DOCUMENT SUMMARY

This paper provides a powerful, mechanistic look into the neurobiological underpinnings of neurodiversity using the 15q11-q13 imprinted locus (associated with Angelman and Prader-Willi Syndromes) as a model. By demonstrating the complex and dynamic interplay of DNA methylation, chromatin looping, and transcription required for normal neuronal development, it supplies robust scientific evidence that human brains are not static but are shaped by intricate epigenetic processes. The research explicitly critiques the limitations of non-human and other in vitro models, paralleling Enlitens' critique of inadequate assessment tools, and validates the use of a more appropriate human model (LUHMES cells) to understand these uniquely human developmental dynamics.

FILENAME

FUGON_2024_Epigenetic_Imprinting_in_Human_Neurons_Mechanisms_of_Neurodiversity.md

METADATA

- **Primary Category**: RESEARCH
- **Document Type**: research_article
- **Relevance**: Core
- **Key Topics**: epigenetics, neurodevelopment, gene expression, imprinting, Angelman Syndrome, neurodiversity, assessment models
- **Tags**: #epigenetics, #neurodiversity, #gene_regulation, #imprinting, #Angelman_Syndrome, #PWS, #neuronal_development, #CTCF, #DNA_methylation, #human_cell_models

CRITICAL QUOTES FOR ENLITENS

- "Human in vitro models are essential for understanding neurodevelopmental disorders linked to the 15q11.2-13.3 region due to interspecies genetic and epigenetic differences."
- "Specifically, the transcript that silences paternal UBE3A in neurons exhibits different splicing and termination points in non-neuronal cells when comparing mice to humans [2]."
- "This distinction underscores the necessity of human-specific models to accurately explore the epigenetic landscape and inform therapeutic development."
- "The sequential molecular events that lead to developmental regulation of transcript elongation remains a central question in the epigenetics of the 15q11-q13 region."
- "A major challenge to the field is that no in vitro model can fully replicate the dynamic processes that occur during neurodevelopment in the human brain."

- "Moreover, epigenetic modifications crucial for the regulation of UBE3A expression may not be fully established or maintained in these in vitro systems."
- "These results provide an integrated view of the 15q11-q13 epigenetic landscape during LUHMES neuronal differentiation, underscoring the complex interplay of transcription, chromatin looping, and DNA methylation."
- "In this comprehensive study, we characterized and integrated genome-wide DNA methylation with CTCF loops and RNA expression of LUHMES cells to shed light on their relationship, with a particular emphasis on evaluating their potential as a model for AS."
- "These results provide an integrative multi-omic atlas of neuronal differentiation in LUHMES that could be useful for investigations of multiple neurodevelopmental disorders."

KEY STATISTICS & EVIDENCE

- During neuronal differentiation of LUHMES cells, a genome-wide transcriptome analysis revealed changes in 11,834 genes.
- Of these, 5,379 genes were upregulated and 6,455 genes were downregulated in neurons compared to undifferentiated cells.
- The study identified two neuron-specific CTCF loops: one between *MAGEL2-SNRPN* and another between *PWAR1-UBE3A*.
- The *UBE3A-ATS* transcript, which silences the paternal UBE3A allele in neurons, was below the level of detection in non-neuronal HEK293T cells and undifferentiated LUHMES cells.
- In contrast, differentiated LUHMES neurons showed high levels of *UBE3A-ATS*, comparable to levels in adult human cerebral cortex tissue.
- Expression of *UBE3A-ATS* progressively increased over a seven-day differentiation period, with the most substantial increases between Days 5 and 7.
- Using a less stringent filtering method, the study identified 36,816 HiChIP interactions unique to neurons, 74,469 unique to undifferentiated cells, and 26,162 shared between them.

METHODOLOGY DESCRIPTIONS

Critique of Existing Research Models

- **Interspecies Differences**: Human in vitro models are essential for understanding neurodevelopmental disorders linked to the 15q11.2-13.3 region due to interspecies genetic and epigenetic differences. Specifically, the transcript that silences paternal UBE3A in neurons exhibits different splicing and termination points in non-neuronal cells when comparing mice to humans [2]. This distinction underscores the necessity of human-specific models to accurately explore the epigenetic landscape and inform therapeutic development.
- **Limitations of In Vitro Models**: A major challenge to the field is that no in vitro model can fully replicate the dynamic processes that occur during neurodevelopment in the

human brain. Differentiation protocols might not accurately recapitulate the complex maturation steps that UBE3A-ATS expressing neurons undergo in vivo. Moreover, epigenetic modifications crucial for the regulation of UBE3A expression may not be fully established or maintained in these in vitro systems.

- **Critique of Specific Cell Lines**: Models for studying the AS/PWS locus include SH-SY5Y cells and human induced pluripotent stem cells (iPSCs) from AS patients [16]. However, SH-SYSY are aneuploid and derive from cancer cells and thus may have an aberrant epigenetic profile. While patient-derived iPSCs hold great promise, full differentiation to mature neurons is a challenging and inconsistent process that can extend beyond seven weeks [16]. Despite their valuable insights, these models might not fully capture the intricate epigenetic complexities inherent in the 15q11.2-q13.3 locus and other disease loci with complex neuronal expression patterns.

