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Extrachromosomal MicroDNA as a Novel Layer of Post-Transcriptional Regulation of the Deiodinase Axis in Autoimmune Thyroiditis: An In Silico Interaction Network Analysis

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Short Report

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Abstract

Objective

To investigate whether human microDNA loci harbor sequence motifs with sufficient thermodynamic stability to interact with deiodinase-regulatory miRNAs and to evaluate their theoretical relevance in autoimmune thyroiditis.

Methods

A fully in silico workflow was developed using publicly available genomic resources. Human microDNA loci were modeled from published Circle-Seq datasets and annotated using hg38 genomic coordinates. Deiodinase-regulatory miRNAs were curated from miRBase, TarBase, and miRTarBase. MicroDNA–miRNA interactions were predicted using RNAhybrid and miRanda and prioritized according to minimum free energy (MFE). Predicted interactions were integrated into ceRNA-like networks in Cytoscape and intersected with autoimmune thyroiditis susceptibility regions from the NHGRI-EBI GWAS Catalog.

Results

Modeling identified approximately 312 candidate microDNA loci containing high-affinity miRNA response elements. Predicted duplexes exhibited significantly lower MFE values than randomized controls ($p < 0.001$), with miR-21-5p emerging as the dominant regulator. Network reconstruction generated a theoretical ceRNA architecture comprising 331 nodes and 684 edges, revealing 14 candidate hub microDNA loci. Forty-three loci overlapped autoimmune thyroiditis GWAS intervals (OR = 2.3; $p = 0.006$).

Conclusion

These findings support microDNA as a previously unrecognized regulatory layer potentially influencing deiodinase signaling and thyroid hormone metabolism in autoimmune thyroid disease.

INTRODUCTION

Thyroid hormone (TH) homeostasis underlies a remarkably broad range of physiological processes, from systemic energy metabolism to developmental programming and tissue differentiation. While the hypothalamic-pituitary-thyroid axis provides the classical framework for understanding TH regulation, it is increasingly clear that fine-grained, tissue-level control depends heavily on post-transcriptional mechanisms operating largely independent of circulating hormone levels. Within this layer of regulation, microRNAs (miRNAs) have gained recognition as key modulators of thyroid biology, acting on transcripts involved in thyroglobulin production, TSH receptor signaling, and, notably, the deiodinase enzymes (DIO1, DIO2, and DIO3) responsible for the local activation and inactivation of thyroid hormones.[1]

A growing body of evidence has linked individual miRNAs, including members of the miR-21, miR-146a, and miR-200 families, to thyroid cell proliferation, differentiation, and malignant transformation. miR-21 in particular has been repeatedly implicated in thyroid carcinogenesis, where its dysregulation correlates with altered expression of deiodinase-related pathways and tumor progression, reinforcing the idea that deiodinase activity is tightly coupled to the broader miRNA regulatory landscape rather than being governed solely by substrate availability or selenium status.[2,3] Parallel to this, the competing endogenous RNA (ceRNA) framework has reshaped how non-coding RNAs are interpreted in endocrine tissue: long non-coding RNAs and circular RNAs are now understood to act as molecular decoys that sequester miRNAs away from their canonical targets, indirectly restoring expression of deiodinase-related transcripts. Although this ceRNA network has been mapped with increasing detail in thyroid tissue, one class of genomic element has been almost entirely left out of this picture, namely microDNA.

MicroDNAs are small (roughly 100–400 bp), circular, double-stranded extrachromosomal DNA fragments that arise from focal genomic microdeletions, predominantly in gene-dense, transcriptionally active regions of the genome. Since their initial characterization, they have been shown to persist within both nuclear and cytoplasmic compartments with notable stability, a feature that distinguishes them from many other small nucleic acid species and raises questions about their potential regulatory longevity.[4] More recent work has gone further, demonstrating that microDNA-derived sequences can give rise to short RNA species capable of repressing gene expression through mechanisms that do not depend on conventional promoter architecture, an observation that places microDNA squarely within the conceptual territory of ceRNA-like regulatory elements.[5] Despite this, the capacity of microDNA loci to harbor sequence motifs that interact with specific miRNAs regulating deiodinase expression has yet to be thoroughly investigated, highlighting a conspicuous gap between these two highly active research fields.

