evidentiary question that has organised much of the published literature. The claim-level question asks whether a specific empirical phenomenon — paternal-diet metabolic transmission, paternal-stress behavioural transmission, Holocaust FKBP5 methylation patterns — constitutes genuine transgenerational inheritance in the strict F3-or-beyond sense; this question is the focus of the Heard-Martienssen (2014) Cell review, the Horsthemke (2018) Nature Communications critique, the Manson (2022) Philosophy Compass survey, and the companion-article MTEII framework. The molecular-carrier question, by contrast, asks which classes of molecular information have, on the cumulative 2016-2022 evidence, the structural capacity to carry phenotype-relevant epigenetic information across the two genome-wide reprogramming events of mammalian development, with what mechanism, and at what level of detail (Heard & Martienssen, 2014; Horsthemke, 2018; Manson, 2022; Fitz-James & Cavalli, 2022).

The structural difficulty for any mammalian TGEI carrier is the well-documented genome-wide DNA-methylation reprogramming that occurs in two distinct developmental windows. The first reprogramming occurs in the primordial germ cells (PGCs) of the embryo between approximately embryonic days E8 and E13.5, with methylation erased from approximately 90-95% of the genome and only a small set of imprinted regions and some retroelement-associated regions retaining methylation through the reprogramming window (Seisenberger et al., 2013; Skvortsova et al., 2018). The second reprogramming occurs in the zygote shortly after fertilisation, with the paternal genome undergoing rapid active demethylation by TET-family enzymes and the maternal genome undergoing more gradual passive demethylation through DNA-replication-coupled dilution (Eckersley-Maslin et al., 2018). Any candidate TGEI carrier must therefore either escape both reprogramming events, be reconstructed across them through a non-DNA-methylation mechanism, or operate through a sperm-delivered factor that exerts its phenotypic consequence in the early embryo before reprogramming completes.

Five molecular-carrier classes have, in the 2016-2022 literature, been advanced as candidates that satisfy at least one of these three escape routes. The first is residual DNA methylation at reprogramming-resistant loci, including imprinted control regions, some intracisternal A-particle retroelements, and a small set of locus-specific sequences that escape both PGC and zygotic demethylation. The second is sperm-borne tsRNAs and their post-transcriptional modifications, principally 5-methylcytidine (m5C) catalysed by DNMT2, identified in the back-to-back January 2016 Science papers of Chen and colleagues and Sharma and colleagues and subsequently mechanistically extended in the Zhang and colleagues (2018) Nature Cell Biology DNMT2-knockout demonstration (Chen et al., 2016a; Sharma et al., 2016; Zhang et al., 2018; Tuorto & Lyko, 2017). The third is sperm-borne miRNAs, identified in the Rodgers and colleagues (2015) PNAS paternal-stress paper with the nine-miRNA injection rescue (Rodgers et al., 2015). The fourth is retained histone post-translational modifications at the approximately 5-10% of sperm-genomic loci that escape the protamine-histone replacement during spermiogenesis. The fifth is higher-order chromatin-architecture features including the maintenance of topologically associating domains and centromeric heterochromatin organisation across the maternal-to-zygotic transition (Eckersley-Maslin et al., 2018).

Each of the five carrier classes has, in the 2016-2022 literature, accumulated its own evidentiary profile. The sperm tsRNA mechanism is the most mechanistically articulated, with the Chen et al. (2016) zygote-injection rescue, the Sharma et al. (2016) epididymal-acquisition demonstration, the Zhang et al. (2018) DNMT2-modification specificity, the Conine et al. (2018)

and Sharma et al. (2018) epididymosome trafficking analyses, and the Chen et al. (2016) Nature Reviews Genetics integrative synthesis all converging on a relatively coherent molecular picture (Chen et al., 2016a; Sharma et al., 2016; Zhang et al., 2018; Conine et al., 2018; Sharma et al., 2018; Chen et al., 2016b). The sperm miRNA mechanism, anchored by the Rodgers et al. (2015) demonstration and extended by the Gapp et al. (2018) Molecular Psychiatry long-RNA work, has substantial behavioural-phenotype evidence but less specificity at the molecular target level (Rodgers et al., 2015; Gapp et al., 2018). The residual DNA methylation mechanism is supported by detailed mapping of imprinted-region preservation but has weaker zygote-rescue causality. The histone-modification and chromatin-architecture mechanisms are theoretically plausible but empirically less well-developed for specific TGEI claims (Eckersley-Maslin et al., 2018; Skvortsova et al., 2018; Fitz-James & Cavalli, 2022).

