**Title**: Phenotypic differences in Okur-Chung Neurodevelopmental Syndrome (OCNDS) dependent on location of mutation in the *CSNK2A1* gene

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**Introduction**: OCNDS is typically caused by *de novo* mutations in the casein kinase alpha (CK2α) subunit of the protein, with some autosomal dominant inherited cases noted in literature1. Typical clinical characteristics associated with OCNDS are global developmental delay, distinct facial features, speech delay, hypotonia (low muscle tone), mild-to-moderate intellectual disabilities, and difficulty feeding2. There are also broadly reported nonspecific clinical features reported in the literature, such as behavioral problems, disrupted sleep patterns, seizures, and abnormal growth (i.e., short stature)3,4. To date, there are no direct therapeutic treatments for OCNDS, however treatments often used focus on symptom management. The goal of this research is to provide insight into the phenotypic spectrum of OCNDS patients with the goal of enhancing prospective studies and uncovering unique features. Previous reviews have noted that the majority of CK2α variants observed in OCNDS are missense mutations that cluster in highly conserved functional domains and key structural regions, including the Glycine (Gly)-rich loop, activation loop, and P+1 loop5. Since such key regions are highly conserved across kinases, we aimed to explore how regional mutations have varying effects on OCNDS phenotypes.

**Method**: We analyzed natural history data about individuals with OCNDS from Simons Searchlight. Activities were determined to not constitute regulated research involving human subjects (Salus IRB, Study ID 23134-01). We cross-referenced included patients with existing literature to identify the specific *CSNK2A1* gene segment represented by each individual. Using the Simons Searchlight dataset representing the medical history intake, we generated pivot tables in Microsoft Excel specific to each organ system to identify associations between genetic mutations and phenotypic expressions. We then categorized the symptoms into loop or non-loop variant categories to compare the total unique symptom count per individual as well as the total symptom count per individual for each organ system analyzed. GraphPad PRISM software was used for all statistical analyses. For comparisons of loop vs. non-loop variants, Mann Whitney tests were utilized. The cumulative organ anomaly score and NPD score among *CSNK2A1* variants in different regions of CK2α were presented as means ± SD, and differences between two cumulative scores were compared using Mann–Whitney *U* tests. At the time of data export (May 2024), the CSNK2A1 dataset held information on 220 individuals including individuals with OCNDS and unaffected family members. Out of 220, only 92 individuals had a confirmed genetic report. Since the majority of reported OCNDS cases are missense mutations5, we narrowed our analyses to include only individuals with missense variants that had a classification of pathogenic or likely pathogenic (n=53), excluding variants of uncertain significance, deletions, splice variants, and frameshift variants

**Results**: In this cohort, the top reported symptoms were non-seizure neurological (n=44, 83%) and gastrointestinal (n=39, 76%). We classified the patients’ mutations according to location along the different domains and calculated the ratio of mutations to residues. We observed missense mutations in every segment except the hinge + helixαD and c-terminal segment, with the p+1 loop illustrating the highest number of patients (ratio of observed patients/residue: 2.3). We first attempted to analyze variants across all *CSNK2A1* gene segments. Differences were not significant (p=0.38) likely due to the large distribution of sample sizes across gene segments and our limited total sample size. This illustrated that regions classified as having loops displayed more patients with a higher number of symptoms. When we analyzed this data comparing loop vs. non-loop variants, we observed that individuals in loop regions reported a higher number of symptoms (0.03). We did not observe a difference in the total number of seizure symptoms reported between loop and non-loop variants (p=0.39). Individuals with loop variants reported a higher number of non-seizure neurological symptoms (p=0.03). The most common non-seizure neurological symptom was low muscle tone (n=42). When comparing low muscle tone, there was a significant difference wherein individuals with loop variants reported a higher frequency of low muscle tone symptoms (p=0.01). Individuals with loop variants reported a higher number of gastrointestinal symptoms, however this difference was not statistically significant (p=0.09). The most reported GI symptoms in our cohort were constipation (n=32) and gastroesophageal reflux disease (GERD, n=10). All 10 patients that reported GERD as a symptom had loop variants however, no significant differences were observed for constipation. No significant differences were observed between loop and non-loop variants for cognitive phenotypes, including speech/language delay and Vineland-3 scores, sleep issues, or total medication use. We then analyzed the reported age at diagnosis – for OCNDS, this is age at genetic testing as this is the only method for diagnosis. We had a recorded age at genetic diagnosis for 51/53 (96%) of missense variants characterized (n=9 non-loop, n=42 loop). We observed that individuals with loop variants in *CSNK2A1* are diagnosed at a younger age than individuals with non-loop variants (p=0.04).

**Discussion:** A recent literature review noted that individuals harboring *CSNK2A1* null variants (i.e., no protein is produced) presented with a milder phenotype than individuals with missense variants, specifically annotating reduced frequency of symptoms associated with dysmorphic facial features, language deficits, and intellectual disability. We did not have sufficient representation of protein-truncating variants in the dataset to investigate this difference6. Together, our results expand on previous genotype-phenotype correlations and highlight the need for additional studies to ascertain functional changes that particular mutations exert on the protein. Since no phenotypic differences were observed for constipation, sleep, or speech/language delay, this suggests a set of common symptoms to focus on for therapeutic development that could benefit OCNDS patients regardless of mutation location. The age at diagnosis result was not surprising given that individuals with loop variants report a higher number of overall symptoms and thus, may be more likely to see a specialist or be recommended for genetic testing. This further highlights the need for careful considerations of symptom presentation at early ages and the significance of access to genetic testing early when symptoms first arise. We were limited by the number of individuals enrolled in Simons Searchlight. There is a need for further investigation into deletions, duplications, frameshift, and splice site variants in *CSNK2A1*. This data is reported by caregivers of individuals with OCNDS. Therefore, we are limited by the questions asked of caregivers within the surveys and the memory recall of caregivers covering many years of symptoms and treatments. An option for future studies would be to analyze medical records of OCNDS patients directly to ensure we do not miss currently unrecognized symptoms. The CSNK2A1 Foundation is currently partnering with Citizen Health to pursue this type of analysis7,8. We aim to enroll additional OCNDS patients in our Simons Searchlight natural history study to further assess genotype-phenotype differences across all mutation types in the future.

**References:**

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