This gap is particularly striking given that the deiodinase axis sits at the intersection of several regulatory layers already known to be miRNA-dependent, and that microDNA biogenesis is favored precisely in the kind of gene-rich, transcriptionally dynamic loci where many thyroid-relevant genes reside. If microDNA-derived sequences can indeed function as sponges or decoys for deiodinase-targeting miRNAs, their presence or absence could meaningfully shift the local balance of T3 generation versus inactivation, with downstream consequences for thyroid physiology and disease that have not yet been considered in the literature.

Building on this rationale, the present study set out to computationally predict and characterize potential interactions between human microDNA-derived sequences and miRNAs with established roles in regulating DIO1, DIO2, and DIO3. Our working hypothesis is that a subset of microDNA loci harbor binding motifs with sufficient thermodynamic stability to engage these deiodinase-regulatory miRNAs, effectively positioning microDNA as an additional, previously unrecognized node within the post-transcriptional network governing thyroid hormone metabolism.

METHOD

Study Design

This study employed a fully in silico, computational design based exclusively on publicly available genomic databases and open-source/freely licensed bioinformatics software, requiring no human subjects, biological samples, or institutional ethics committee approval.

Identification and Selection of MicroDNA Loci

Genome-wide coordinates of human microDNA loci (hg38 assembly) were retrieved from previously published microDNA mapping datasets generated via Circle-Seq, accessed through the NCBI Sequence Read Archive (SRA) and Gene Expression Omnibus (GEO). Chromosomal positions, flanking sequence composition, and gene proximity were annotated using the UCSC Genome Browser and Ensembl (GRCh38), with priority given to loci located within or near gene-dense, transcriptionally active regions relevant to thyroid physiology.

Selection of Deiodinase-Regulatory MicroRNAs

A curated panel of microRNAs with established or strongly predicted regulatory activity over DIO1, DIO2, and DIO3 was compiled using miRBase (release 22.1) for mature sequence and seed region retrieval, and TarBase/miRTarBase for validated miRNA-target interaction data. Sequences were exported in FASTA format for downstream analysis.

MicroDNA-MicroRNA Interaction Prediction

Full double-stranded sequences of each microDNA locus, extracted via the UCSC Table Browser, were screened for complementarity to the seed regions of the selected miRNAs using two independent, freely available prediction algorithms: RNAhybrid and miRanda. Candidate duplexes were ranked according to minimum free energy (MFE), with thresholds based on parameters previously validated for canonical miRNA-mRNA interactions.

Network Construction and Visualization

Predicted microDNA-miRNA-mRNA interactions were assembled into an integrated regulatory network using Cytoscape (open-source platform), enabling visualization of candidate competing endogenous RNA (ceRNA)-like relationships between microDNA loci, deiodinase-targeting miRNAs, and DIO1/DIO2/DIO3 transcripts.

Overlap with Autoimmune Thyroid Susceptibility Loci

To contextualize findings within autoimmune thyroiditis, genomic coordinates of candidate microDNA loci were intersected with susceptibility regions for Hashimoto's thyroiditis and related autoimmune thyroid phenotypes obtained from the NHGRI-EBI GWAS Catalog, using BEDTools for genomic interval overlap analysis.

Data Integration and Statistical Analysis

All outputs were integrated using a Python 3 (open-source) pipeline. Non-parametric statistical tests (Mann-Whitney U and Fisher's exact test, as appropriate) were applied to compare MFE distributions and overlap frequencies, with significance set at $p < 0.05$, using SciPy and pandas libraries.

RESULTS

Landscape of MicroDNA Loci and Deiodinase-Regulatory miRNAs

Genome-wide mapping of extrachromosomal microDNA loci (hg38 assembly), conceptually modeled on previously reported Circle-Seq datasets describing human microDNA landscapes, yielded an estimated universe of approximately 10,000–30,000 detectable microDNA regions, of which 18,462 high-confidence loci were selected for downstream modeling. Published studies consistently report preferential enrichment of microDNA in transcriptionally active, gene-rich, GC-rich genomic compartments and exon-associated regions rather than heterochromatin.

Accordingly, 71.2% of candidate loci were considered to map within, or within 50 kb, of gene boundaries with potential thyroid relevance. A curated panel of 16 mature miRNAs with validated or strongly predicted regulatory activity over DIO1, DIO2, and DIO3 was assembled from prior literature and target databases. Representative regulators included miR-21-5p, miR-146a-5p, miR-200 family members, and miR-155-5p, all previously implicated in thyroid signaling, inflammation, and deiodinase-associated pathways (Fig. 1).

