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Exploring the Placental Methylome: Identifying Genetic Markers for Severe Neonatal Opioid Withdrawal Syndrome

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Every 25 minutes, a baby is born with neonatal opioid withdrawal syndrome (NOWS) in the United States due to in utero opioid exposure. The diagnosis and severity of NOWS remain unpredictable, with unknown influencing factors. This study aimed to assess the placental methylome in pregnancies exposed to medication for opioid use disorder (MOUD), specifically methadone or buprenorphine, to identify genes associated with severe NOWS risk. We conducted a secondary analysis of a multi-center prospective cohort study involving 33 pregnancies on MOUD from 2016 to 2018, along with two pregnancies without opioid exposure (controls). Villous fragments were collected within one hour of delivery. Methylation was measured using the Illumina Infinium MethylationEPIC BeadChip, with differentially methylated probes (DMPs) at $p<0.005$ considered significant. Gene expression was measured using qPCR. Severe NOWS was diagnosed with Finnegan scoring. Severe NOWS was observed in 17 neonates (52%), while 16 neonates (48%) experienced non-severe NOWS. A total of 338 DMPs were identified between the severe and non-severe NOWS groups. A total of 338 DMPs were identified between the groups, with CST3, INTU, ACP6, MYL10, and TIMELESS as the top differentially methylated loci. CST3 showed a 26% increase in methylation in severe NOWS cases and was downregulated in opioid-exposed placentas. Pathway analysis implicated downregulation of the sphingolipid pathway and upregulation of the mRNA surveillance pathway in severe NOWS. Placental CST3 has been associated with developmental delay at 6 months of life in other studies. We aim to validate these findings in our recently recruited prospective cohort.