A novel assay for potential mass screening of Turner syndrome based on Oxford Nanopore Technology

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Abstract

Turner Syndrome (TS), a rare chromosomal disorder, poses significant challenges for affected individuals and healthcare providers alike. Despite its prevalence (1:2000 – 1:2500) and recognition of being the only monosomic karyotype, TS often remains undetected until later stages of life, leading to neglected opportunities for early intervention and clinical management.

The aim of this study is to evaluate the diagnostic performance of the Screening Chromosomal ANueploidie (SCAN) assay, originally developed for Klinefelter Syndrome, in detecting TS. Utilizing a two-step PCR system and machine learning-based classification, SCAN demonstrated promising results in accurately identifying TS. With high average (± standard deviation) sensitivity (93.75 %), specificity (99.68 %) and accuracy (98.69 %) SCAN distinguished TS from other chromosomal aneuploidies and healthy controls.

SCAN Methodology

1. **Wet-lab protocol:**
   - Coriell Sample Collection, gDNA extraction and sample preparation.
   - Detection of Turner Syndrome using two- step PCR system
   - Sequencing Library Preparation of multilevel multiplexed samples
   - Sequencing and first level demultiplexing with Oxford Nanopore Technologies
2. **Data preprocessing and model training:**
   - Second level demultiplexing with Torchlex
   - Training a binary classifier (Support Vector Classifier) and evaluating

Data Explanation

The dataset consists of **384 female samples** distributed on four experimental set-up, covering the karyotype of:

- Trisomy 21 (47,XX+21)
- Trisomy 18 (47,XX+18)
- Trisomy 13 (47,XX+13)
- Turner Syndrome (45,X0)
- Healthy female (46,XX)