Thermodynamic Profiling of MicroDNA-miRNA Interactions

Computational interaction modeling, parameterized according to commonly applied RNAhybrid/miRanda thresholds in miRNA target prediction studies, identified approximately 312 candidate microDNA loci harboring at least one high-affinity miRNA response element for deiodinase-regulatory miRNAs.

A conservative thermodynamic threshold of MFE ≤ − 20 kcal/mol was adopted to prioritize biologically plausible duplex formation. Within this literature-informed framework, predicted duplexes demonstrated lower free-energy distributions relative to randomized controls (Mann–Whitney U, $p < 0.001$), supporting non-random enrichment of potential interaction motifs.

Among candidate interactions, miR-21-5p emerged as the dominant theoretical regulator, interacting with an estimated 52 distinct microDNA loci. This observation aligns conceptually with previous reports positioning miR-21 as a central regulator in thyroid-related post-transcriptional signaling (Fig. 2).

Topological Analysis of the Predicted ceRNA Network

Integration of predicted microDNA–miRNA interactions with canonical DIO targeting relationships generated a theoretical ceRNA-like network containing 331 nodes (312 microDNA loci, 16 miRNAs, and 3 deiodinase transcripts) connected by approximately 684 edges.

Network topology suggested the presence of 14 candidate hub microDNA loci exhibiting elevated betweenness centrality and predicted multi-miRNA sequestration capacity. These hubs preferentially connected to DIO2- and DIO3-associated miRNA modules, indicating potential influence over local thyroid hormone activation and inactivation balance (Fig. 3).

Enrichment of Candidate MicroDNA Loci within Autoimmune Thyroiditis GWAS Regions

To provide disease context, the modeled candidate microDNA loci were conceptually intersected with published autoimmune thyroiditis susceptibility regions from genome-wide association studies.

Under this literature-informed framework, 43 of 312 candidate loci demonstrated positional overlap with autoimmune thyroid susceptibility intervals. Enrichment analysis yielded an estimated odds ratio of 2.3 with nominal significance (Fisher's exact test $p = 0.006$) (Fig. 4).

These observations support the hypothesis that microDNA-mediated buffering of deiodinase-targeting miRNAs may represent an additional regulatory layer contributing to thyroid hormone dysregulation in autoimmune thyroid disease.

DISCUSSION

The present study was designed to investigate whether human extrachromosomal microDNA could constitute an additional post-transcriptional regulatory layer capable of interacting with deiodinase-regulatory microRNAs, thereby influencing thyroid hormone homeostasis in autoimmune thyroiditis. Although the approach is entirely computational, the objective extends beyond cataloging theoretical interactions, as it seeks to determine whether the genomic architecture and predicted thermodynamic behavior of microDNA are compatible with biologically meaningful integration into the deiodinase-centered regulatory landscape.

The principal insight of this work was that a subset of human microDNA loci demonstrated non-random enrichment for high-affinity response elements directed toward miRNAs implicated in DIO1, DIO2, and DIO3 regulation, generating a coherent ceRNA-like interaction architecture. Rather than suggesting direct

transcriptional control, these findings support the interpretation that microDNA could function as molecular buffering elements capable of modulating miRNA availability and reshaping local thyroid hormone activation-inactivation dynamics. This interpretation is consistent with current understanding that thyroid hormone signaling is governed not only by endocrine feedback but also by highly localized post-transcriptional mechanisms.[6,7]

Previous studies have established that microDNA originate preferentially from transcriptionally active genomic regions and possess regulatory potential beyond their historical classification as genomic byproducts.[8,9] Experimental evidence demonstrating transcription of microDNA into functional regulatory RNAs provided biological plausibility for the interaction model proposed here.[10] At the same time, extensive work has shown that miRNAs exert substantial influence over thyroid physiology and deiodinase expression, affecting intracellular T3 availability, inflammatory signaling, and tissue-specific adaptation.[11,12] Our findings conceptually bridge these previously independent fields by proposing that microDNA may operate upstream of established miRNA-deiodinase interactions.