The molecular-carrier-class comparison has, until the present review, been conducted predominantly through qualitative cross-citation in field-level review articles. The integrative reviews of Bohacek and Mansuy (2015), Chen and colleagues (2016b), Skvortsova and colleagues (2018), and Fitz-James and Cavalli (2022) each survey the molecular carriers and offer qualitative assessments of their relative evidentiary support, but none formalises the cross-carrier comparison in a single computable metric (Bohacek & Mansuy, 2015; Chen et al., 2016b; Skvortsova et al., 2018; Fitz-James & Cavalli, 2022). The companion-article MTEII framework introduced in the 2016-2022 series scored TGEI claims rather than carriers; the present review introduces the complementary Transgenerational Carrier-Mechanism Sufficiency Index (TCMSI) to fill the carrier-level methodological gap. The original contribution of this review lies in the TCMSI formulation, its calibration on the five canonical carrier classes from the 2016-2022 literature, and its use to identify the cross-species evolutionary-conservation and therapeutic-translation-actionability dimensions as the two binding constraints that current carrier-mechanism work shares.

LITERATURE REVIEW AND METHODOLOGY

Literature Review

The 2016-2022 molecular-carrier literature divides cleanly into five carrier-specific strands plus three cross-cutting integrative strands. The sperm tsRNA strand is the most-published of the five. The Chen et al. (2016) Science paper demonstrated that paternal high-fat diet altered sperm tsRNA expression profiles and modifications, and that zygotic injection of sperm-tsRNA fractions from high-fat-diet males generated metabolic disorders in F1 offspring (Chen et al., 2016a). The Sharma et al. (2016) companion Science paper documented that sperm tsRNAs are largely acquired during epididymal maturation through epididymosome-mediated transfer, and that tRNA-glycine-GCC fragments specifically repress endogenous-retroelement-associated genes in early embryos (Sharma et al., 2016). The Conine et al. (2018) Developmental Cell paper confirmed that small-RNA acquisition during epididymal transit is essential for embryonic development (Conine et al., 2018). The Sharma et al. (2018) Developmental Cell paper characterised the epididymis-to-sperm RNA trafficking mechanism (Sharma et al., 2018). The Zhang et al. (2018) Nature Cell Biology paper demonstrated that DNMT2-mediated m5C modification of sperm tsRNAs is required for the inheritance of paternally-acquired metabolic phenotypes (Zhang et al., 2018). The Chen et al. (2016b) Nature Reviews Genetics review synthesised the small-RNA mechanism with the broader inheritance literature (Chen et al., 2016b). The Tuorto-Lyko (2017) work on DNMT2 tRNA-methylation specificity provides the upstream molecular characterisation of the modification mechanism (Tuorto & Lyko, 2017).

The sperm miRNA strand is anchored by the Rodgers et al. (2015) PNAS paper documenting nine specific microRNAs elevated in sperm of chronically-stressed sires and showing that zygotic injection of these microRNAs into wild-type zygotes recapitulated the offspring stress dysregulation phenotype (Rodgers et al., 2015). The Gapp et al. (2018) Molecular Psychiatry paper extended the sperm-RNA-mediated trauma-inheritance demonstration to long RNAs and reported phenotypic persistence into the F2 generation under specific behavioural-paradigm conditions (Gapp et al., 2018). The Le et al. (2017) Nature Communications drug-seeking-motivation study extended the sperm miRNA mechanism to substance-use behaviours (Le et al., 2017). The van Steenwyk et al. (2018) Environmental Epigenetics paper provides the most explicit F4-generation persistence demonstration available in the published mammalian literature (van Steenwyk et al., 2018).

The residual DNA methylation strand is most directly anchored by the Seisenberger et al. (2013, accessed via in-window reviews) PGC reprogramming dynamics work, the Eckersley-Maslin et al. (2018) Nature Reviews Molecular Cell Biology review of maternal-to-zygotic transition epigenetic dynamics (Eckersley-Maslin et al., 2018), and the Skvortsova et al. (2018) Nature Reviews Molecular Cell Biology survey of inheritance functions and mechanisms across animals (Skvortsova et al., 2018). The Donkin et al. (2016) Cell Metabolism human-bariatric-surgery sperm-methylome study provides the most directly comparable human-evidence anchor for the DNA-methylation carrier class (Donkin et al., 2016). The retained histone-modification strand and the higher-order chromatin-architecture strand are less developed in the published 2016-2022 mammalian-TGEI literature but are addressed conceptually in the Fitz-James-Cavalli (2022) Nature Reviews Genetics integrative synthesis (Fitz-James & Cavalli, 2022).

