QUANTIFICATION OF PTCHD1-AS DOSAGE AT XP22.11 IN AUTISM SPECTRUM DISORDER: QPCR IN HUMAN CELL MODELS AND A CRISPRA VALIDATION FRAMEWORK

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ABSTRACT

AUTISM SPECTRUM DISORDER (ASD) FREQUENTLY INVOLVES DOSAGE-SENSITIVE GENOMIC LOCI THAT INFLUENCE SYNAPTIC DEVELOPMENT. AT XP22.11, DELETIONS THAT TRUNCATE THE LONG NON-CODING RNA PTCHD1-AS ARE ROBUST ASD RISK FACTORS; PATIENT IPSC-DERIVED NEURONS CARRYING THESE DELETIONS DISPLAY EXCITATORY SYNAPTIC DEFECTS CONSISTENT WITH NMDA-RECEPTOR HYPOFUNCTION. IN THIS LAIDLAW SUMMER PROJECT, I SUPPORTED CRISPR INTERFERENCE (CRISPRI) EXPERIMENTS TARGETING PTCHD1-AS AND QUANTIFIED LOCUS-PROXIMAL AND SYNAPTIC TRANSCRIPTS USING TAQMAN QPCR. TWO MAIN OBSERVATIONS EMERGED. FIRST, ACROSS MATCHED CRISPR-CORRECTED (CC) AND NON-CORRECTED (CNC) 1134 IPSC LINES AND THEIR NEURONAL DERIVATIVES, ASH1L MRNA WAS LOWER IN CORRECTED MATERIAL FOR TWO INDEPENDENT EXON WINDOWS (EXONS 1–2 AND 3–4). SECOND, IN SK-N-BE(2) NEUROBLASTOMA CELLS, BASELINE EXPRESSION FOR DDX53, PTCHD1-AS3 (EXON 1), AND PTCHD1 WAS STABLE WHEN CALIBRATED TO TBP, PROVIDING A PRACTICAL REFERENCE FOR FUTURE STUDIES. THESE DATA MOTIVATE AN ACADEMIC-YEAR CRISPRA OVEREXPRESSION STUDY TO DEFINE PTCHD1-AS DOSAGE–RESPONSE RELATIONSHIPS AND TEST FOR CIS EFFECTS ON PTCHD1/DDX53 ALONGSIDE SYNAPTIC RELATED GENES.

INTRODCUTION

WHY XP22.11?

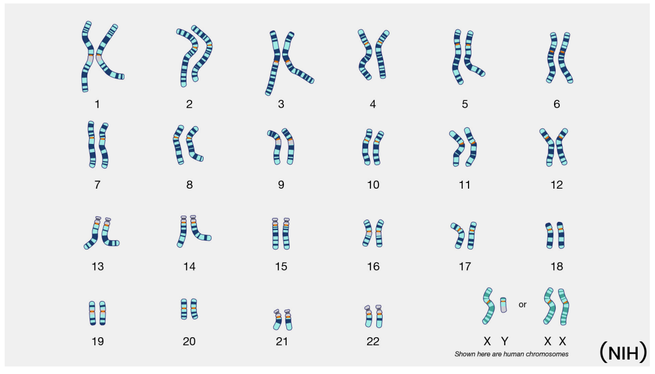
THE PTCHD1/PTCHD1-AS/DDX53 LOCUS IS REPEATEDLY LINKED TO AUTISM SPECTRUM DISORDER (ASD). TRUNCATING DELETIONS THAT REDUCE PTCHD1-AS ARE ASSOCIATED WITH SYNAPTIC DEFICITS AND NMDA-RECEPTOR HYPOFUNCTION IN PATIENT IPSC-NEURONS (NOOR ET AL., 2010; ROSS ET AL., 2020).

KEY GENES.

PTCHD1 ENCODES A POSTSYNAPTIC PROTEIN IMPLICATED IN EXCITATORY SYNAPSES; DDX53 IS A NEARBY SINGLE-EXON HELICASE WITH EMERGING ASD RELEVANCE (UNG ET AL., 2017; PASTORE ET AL., 2022; SCALA ET AL., 2025).