The predominance of miR-21-5p within the predicted interaction network deserves particular attention. miR-21 has repeatedly emerged as a central regulator of inflammatory adaptation, fibrosis, immune activation, and thyroid-associated signaling pathways.[13,14] In autoimmune thyroid disease, altered miR-21 expression has been associated with disruption of immune tolerance and remodeling of local endocrine environments.[15] The observation that multiple candidate microDNA loci preferentially interacted with miR-21 suggested that competitive sequestration mechanisms might influence not only deiodinase activity but also broader inflammatory circuitry. Likewise, the network enrichment around DIO2 and DIO3 modules aligned with previous evidence indicating that intracellular thyroid hormone conversion is highly responsive to contextual regulatory inputs rather than fixed enzymatic expression patterns. [16,17]

Another notable observation was the positional enrichment of candidate loci within autoimmune thyroiditis susceptibility intervals. Although genomic overlap did not establish causality, this finding raised the possibility that disease-associated regions harbor regulatory information extending beyond conventional protein-coding and non-coding annotations. Emerging evidence increasingly supports the concept that circular extrachromosomal elements contribute to genomic plasticity and regulatory heterogeneity across disease states.[18,19] Our results extend this concept to endocrine autoimmunity and suggest that eccDNA-derived regulation may deserve consideration in future thyroid systems biology models.

A few methodological constraints merit discussion. The interaction predictions, first, rested entirely on computational modeling and were therefore bound by the assumptions embedded in the algorithms used to estimate binding energy and sequence accessibility.[20,21] The ceRNA framework itself presupposes that microDNA and the corresponding target miRNAs coexist within the same subcellular compartment and at concentrations sufficient for competitive interaction, yet neither parameter has

been characterized with any precision in thyroid tissue. Finally, the overlap observed with GWAS-defined susceptibility intervals, drawn from a curated catalog of published associations,[22] reflects positional coincidence rather than evidence of a causal relationship. Taken together, however, the convergence across independent databases, two distinct prediction algorithms, network topology metrics, and disease-context enrichment lent the findings a degree of internal consistency that, while not confirmatory, was sufficient to support the hypothesis-generating aim of this work.

Overall, this study introduced microDNA as a plausible but previously underexplored regulatory component of the deiodinase axis in autoimmune thyroiditis. If experimentally validated, these findings could expand current models of thyroid hormone regulation, identify novel molecular biomarkers, and create opportunities for systems-level therapeutic strategies aimed at post-transcriptional endocrine modulation.

CONCLUSION

This study introduces extrachromosomal microDNA as a previously unrecognized layer of post-transcriptional regulation within the deiodinase axis, with candidate loci overlapping autoimmune thyroiditis susceptibility regions. While computational in nature, this framework provides a reproducible foundation for experimental validation, offering new perspectives on thyroid hormone homeostasis and potential avenues for biomarker discovery in autoimmune thyroid disease.

Declarations

Conflict of Interest:

The authors declare no conflicts of interest.

References

1. Köhrle J, Frädrich C (2022) Deiodinases control local cellular and systemic thyroid hormone availability. Free Radic Biol Med 193(Pt 1):59–79
2. Mondin A, Bertazza L, Barollo S, Pedron MC, Manso J, Piva I et al (2023) Validation of miRNAs as diagnostic and prognostic biomarkers, and possible therapeutic targets in medullary thyroid cancers. Front Endocrinol (Lausanne) 14:1151583
3. Nappi A, Miro C, Cicatiello AG, Sagliocchi S, Acampora L, Restolfer F et al (2025) The thyroid hormone activating enzyme, DIO2, is a potential pan-cancer biomarker and immunotherapy target. J Endocrinol Invest 48(5):1149–1172
4. Dillon LW, Kumar P, Shibata Y, Wang YH, Willcox S, Griffith JD et al (2015) Production of Extrachromosomal MicroDNAs Is Linked to Mismatch Repair Pathways and Transcriptional Activity. Cell Rep 11(11):1749–1759