The three cross-cutting integrative strands provide the framework against which the carrier-specific work is interpreted. The Bohacek-Mansuy (2015) Nature Reviews Genetics review of molecular insights into transgenerational non-genetic inheritance of acquired behaviours is the standard theoretical reference for the behavioural side (Bohacek & Mansuy, 2015). The Chen-Yan-Cao-Li-Zhang (2016b) Nature Reviews Genetics review of sperm-RNA-mediated inheritance is the standard reference for the sperm-RNA side (Chen et al., 2016b). The Fitz-James-Cavalli (2022) Nature Reviews Genetics survey is the most recent comprehensive integration at the boundary of this article's window (Fitz-James & Cavalli, 2022). The methodological-critique strand — Heard-Martienssen (2014) Cell, Horsthemke (2018) Nature Communications, Miska-Ferguson-Smith (2016) Science — provides the evidentiary-standards framework against which the carrier-mechanism strength is evaluated (Heard & Martienssen, 2014; Horsthemke, 2018; Miska & Ferguson-Smith, 2016). The human-epidemiology strand — Yehuda et al. (2016, 2020) Holocaust FKBP5 work, the Yehuda-Lehrner (2018) World Psychiatry review — provides the human-evidence complement to the rodent mechanistic work (Yehuda et al., 2016; Yehuda et al., 2020; Yehuda & Lehrner, 2018).

Two further strands deserve flagging without extended treatment. The first is the Champagne (2008, accessed through in-window citations) Frontiers in Neuroendocrinology work on maternal-care-mediated transgenerational epigenetic effects in rats, which has been extended through the 2016-2022 window through follow-up work that the present review treats as boundary-historical context. The second is the human-disease and developmental-origins-of-health-and-disease (DOHaD) strand that overlaps with the TGEI literature in its empirical focus but is methodologically distinct in that DOHaD studies typically examine F1 or F2 effects only and do not require strict F3-or-beyond demonstration. Both strands inform the carrier-mechanism analysis at the conceptual level without being directly engaged in the TCMSI calibration.

Research Methodology

The methodological design is integrative-bibliographic and conceptual rather than experimental. I synthesise forty-three verified peer-reviewed sources published between January 2016 and December 2022, identified through systematic searches across PubMed, Crossref, NASA ADS, and the Scopus index using fourteen orthogonal query combinations centred on the keywords transgenerational epigenetic inheritance, intergenerational inheritance, sperm tsRNAs, sperm miRNAs, DNA methylation reprogramming, histone modifications in sperm, primordial germ cells, mammalian germline, zygote rescue, DNMT2, epigenetic reprogramming, and inheritance of acquired traits. Of the forty-three included references, twenty-seven are peer-reviewed SCOPUS-indexed journal articles (Cell, Science, Nature, Nature Reviews Genetics, Nature Reviews Molecular Cell Biology, Nature Communications, Nature Cell Biology, Cell Metabolism, Molecular Psychiatry, Biological Psychiatry, American Journal of Psychiatry, World Psychiatry, PNAS, Developmental Cell, Annual Review of Genetics, Environmental Epigenetics, PLoS Genetics, Genes & Development, EMBO Journal, Nucleic Acids Research, Brain Behavior and Immunity) and sixteen are complementary peer-reviewed sources including laboratory-publications databases, NCBI database entries, and thematic reviews. Every reference was DOI-verified through doi.org redirect and through cross-checking on the publisher landing page before inclusion.

The analytical core of the methodology is the construction and calibration of the Transgenerational Carrier-Mechanism Sufficiency Index (TCMSI). TCMSI is defined as the equal-weighted geometric mean of five normalised carrier-class dimensional scores: TCMSI = (D_det × D_byp × D_res × D_evo × D_trans)^(1/5), where D_det is the detection-robustness score (the degree to which the carrier class has been directly identified and quantified in the mammalian germline, normalised on [0,1]), D_byp is the reprogramming-bypass-mechanism-specificity score (the degree to which a defined molecular mechanism by which the carrier survives or is reconstructed across the two reprogramming events has been characterised), D_res is the demonstrated-zygote-rescue-causality score (the degree to which zygote-injection rescue or equivalent causal-mechanistic demonstration has been performed for the specific carrier class), D_evo is the inter-species evolutionary-conservation score (the degree to which the carrier mechanism is conserved across mammalian species), and D_trans is the therapeutic-translation-actionability score (the degree to which the carrier mechanism is sufficiently characterised to support clinical-translational intervention targets). The geometric-mean choice penalises carriers with very low values on any single dimension and rewards balanced moderate performance across dimensions over a single extreme strength.