Visual Analysis

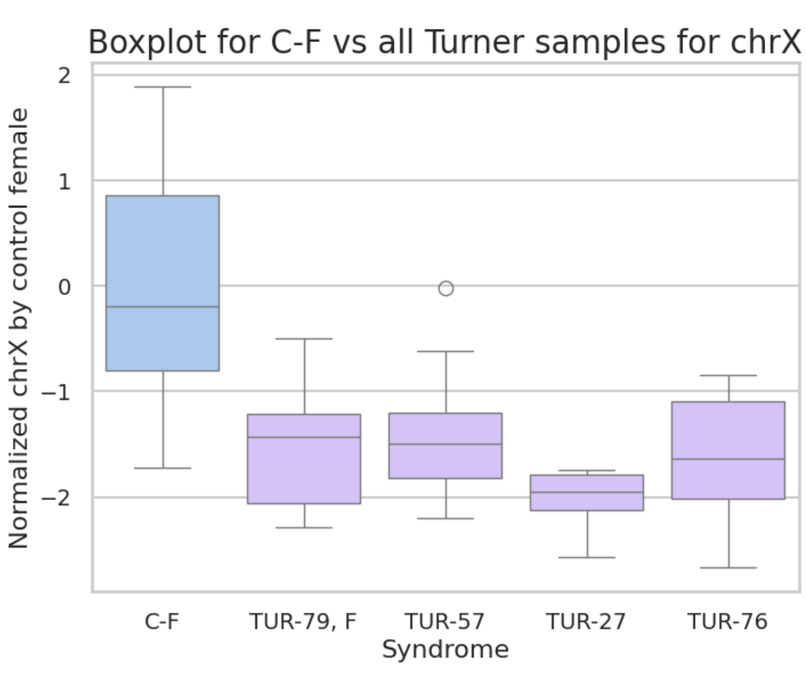


Figure 1: Boxplot of the distribution of chromosome X

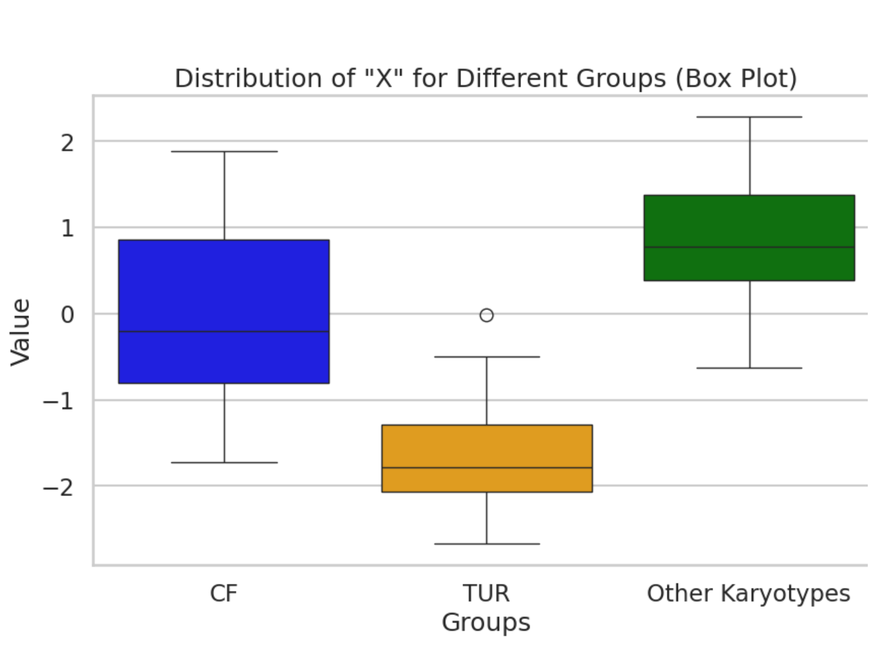


Figure 2: Boxplot of the distribution of chromosome X

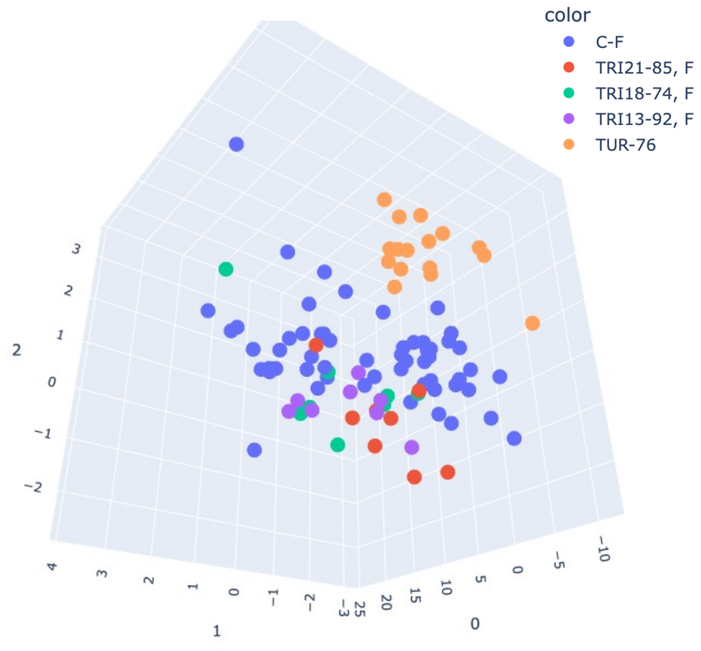


Figure 3: PCA plot for run 01

Figure1 shows four different Turner Syndrome patients (TUR-79, TUR-57, TUR-27 and TUR-76) plotted with healthy female (CF). The plot shows a significant lower amount of chromosome X for Turner Syndrome patients. Figure2 shows the distribution of chromosome X across healthy female (CF), Turner Syndrome samples (TUR groups) and other trisomies (Other karyotypes). Figure3 represents a PCA plot for run01 with a clear significant cluster-tendency of Turner Syndrome samples amongst other karyotypes.

Results

	p-value	Hotelling T-square test
CF vs all others	1.11E-16	377.05
TRI21 vs all others	4.44E-16	134.92
TRI18 vs all others	1.11E-16	786.98
TRI13 vs all others	1.97E-14	90.38
TUR vs all others	1.11E-16	1210.68

Training set	Test set	FN	FP	TN	TP	Sensitivity	Specificity	Accuracy
run01,02,03	run04	1	-	80	15	93.75	100	98.96
run01,02,04	run03	2	-	80	14	87.5	100	97.92
run01,03,04	run02	1	-	80	15	93.75	100	98.95
run02,03,04	run01	-	1	79	16	100	98.75	98.96

Statistical analysis was performed using Hotelling T-square test.

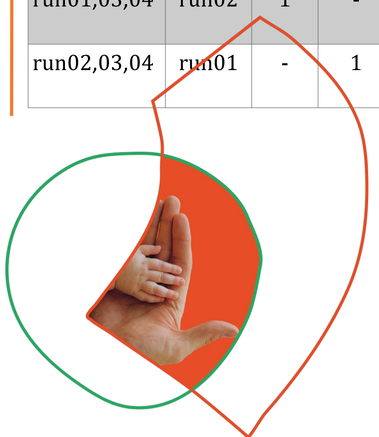
- The results obtained for the p-values show that there are statistically significant differences in the multivariate means between each syndrome and the combined group of all other syndromes, with p-values indicating extremely low probabilities.

Conclusion

- The findings of this study demonstrate the feasibility and effectiveness of the SCAN assay in detecting Turner Syndrome amongst other chromosomal aneuploidies.

-With its high diagnostic accuracy of 98.69 % on sample level, and a 100 % on a patient level (on four technical replicates), SCAN consist of the possibility to be extrapolated as an assisting decision supporting tool for detection and classification of Turner Syndrome.

-Furthermore, SCAN possess the ability to detect mosaic karyotypes (LoD = 55.1 %). Though, this LoD might not seem equivalent to karyotyping, however, by further exposing the model to a larger dataset, and exposure to mosaic karyotypes, we believe that SCAN can improve, and reach a lower level of LoD.



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