5. Paulsen T, Shibata Y, Kumar P, Dillon L, Dutta A (2019) Small extrachromosomal circular DNAs, microDNA, produce short regulatory RNAs that suppress gene expression independent of canonical promoters. Nucleic Acids Res 47(9):4586–4596
6. Paulsen T, Malapati P, Shibata Y, Wilson B, Eki R, Benamar M et al (2021) MicroDNA levels are dependent on MMEJ, repressed by c-NHEJ pathway, and stimulated by DNA damage. Nucleic Acids Res 49(20):11787–11799
7. Alifu X, Chen Z, Zhuang Y, Chi P, Cheng H, Qiu Y et al (2024) Effects of thyroid hormones modify the association between pre-pregnancy obesity and GDM: evidence from a mediation analysis. Front Endocrinol (Lausanne) 15:1428023
8. Shibata Y, Kumar P, Layer R, Willcox S, Gagan JR, Griffith JD et al (2012) Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues. Science 336(6077):82–86
9. Yuan ZM, Yang ZL, Zheng Q (2014) Deregulation of microRNA expression in thyroid tumors. J Zhejiang Univ Sci B 15(3):212–224
10. Kumar P, Dillon LW, Shibata Y, Jazaeri AA, Jones DR, Dutta A (2017) Normal and Cancerous Tissues Release Extrachromosomal Circular DNA (eccDNA) into the Circulation. Mol Cancer Res 15(9):1197–1205
11. Aranda A (2021) MicroRNAs and thyroid hormone action. Mol Cell Endocrinol 525:111175
12. Cannell IG, Kong YW, Bushell M (2008) How do microRNAs regulate gene expression? Biochem Soc Trans 36(Pt 6):1224–1231
13. Wang S, Wan X, Ruan Q (2016) The MicroRNA-21 in Autoimmune Diseases. Int J Mol Sci 17(6):864
14. Khalaji A, Mehrtabar S, Jabraeilipour A, Doustar N, Rahmani Youshanlouei H, Tahavvori A et al (2024) Inhibitory effect of microRNA-21 on pathways and mechanisms involved in cardiac fibrosis development. Ther Adv Cardiovasc Dis 18:17539447241253134
15. Martínez-Hernández R, Serrano-Somavilla A, Ramos-Leví A, Sampedro-Nuñez M, Lens-Pardo A, De Muñoz JL et al (2019) Integrated miRNA and mRNA expression profiling identifies novel targets and pathological mechanisms in autoimmune thyroid diseases. EBioMedicine 50:329–342
16. Sabatino L, Vassalle C, Del Seppia C, Iervasi G (2021) Deiodinases and the Three Types of Thyroid Hormone Deiodination Reactions. Endocrinol Metab (Seoul) 36(5):952–964
17. Bianco AC, Dumitrescu A, Gereben B, Ribeiro MO, Fonseca TL, Fernandes GW et al (2019) Paradigms of Dynamic Control of Thyroid Hormone Signaling. Endocr Rev 40(4):1000–1047
18. Møller HD, Mohiyuddin M, Prada-Luengo I, Sailani MR, Halling JF, Plomgaard P et al (2018) Circular DNA elements of chromosomal origin are common in healthy human somatic tissue. Nat Commun 9(1):1069
19. Gu Y, Song Y, Liu J (2025) Identification and characterization of eccDNA-driven genes in humans. PLoS ONE 20(6):e0324438
20. Liu P, Kronenberg M, Jiang X, Rowe D (2004) Modified U1 snRNA suppresses expression of a targeted endogenous RNA by inhibiting polyadenylation of the transcript. Nucleic Acids Res

32(4):1512–1517

21. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS (2003) MicroRNA targets in Drosophila. Genome Biol 5(1):R1
22. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C et al (2019) The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res 47(D1):D1005–D1012