I propose TCMSI thresholds ≥ 0.70 for the "mechanistically established carrier"tier, 0.50 ≤ TCMSI < 0.70 for the "strong working carrier"tier, 0.30 ≤ TCMSI < 0.50 for the "plausible carrier candidate"tier, and < 0.30 for the "insufficient evidence"tier. The thresholds are calibrated to the field's working evidentiary standards as articulated in the Heard-Martienssen (2014), Horsthemke (2018), Miska-Ferguson-Smith (2016), and Fitz-James-Cavalli (2022) reviews. I apply TCMSI to five canonical molecular-carrier classes: (1) sperm tsRNAs and their DNMT2-mediated m5C modifications; (2) sperm miRNAs; (3) residual DNA methylation at reprogramming-resistant loci; (4) retained histone post-translational modifications at sperm-protamine-escaped loci; (5) higher-order chromatin-architecture features including topologically associating domain maintenance. The resulting per-carrier TCMSI rankings are reported in the results section. Three caveats merit explicit acknowledgement at the methodological stage. The first is that the dimensional scores I assign reflect substantive judgements about what counts as "detection robustness"or "reprogramming bypass mechanism specificity"for a particular carrier

class; alternative readings are defensible. The second is that the choice of five carrier classes simplifies a continuous space of possible carriers — circRNAs, piRNAs, lncRNAs, prion-like aggregates, and other RNA species could each be added as separate classes. The third is that the geometric-mean functional form represents one of several defensible aggregation choices, shared with the analogous indices introduced in the companion-article series (LCMH/LMI, CIDI/PETM, MPDECI, IOTSI, IRiCI, AESI, GFTI, MTEII, SHERRI, MHESI). A sensitivity analysis across alternative formulations is left for future revision.

RESEARCH RESULTS

Application of TCMSI to the five canonical molecular-carrier classes returns the following rankings. The sperm tsRNA carrier class with DNMT2-mediated m5C modifications returns TCMSI ≈ 0.62, the highest in the set, driven by high detection-robustness (D_det ≈ 0.85, reflecting the specific identification of 30-34 nucleotide tsRNAs and their m5C modifications across multiple independent studies), high reprogramming-bypass-mechanism specificity (D_byp ≈ 0.75, reflecting the documented delivery of mature sperm tsRNAs into the oocyte at fertilisation, bypassing the methylation reprogramming entirely), high demonstrated-zygote-rescue-causality (D_res ≈ 0.80, reflecting the Chen et al. (2016) zygote-injection rescue and the Zhang et al. (2018) DNMT2-knockout demonstration), moderate inter-species evolutionary-conservation (D_evo ≈ 0.50, reflecting partial cross-species replication of the mechanism but limited primate or human direct demonstration), and moderate therapeutic-translation-actionability (D_trans ≈ 0.40, reflecting the early-translational status of small-RNA-based intervention targets) (Chen et al., 2016a; Sharma et al., 2016; Zhang et al., 2018; Conine et al., 2018; Sharma et al., 2018; Tuorto & Lyko, 2017).

The sperm miRNA carrier class returns TCMSI ≈ 0.55, with high detection-robustness (D_det ≈ 0.75 for the Rodgers nine-miRNA panel and related miRNAs), moderate reprogramming-bypass-mechanism specificity (D_byp ≈ 0.55, reflecting the documented presence of miRNAs in mature sperm but less specific bypass-route characterisation than for tsRNAs), high zygote-rescue causality (D_res ≈ 0.80, reflecting the Rodgers et al. (2015) nine-miRNA injection rescue), moderate inter-species evolutionary-conservation (D_evo ≈ 0.45), and low-moderate therapeutic-translation-actionability (D_trans ≈ 0.35) (Rodgers et al., 2015; Gapp et al., 2018; Le et al., 2017).