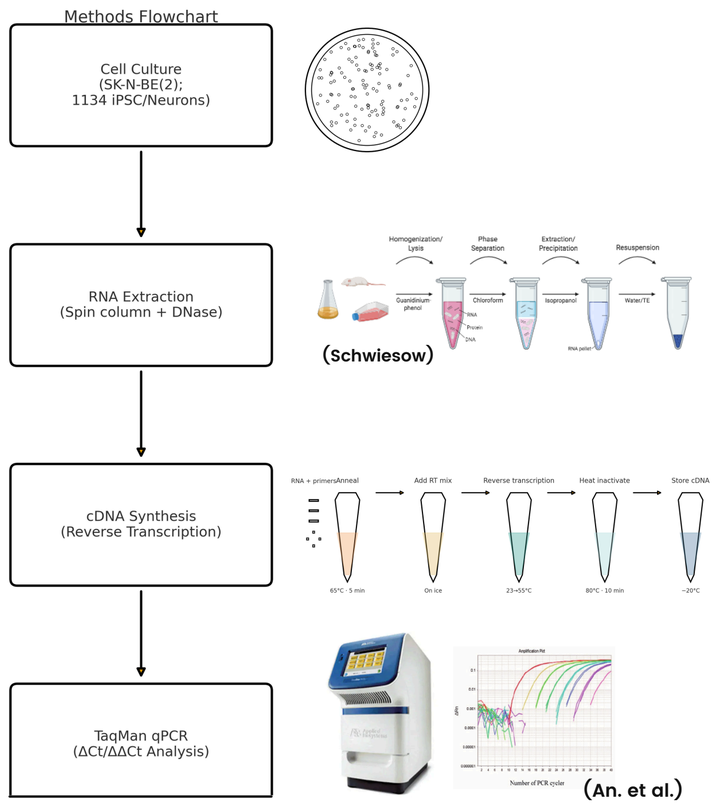
EPIGENETIC READOUT.

ASH1L (KMT2H) IS A HISTONE METHYLTRANSFERASE (H3K36ME2) LINKED TO NEURODEVELOPMENT; CHANGES IN ASH1L EXPRESSION CAN FLAG BROADER DEVELOPMENTAL EFFECTS (QIN ET AL., 2021; GAO ET AL., 2021).



(NIH)

METHODOLOGY



(Schwiesow)

(An. et al.)

Cell culture: Grow SK-N-BE(2) and 1134 iPSC/neurons; harvest at ~80% confluency.

RNA extraction: Lyse cells; column-purify RNA with on-column DNase; elute in RNase-free water.

cDNA synthesis: Reverse-transcribe RNA (SuperScript IV; random hexamers + oligo(dT)); include no-RT control.

qPCR (TaqMan): Quantify targets in triplicate; normalize to TBP or TFRC; report 2^-ΔΔCt.

RESULTS

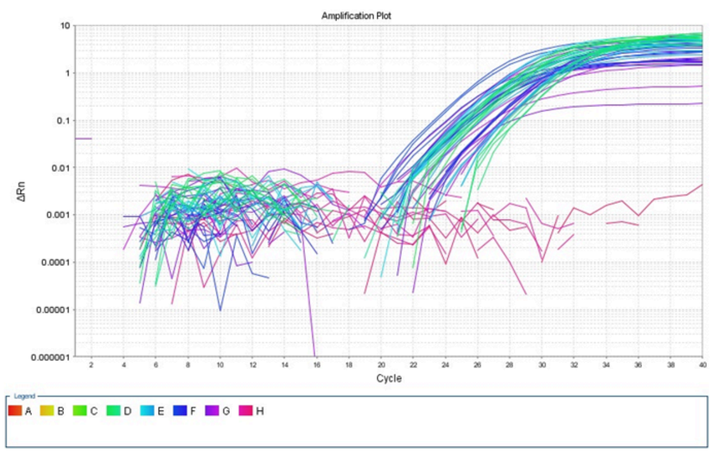


Figure 1. Amplification plot of ASH1L Exon 1-2 and 3-4, indicating the number of cycles the given sample took to double in expression.

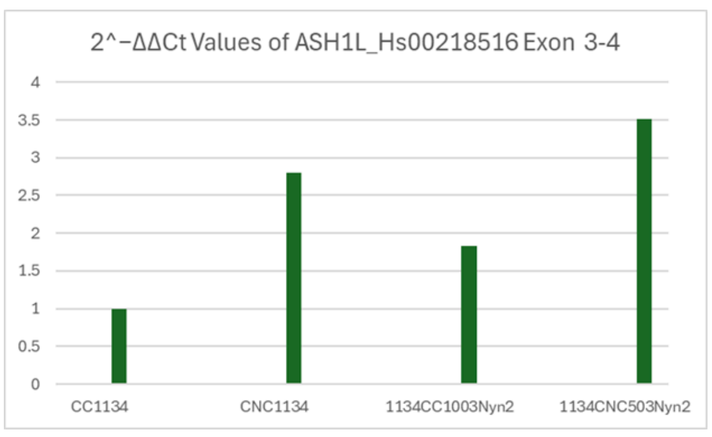


Figure 3. ASH1L Exon 3–4 fold change (calibrator CC1134): CNC1134 = 2.8×; 1134CC1003Nyn2 = 1.8×; 1134CNC503Nyn2 = 3.5×.