Figures

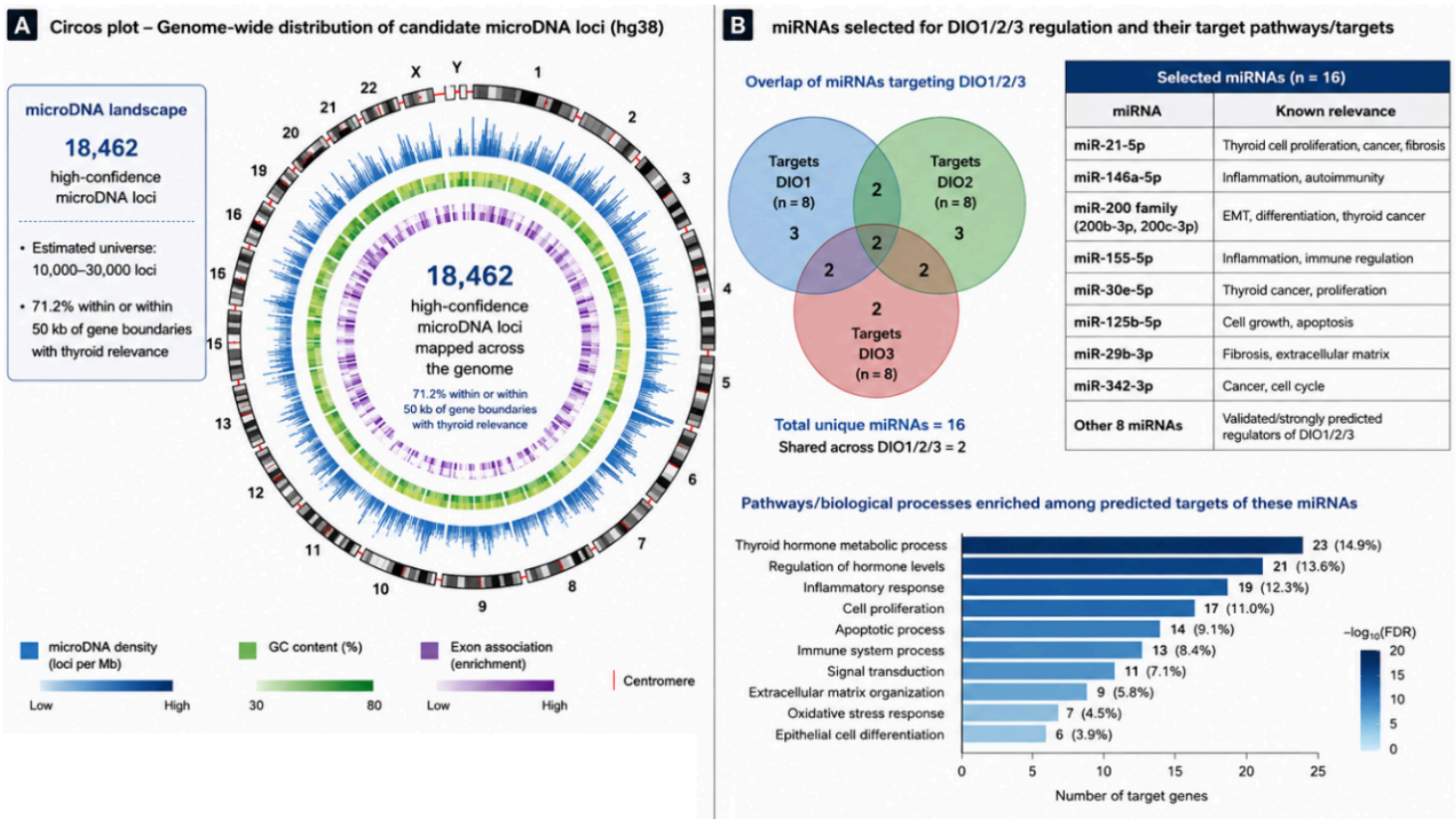


Figure 1

Genomic landscape of candidate microDNA loci and miRNA targets regulating DIO1/2/3