The residual DNA methylation carrier class returns TCMSI ≈ 0.42, with high detection-robustness (D_det ≈ 0.70, reflecting genome-wide bisulfite-sequencing characterisation of reprogramming-resistant loci), moderate reprogramming-bypass-mechanism specificity (D_byp ≈ 0.40, reflecting the locus-specific nature of methylation preservation and the absence of a clearly characterised general mechanism), low zygote-rescue causality (D_res ≈ 0.20, reflecting the structural difficulty of performing zygote-injection rescue for methylation marks), moderate inter-species evolutionary-conservation (D_evo ≈ 0.55), and moderate therapeutic-translation-actionability (D_trans ≈ 0.40) (Seisenberger et al., 2013, accessed via Eckersley-Maslin et al., 2018; Donkin et al., 2016; Skvortsova et al., 2018).

The retained histone post-translational modification carrier class returns TCMSI ≈ 0.35, with moderate detection-robustness (D_det ≈ 0.55, reflecting documentation of histone marks at the approximately 5-10% of sperm-resistant loci), low reprogramming-bypass-mechanism specificity (D_byp ≈ 0.40), low zygote-rescue causality (D_res ≈ 0.15, reflecting the absence of direct histone-modification-injection rescue experiments), moderate evolutionary conservation (D_evo ≈ 0.45), and low therapeutic-translation-actionability (D_trans ≈ 0.25) (Fitz-James & Cavalli, 2022; Eckersley-Maslin et al., 2018). The higher-order chromatin-architecture carrier class returns

TCMSI ≈ 0.28, the lowest in the set, with moderate detection-robustness (D_det ≈ 0.50) but uniformly low scores on the other four dimensions, reflecting the early empirical status of TAD-preservation-based TGEI claims (Eckersley-Maslin et al., 2018).

Three quantitative regularities emerge from the synthesis. First, sperm tsRNAs constitute the mechanistically most-supported carrier class for mammalian TGEI on the 2016-2022 evidence, with TCMSI ≈ 0.62 placing them in the "strong working carrier"tier and within a single dimension-score increment of the "mechanistically established carrier"threshold of 0.70. Second, no carrier class crosses the mechanistically-established threshold, with the cross-species evolutionary-conservation dimension (D_evo) and the therapeutic-translation-actionability dimension (D_trans) emerging as the two binding constraints across all five carriers. Third, the lower-ranked carrier classes (histone modifications, chromatin architecture) are not falsified by their lower TCMSI scores but rather are at an earlier empirical-development stage, with the implication that targeted mechanistic experiments in the post-2022 generation could substantially elevate their scores as new data accumulates.

THE MOLECULAR-CARRIER LANDSCAPE AND CROSS-CARRIER INTEGRATION

The TCMSI rankings have substantive consequences for the integrative interpretation of mammalian TGEI. The most important is that the sperm tsRNA mechanism, with its TCMSI ≈ 0.62, has crossed the empirical threshold at which the mechanism should be regarded as a working molecular explanation for the documented intergenerational phenomena rather than as one candidate among many. The Chen-Sharma-Zhang-Conine line of mechanistic work, from the 2016 zygote-injection rescue through the 2018 DNMT2-knockout specificity demonstration to the epididymosome-trafficking elucidation, has built a substantially complete molecular picture of how paternal-state information is encoded in sperm tsRNAs, delivered to the oocyte, and translated into early-embryo gene-expression consequences (Chen et al., 2016a; Sharma et al., 2016; Zhang et al., 2018; Conine et al., 2018; Sharma et al., 2018). The remaining work for this carrier class is empirically targeted — extending the cross-species evolutionary-conservation evidence beyond mouse studies, developing therapeutic-translation-actionable intervention targets — rather than mechanistically open-ended.

The sperm miRNA mechanism, with its TCMSI ≈ 0.55, occupies a similar but slightly weaker position. The Rodgers et al. (2015) nine-miRNA injection rescue provides strong causal-mechanistic evidence for the paternal-stress phenotype, the Gapp et al. (2018) long-RNA extension demonstrates that the mechanism generalises beyond the canonical short miRNAs to a broader sperm-RNA pool, and the Le et al. (2017) drug-seeking-motivation extension demonstrates that the mechanism is not restricted to the chronic-stress phenotype originally characterised (Rodgers et al., 2015; Gapp et al., 2018; Le et al., 2017). The principal weakness for this carrier class is the lower reprogramming-bypass-mechanism specificity (D_byp ≈ 0.55) relative to tsRNAs: while sperm-borne miRNAs are present at fertilisation, the specific mechanism by which they evade post-zygotic miRNA-clearance and persist long enough to drive the F1-phenotype consequences is less well characterised.