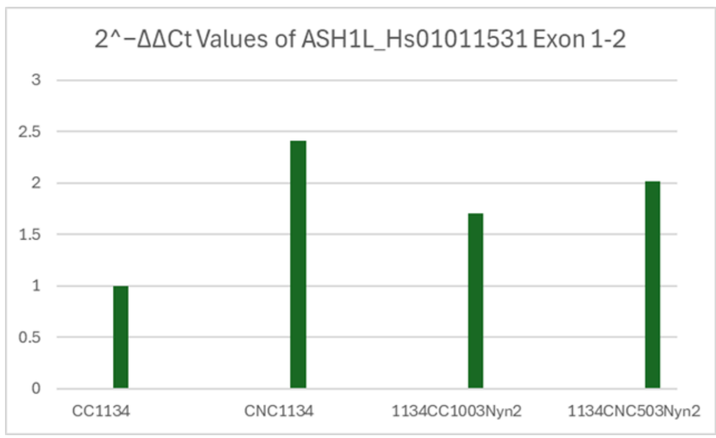


Figure 2. ASH1L Exon 1–2 fold change (calibrator CC1134): CNC1134 = 2.4×; 1134CC1003Nyn2 = 1.7×; 1134CNC503Nyn2 = 2.0×.

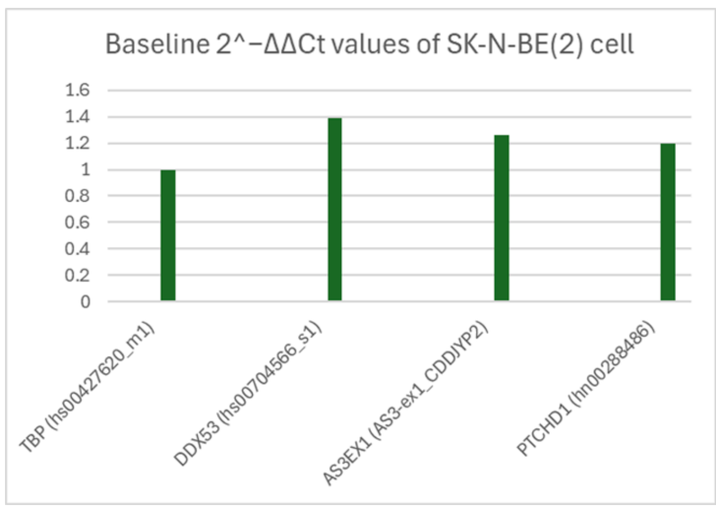


Figure 4. SK-N-BE(2) baseline calibrated to TBP = 1.0: DDX53 = 1.39; PTCHD1-AS3 (exon 1) = 1.27; PTCHD1 = 1.19.

DISCUSSION

Key findings

Reliable workflow: Cell culture → RNA extraction → cDNA → TaqMan qPCR ran cleanly with good replicate agreement.

Stable baselines: SK-N-BE(2) showed consistent detection of PTCHD1, PTCHD1-AS3 exon 1, and DDX53 using TBP as the control.

ASH1L pattern in 1134 lines: Across assays targeting exons 1–2 and 3–4, non-corrected (CNC) samples showed higher ASH1L than corrected (CC) in both iPSCs and neurons.

Interpretation (working model): Signals are consistent with compensation, isoform/regulatory complexity, or cell-state differences, rather than a simple “correction = higher ASH1L” expectation.

Next steps

Replicate and tighten QC: Add biological replicates; include an additional housekeeping gene (e.g., RPLP0 or HPRT1); confirm primer efficiency.

Mechanistic checks: Measure ASH1L protein and relevant H3K36 marks; consider locus-focused chromatin assays (e.g., ChIP-qPCR/CUT&RUN).

CRISPRa plan (HMB496): Overexpress PTCHD1-AS with dCas9-VPR (BPK1520 gRNAs), verify induction, then profile PTCHD1, DDX53, and E/I marker panels. Compare effects with existing CRISPRi data.

Model progression: Optimize in SK-N-BE(2), then move to isogenic iPSC-neurons for disease relevance.

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