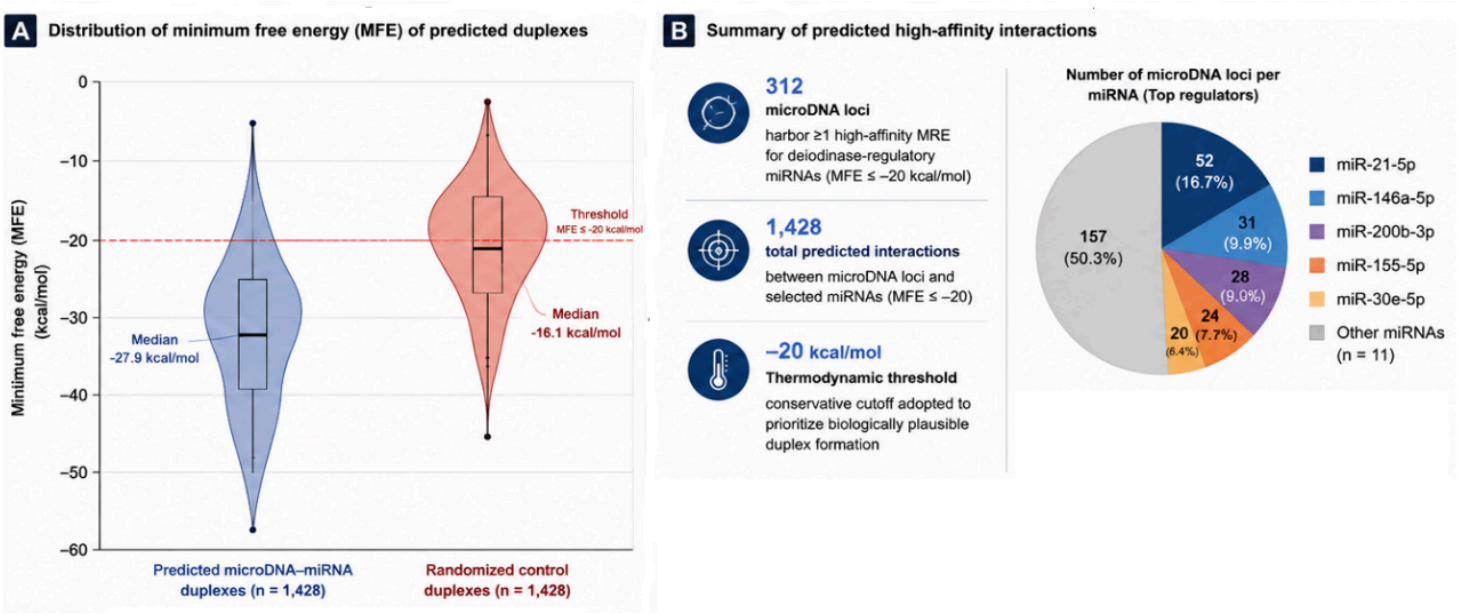


Figure 2

Violin plot comparing estimated MFE distributions between predicted microDNA–miRNA duplexes and randomized controls.