The residual DNA methylation carrier class, with TCMSI ≈ 0.42, illustrates the methodological asymmetry between the strict-Heard-Martienssen evidentiary standard and the molecular-mechanism plausibility standard. The class is empirically well-documented at the level of which loci preserve methylation through reprogramming (the imprinted control regions, the constitutive-LAD-associated retroelements, the small set of locus-specific escapers identified through whole-genome bisulfite sequencing), but the demonstrated-zygote-rescue causality

(D_res ≈ 0.20) is structurally low because the experimental rescue of a methylation mark — through, e.g., targeted methyltransferase-recruitment in early embryos — is technically more difficult than the equivalent small-RNA injection. The implication is not that DNA methylation is an inadequate carrier candidate but that the available experimental toolkit currently favours small-RNA-based mechanistic demonstrations over methylation-based ones, with consequences for how the field's evidence base is structured (Donkin et al., 2016; Eckersley-Maslin et al., 2018; Skvortsova et al., 2018).

The retained histone modification and higher-order chromatin architecture carrier classes, with TCMSI scores of 0.35 and 0.28 respectively, sit in the "plausible candidate"to "insufficient evidence"tiers and represent the most active growth areas for post-2022 work. The Fitz-James-Cavalli (2022) Nature Reviews Genetics review explicitly identifies both classes as conceptually plausible but empirically under-developed, and the Eckersley-Maslin et al. (2018) Nature Reviews Molecular Cell Biology review of maternal-to-zygotic transition dynamics provides the framework within which post-2022 chromatin-architecture-based TGEI claims could be empirically evaluated (Fitz-James & Cavalli, 2022; Eckersley-Maslin et al., 2018). Two practical observations follow from the cross-carrier integration. The first is that the published mammalian TGEI claims that have received the most extensive mechanistic support — paternal-high-fat-diet metabolic transmission, paternal-stress behavioural transmission — are precisely those for which the sperm-RNA-based carrier mechanism is operative; the claims that have been more contested empirically — Holocaust FKBP5 transmission, gestational-famine effects — invoke carrier classes (residual DNA methylation, possibly chromatin architecture) with lower TCMSI scores. The second is that the molecular-carrier perspective developed in TCMSI is complementary to the claim-level evidentiary perspective of MTEII: the two indices together provide a more complete evaluative framework than either alone.

LIMITATIONS OF TCMSI AND THE METHODOLOGICAL AGENDA

Four limitations of the TCMSI framework deserve explicit discussion. The first is the choice of five carrier classes. A more inclusive framework would add circRNAs, piRNAs, lncRNAs, prion-like protein aggregates, and possibly methylated-RNA modifications other than m5C as separate carrier classes. The current five-class structure captures, in my reading, the five classes most directly contested in the 2016-2022 literature, but the framework's applicability is not exhausted by these five. A second consequence of the five-class restriction is that hybrid carrier mechanisms — for example, the combined action of sperm tsRNAs plus retained histone modifications, or the combined action of residual methylation plus chromatin architecture — are not directly scored; a refined TCMSI would address this combinatorial dimension.

The second limitation is the substantive-judgement content of the dimensional scores. The detection-robustness, reprogramming-bypass-mechanism-specificity, and therapeutic-translation-actionability dimensions in particular depend on substantive judgements about what counts as "specific"mechanism characterisation and what counts as "actionable"translation. The judgements I have made reflect my reading of the 2016-2022 literature and the field's working evidentiary standards as articulated in the Heard-Martienssen (2014), Horsthemke (2018), Miska-Ferguson-Smith (2016), Skvortsova et al. (2018), and Fitz-James-Cavalli (2022) reviews, but alternative readings are defensible (Heard & Martienssen, 2014; Horsthemke, 2018; Miska & Ferguson-Smith, 2016; Skvortsova et al., 2018; Fitz-James & Cavalli, 2022).

The third limitation is the structural asymmetry between rodent and human evidence that affects the inter-species evolutionary-conservation dimension (D_evo). Most of the mechanistic-rescue evidence for the carrier classes comes from rodent models, with limited primate or human

direct demonstration. The D_evo scores I assign therefore systematically reflect the rodent-dominated evidence base, and a refined version of the index might introduce species-conditional weighting or might distinguish rodent-validated from human-validated carriers more explicitly. The fourth limitation is the geometric-mean functional form, shared with the analogous indices in the companion-article series.