The LUHMES Cell Model as a Superior Alternative

- **Rationale**: In contrast, the human LUHMES (Lund human mesencephalic) cell line may be an ideal model to study neurodevelopmental disorders with an epigenetic component. LUHMES cells are female human embryonic neuronal precursor cells capable of sustained proliferation, which is attributed to the presence of an engineered tetracycline-inducible (Tet-off) v-myc transgene [29].
- **Advantages**: Compared to pluripotent stem cell lines, they are relatively easy to grow and differentiate into neurons within one week. This rapid expression of UBE3A-ATS, achieved within seven days, underscores the potential of LUHMES cells as a superior model for neuronal studies, particularly over iPSC-derived neurons where differentiation is more protracted. The swift transition of LUHMES cells to mature neuronal functions, alongside their non-cancerous origin, offers a distinct advantage in delineating the boundary region of this loci and assessing the regulatory role of CTCF in UBE3A-ATS expression.

THEORETICAL FRAMEWORKS

Epigenetic Regulation of the 15q11-q13 Locus

- **Paternal Silencing in Neurons**: AS results from loss of maternal *UBE3A* in neurons, where the paternal allele is silenced by a convergent antisense transcript *UBE3A-ATS*, a lncRNA that terminates at *PWAR1* in non-neurons. In neurons, SNHG14 transcription continues beyond PWAR1 through the SNORD115 cluster and further extends antisense to UBE3A (UBE3A-ATS). This antisense transcript has been shown to be responsible for the silencing of the paternal allele in neurons [15].
- **CTCF as a Regulatory Boundary**: The presence of binding sites for the insulator protein CTCF (CCCTC-Binding Factor) at PWAR1 has led to the hypothesis that this boundary may serve as the barrier to transcriptional extension in non-neurons [16], but the role of chromatin topology has not been assayed explicitly.
- **Role of Chromatin Loops**: CTCF associates with cohesin to form chromatin loops which have been shown to regulate tissue and allele-specific differential gene expression [17, 18]. Chromatin loops are formed preferentially by two convergent CTCFs and a stabilizing cohesin ring [24].

- **DNA Methylation and CTCF Binding**: Reduced CTCF binding correlates with CpG hypermethylation at its canonical binding motif [19]. Given that adjacent DNA hypermethylation is known to hinder CTCF binding, this observation may relate to mechanisms similar to those at the Igf2 locus in mice. There, hypermethylation near the Igf2 gene prevents CTCF from binding to its regulatory sequences, which in turn affects the gene's expression [20, 42].
- **Collision Model**: One of the prevailing models describing how UBE3A is silenced by UBE3A-ATS within the AS/PWS locus is the collision model, which proposes that RNA polymerase II from convergent transcripts can disrupt gene expression [27, 28].

POPULATION-SPECIFIC FINDINGS

This study's findings are specific to cell populations (neurons vs. non-neurons) and parental alleles, which provides a biological parallel for population differences in humans.

- **Neuron-Specific Changes**: The study identified two neuron-specific CTCF loops between *MAGEL2-SNRPN* and *PWAR1-UBE3A*. A paternally hypomethylated differentially methylated region (DMR) near the *SNRPN* upstream loop anchor was exclusive to neurons. This corresponded to the start of *SNRPN* transcription specifically in this cell state.
- **Non-Neuron-Specific Changes**: A paternally hypermethylated DMR near the *PWAR1* CTCF anchor was exclusive to undifferentiated cells, consistent with increases in neuronal transcription. This suggests its potential role in the regulation of the transcription boundary in this region for non-neurons. In undifferentiated cells, the forward transcript started at the PWS-ICR within SNRPN and showed an abrupt decrease of forward strand transcription at the 3' end of PWARI.
- **Allele-Specific Methylation**: The study was able to assign parentage for each haplotype based on the well characterized paternal hypomethylation of the PWS-ICR. The region between both neuron-specific chromatin loops was particularly enriched for paternal DMRs and was predominantly hypermethylated. In contrast, the long-read DNA methylation patterns were better indicators of parental allele-specific events than chromatin loops.

PRACTICAL APPLICATIONS

Therapeutic Implications and Future Directions

- **Epigenetic Editing**: Building on these foundational insights, our research supports the use of artificial transcription factors (ATFs) to modulate the epigenetic environment surrounding the UBE3A gene. Consisting of a catalytically inactive Cas (dCas) fused to an effector domain, ATFs can be designed for specific epigenetic modifications, either to demethylate or methylate DNA at targeted sites [51, 52].
- **Testing Hypotheses**: Given our capability to edit DNA methylation in the mammalian genome, we envision employing dCas-TET1 for demethylation at the PWAR1 binding site to explore its role in halting lncRNA expression past this region in non-neuronal cells [53]. Furthermore, the use of dCas-DNMT3AL to hypermethylate the PWAR1 binding

site, alone or in conjunction with the SNRPN CTCF binding site in neurons, aims to replicate the transcriptional boundary characteristic of undifferentiated cells, providing insights into UBE3A silencing mechanisms.

- **Translational Potential**: The LUHMES cell model, with its human derivation and capability for rapid neuronal differentiation, is pivotal for our proposed experiments. It not only validates the biological relevance of our findings but also positions itself as a promising platform for bridging laboratory discoveries to therapeutic applications. This human-based system enhances the translational potential of our work, suggesting a clear path for the transferability of our findings to therapeutic interventions. The advancements of epigenetic editing into clinical trials underscore the timeliness of our approach [56].

LIMITATIONS OF THE STUDY

However, we also realize that there are some limitations to the use of LUHMES, including being more challenging to transfect and single-cell clone. Also, the relatively short life span that LUHMES spend in the neuronal state can limit some applications. Additionally, LUHMES are derived from a single individual female subject, thus limiting the ability to examine different genetic backgrounds or sexes.