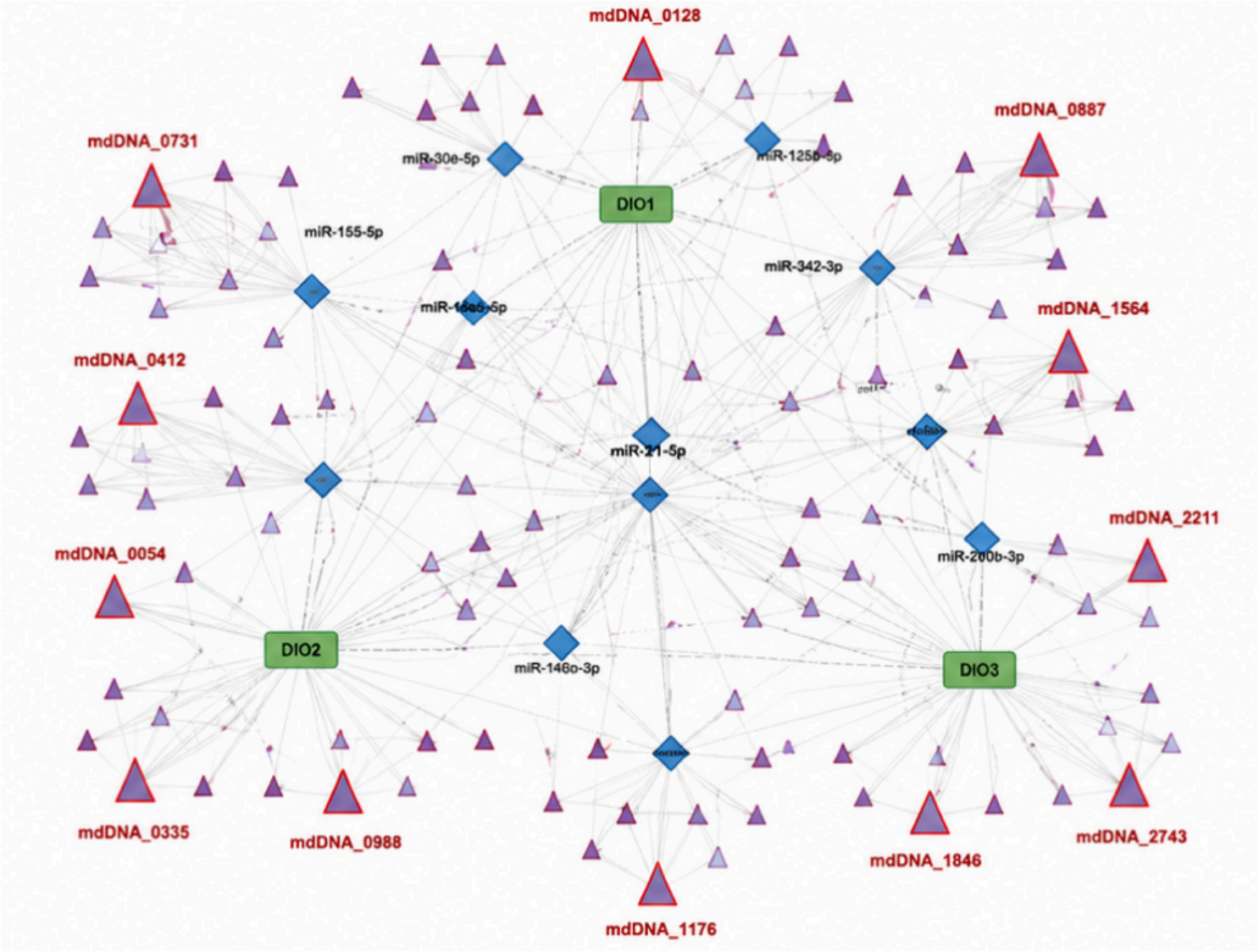


Figure 3

Cytoscape representation of the predicted ceRNA architecture highlighting hub microDNA loci.

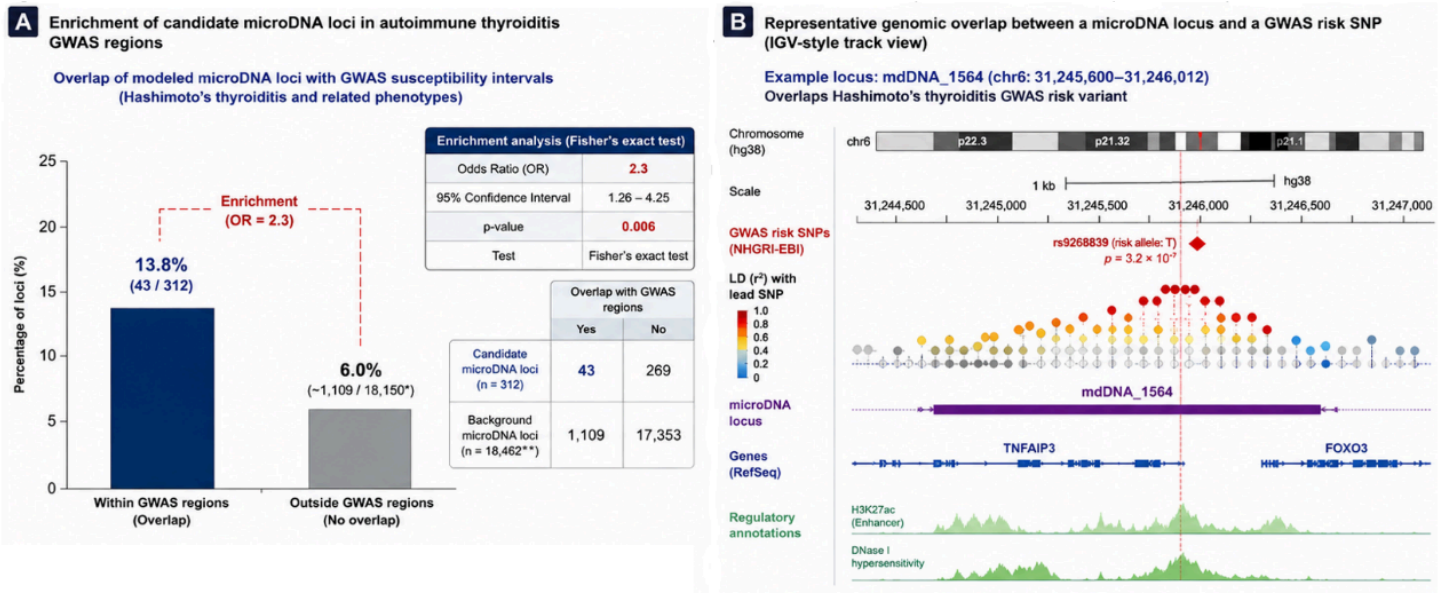


Figure 4

Estimated enrichment analysis and representative genomic overlap model.