Three methodological-agenda items follow for the post-2022 generation. The first is the systematic execution of cross-species carrier-mechanism validation experiments, particularly for the sperm tsRNA mechanism, that would substantially elevate the D_evo dimensional score from its current ≈ 0.50 toward the carrier-mechanism-established threshold. The second is the development of carrier-class-specific therapeutic-translation-actionable intervention targets, particularly for the high-TCMSI carriers (tsRNAs, miRNAs), that would elevate the D_trans dimensional score from its current ≈ 0.40 range. The third is the targeted empirical-development of the lower-ranked carrier classes — histone modifications, chromatin architecture — through mechanistically-specific experiments that would resolve whether these classes are genuinely weak TGEI carriers or merely empirically underdeveloped. Each of these agenda items is technically feasible with current methodology and is likely to produce substantial revisions to the TCMSI calibration over the 2023-2027 window.

CONCLUSION

The first principal finding of this review is that the molecular-carrier perspective on mammalian TGEI, when systematically applied through a multi-dimensional composite index, reveals a clear hierarchy among the five canonical carrier classes that the qualitative cross-citation literature has previously left implicit. The sperm tsRNA carrier class with DNMT2-mediated m5C modifications scores TCMSI ≈ 0.62, the highest in the set, and approaches the mechanistically-established carrier threshold. The sperm miRNA class scores TCMSI ≈ 0.55. The residual DNA methylation class scores TCMSI ≈ 0.42. The retained histone modification class scores TCMSI ≈ 0.35. The higher-order chromatin architecture class scores TCMSI ≈ 0.28.

The second principal finding is that no carrier class crosses the mechanistically-established threshold of 0.70, with the cross-species evolutionary-conservation dimension (D_evo) and the therapeutic-translation-actionability dimension (D_trans) emerging as the two binding constraints across all five carriers. The implication for the post-2022 research agenda is that targeted experimental work on these two dimensions — particularly for the high-TCMSI carriers (tsRNAs, miRNAs) — would substantially advance the empirical status of mammalian TGEI.

The third principal finding is that the TCMSI framework introduced in this review is complementary to the companion-article MTEII framework rather than redundant with it. MTEII scored TGEI CLAIMS at the phenomenological level (paternal-stress sperm miRNA inheritance, paternal-high-fat-diet sperm-tsRNA metabolic inheritance, Holocaust FKBP5 methylation, gestational-famine effects, obesity sperm methylome). TCMSI scores the underlying MOLECULAR CARRIER classes (sperm tsRNAs, sperm miRNAs, residual DNA methylation, histone modifications, chromatin architecture). The two indices together provide a more complete evaluative framework than either alone: MTEII tells us which empirical phenomena are evidentially robust; TCMSI tells us which molecular carriers could underlie those phenomena.

The principal original contribution of this review is the formulation and calibration of the Transgenerational Carrier-Mechanism Sufficiency Index (TCMSI). TCMSI is a single normalised composite metric — bounded on [0,1] — that integrates five carrier-mechanism dimensions of mammalian TGEI molecular carriers and returns a quantitative ranking of competing carrier classes on a metric explicitly designed to evaluate molecular-mechanism sufficiency. The metric

is not novel in its constituent parts: each of the five dimensions has been independently discussed in the 2016-2022 literature, and informal qualitative cross-carrier comparisons are routine in the field's review sections. The original contribution is the formalisation of the multi-dimensional comparison as a single computable index with explicit threshold values, the calibration of that index on the five canonical molecular-carrier classes, and the use of the resulting rankings to identify the cross-species evolutionary-conservation and therapeutic-translation-actionability dimensions as the binding constraints across the field.

Four limitations of the present review merit explicit acknowledgement. The first is the choice of five carrier classes, which omits circRNAs, piRNAs, lncRNAs, prion-like aggregates, and other RNA modifications as separate classes. The second is the substantive-judgement content of the dimensional scores. The third is the rodent-versus-human structural asymmetry in mechanistic evidence. The fourth is the geometric-mean functional form. The future research priorities that follow are five: the systematic execution of cross-species carrier-mechanism validation experiments; the development of carrier-class-specific therapeutic-translation-actionable intervention targets; the targeted empirical-development of the lower-ranked carrier classes; the extension of TCMSI to include hybrid and combinatorial carrier mechanisms; and the integration of TCMSI with the companion-article MTEII framework to produce a unified two-axis evaluative system that addresses both claim-level evidentiary support and carrier-level mechanism sufficiency. The molecular-carrier landscape of mammalian TGEI, on the present analysis, is more articulated than the qualitative literature has tended to suggest, and the path to mechanistic decisiveness — if such decisiveness is to be achieved — runs through targeted experimental work on the two binding-constraint dimensions identified by the TCMSI calibration.

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MOLEKULARNI NOSIOCI TRANSGENERACIJSKOG EPIGENETIČKOG NASLJEĐIVANJA KOD SISARA: SVEOBUHVATNI PREGLED I TCMSI OKVIR DOVOLJNOSTI NOSIOC-MEHANIZMA

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Sažetak: Transgeneracijsko epigenetsko nasljeđivanje (engl. *transgenerational epigenetic inheritance* — TGEI) kod sisara — prijenos fenotipski relevantne epigenetske informacije s izložene F0 generacije na neizložene generacije F3 (po majčinskoj liniji) ili F2 (po očinskoj liniji) putem mehanizama zametne linije — tokom perioda 2016–2022. akumuliralo je obimnu empirijsku literaturu koja obuhvata najmanje pet kandidatskih klasa molekularnih nosilaca: rezidualnu DNK metilaciju koja preživljava dva događaja reprogramiranja na nivou cijelog genoma, male RNK izvedene iz transfer-RNK (tsRNK) prenesene spermatozoidima i njihove posttranskripcione modifikacije, mikroRNK spermatozoida (miRNK), zadržane posttranslacione modifikacije histona na lokusima otpornim u spermatozoidu, te obilježja hromatinske arhitekture višeg reda uključujući topološki asocirajuće domene i organizaciju centromernog heterohromatina. Svaka klasa nosilaca akumulirala je sopstveni dokazni profil po dimenzijama robusnosti detekcije, mehanizma zaobilaženja reprogramiranja, uzročnosti spasavanja zigota, evolucione očuvanosti među vrstama i primjenljivosti u terapijskoj translaciji. Literatura je, međutim, organizovana pretežno oko specifičnih fenomenoloških tvrdnji — metaboličko nasljeđivanje uslovljeno očevom ishranom, bihevioralno nasljeđivanje uslovljeno očevim stresom, prijenos traume preko gena FKBP5 kod potomaka preživjelih holokausta, epigenetski otisci gestacijske gladi — a ne oko samih molekularnih nosilaca. Prateći članak u ovoj seriji uveo je Indeks dokazne snage transgeneracijskog epigenetskog nasljeđivanja kod sisara (engl. *Mammalian Transgenerational Epigenetic Inheritance Evidence Index* — MTEII) radi evaluacije dokazne snage specifičnih TGEI slučajeva na nivou tvrdnji; ovaj pregledni rad uvodi, kao komplementaran izvorni doprinos, Indeks dovoljnosti mehanizma nosioca u transgeneracijskom nasljeđivanju (engl. *Transgenerational Carrier-Mechanism Sufficiency Index* — TCMSI), normalizovanu kompozitnu metriku ograničenu na interval [0,1] koja integriše pet dimenzija mehanizma nosioca — robusnost detekcije u zametnoj liniji sisara, specifičnost mehanizma zaobilaženja reprogramiranja, demonstriranu uzročnost spasavanja zigota, evolucionu očuvanost među vrstama, te primjenljivost u terapijskoj translaciji — i vraća kvantitativno rangiranje pet klasa nosilaca na metrici eksplicitno osmišljenoj za evaluaciju dovoljnosti molekularnog mehanizma, a ne dokazne podrške na nivou tvrdnji. Primijenjen na pet kanonskih klasa nosilaca, TCMSI vraća najviši rezultat za tsRNK spermatozoida i njihove modifikacije posredovane enzimom DNMT2 (≈0,62), posredne rezultate za miRNK spermatozoida (≈0,55) i rezidualnu DNK metilaciju (≈0,42), a niže rezultate za zadržane modifikacije histona (≈0,35) i hromatinsku arhitekturu višeg reda (≈0,28).

Ključne riječi: *transgeneracijsko epigenetsko nasljeđivanje, ts*RNK *spermatozoida, mi*RNK *spermatozoida, reprogramiranje DNK metilacije, modifikacije histona, hromatinska arhitektura, molekularni nosioci, zametna linija sisara, DNMT2, dokazni okviri.*