HELSE SØR-ØST

Title: TRICXY-MINDS: Clinical insight into sex TRIsomies for enhanced psychiatric Care in individuals with X and Y chromosomal aneuploidies

1. Introduction

Mental and behavioural difficulties – a challenge for individuals with sex chromosome aneuplodies

The high heritability of mental disorders signals that improved understanding of the underlying genetics is key to optimizing mental health care. TRICXY-MINDS (Fig 1) focuses on specific genetic disorders with increased risk of mental disease. Using complimentary well-established population and clinical samples, we will identify health profiles throughout the lifespan, from childhood to adulthood, and provide innovative personalized intervention for a larger group of rare disorder patients to enable improved health outcome.

1 in ~400 babies is born with an atypical number of sex chromosomes (termed sex chromosome aneuplodies, SCAs)[2], making SCAs the most common chromosome abnormalities in humans. SCA carriers often display complex disease patterns with somatic challenges including syndromic features and infertility[2]. Additionally, many experience behavioral and cognitive challenges reflected in odds ratios of 2 or higher for several mental disorders[3]. This indicates a substantial subgroup of patients in the psychiatric clinics (1-2 in 200 patients) that may have specific characteristics that could inform tailored treatment. This project represents an opportunity to improve health outcome of these individuals. To this end, we will address these questions:

1. **Do early life SCA health trajectories and standard of care differ between those with and without a genetic diagnosis?**

Less than 25% of SCAs are genetically detected[4] since the majority are not recognized and thus never referred for genetic testing. When detected, this is usually due to syndromic features in adolescence or later[2]. This leaves a considerable knowledge gap on early disorder trajectories, which is an essential period for shaping outcome. Population studies suggest the same level of psychiatric diagnoses in those without knowledge of their SCA[3,6] but the evidence is sparse. A genetic Klinefelter diagnosis (men with an extra X-chromosome) has been associated with lower medical comorbidity than in those with undetected Klinefelter[6], indicating a benefit in detection. More knowledge is needed on genetic and environmental factors that influence if and how detection benefits comorbidity.

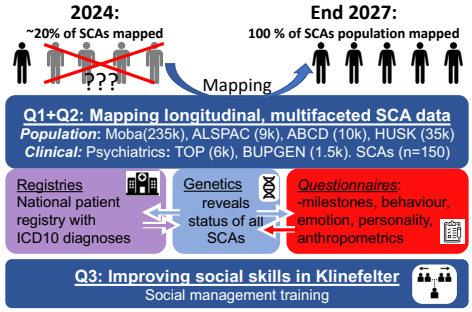

Fig. 1: TRICXY-MINDS' ambition – map health trajectories of SCA carriers (detected and undetected) in Norwegian multi-faceted cohorts and pilot a social management intervention for individuals with Klinefelter for better clinical care.

To improve knowledge on SCA outcome, we will map health trajectories of young SCA carriers, compare with relevant psychiatric patient groups and examine if health outcome differs between those with and without a genetically detected SCA. Such distinction is increasingly important in a healthcare setting where the benefits of genetic testing is under debate.

2. **What are the health and symptomatic profiles of adult SCA carriers in Norway?**

The different groups of SCA carriers have overlapping but also distinct profiles[8]. To provide a road map for better health care for adults in Norway with SCAs, we will map the symptomatic profile of each clinical SCA carrier group. Our preliminary analyses showed that adolescents with detected Klinefelter had similar or higher levels of anxiety and externalizing problems when compared to adolescents under treatment for their anxiety in the clinics[9]. Likewise, case reports exist of SCA carriers displaying treatment resistance[10-12] – but whether such patterns is the norm or the exception is unknown. We will examine whether function and symptom profiles differ between psychiatric patients with or without a SCA since adults with SCAs in the psychiatric clinic may have distinct characteristics. This may help psychiatrists to identify specific focus areas when treating a patient diagnosed with SCA.

3. **Can social management training improve mental health for men with Klinefelter?**

Interventions for SCA carriers have primarily focused on the effects of somatic treatment (e.g., testosterone supplements) whereas cognitive and social interventions remain largely unexplored,

although showing efficacy for other diagnoses[13]. One key challenge across sex trisomies including Klinefelter is social interactions[14]. A manual-based intervention 'Social Management Training (SMT)' adapted for men with Klinefelter syndrome, the first of its kind, recently targeted cognitive functions relevant for social competence[13,15]. Post-trial, participants reported improved emotional stability and informants described improvement in self-regulation and increased awareness of social challenges. Thus, SMT may improve emotional stability, self-regulation, and self-reflection in males with Klinefelter. Demonstrating the efficacy of social training can shift treatment paradigms towards a more personalized holistic approach, integrating psychosocial therapies with existing medical (hormone) interventions. We will implement this SMT directly targeted at improving the social skills of men with Klinefelter[15] and test the transferability and efficacy in a Norwegian setting in our clinical SCA cohort.

Feasibility

We will synergistically leverage **both large population and clinical samples already in-house** with *genetic and extensive registry and questionnaire information.* This unique combination allows for a comprehensive, potentially representative overview of SCA-related disease, behavioral and emotional trajectories in Norway. The data includes ~500 SCA carriers (overview in Table 1, details in Boxes 1-3) effectively representing ~3-4 % of the entire Norwegian SCA population across a broad lifespan and context: *A) Children/Adolescents*[1,16], *B) Adults*, *C) Neuropsychiatric patients* and *D) clinical SCA sample*. The feasibility of the SMT is highlighted by our prior experience with interventions[17,18], our enduring research collaboration with the Norwegian Klinefelter Association[9,19,20], and the acquisition of research consents from 53 men with Klinefelter collected in just the past six months.

Table 1: TRICXY-MINDS cohorts.

		n (in 1000)	SCA, est*
A. Adolescents	MoBa children	90	160
	ALSPAC	11	22
	ABCD	10	20
B. Adults	MoBa parents	140	59
	HUSK	35	70
C. Neuropsychiatric	TOP	6	30
	BUPGEN	1,5	7
D. Clinical SCAs			150
			518

n=actual cohort size, *= estimate for number of carriers based on population frequency of SCAs[2] but corrected for ascertainment bias (due to over-representation in psychiatric samples and under-representation due to e.g. infertility in MoBa parents and volunteer bias in population samples that bias against individuals with health challenges).

Target: TRICXY-MINDS will focus on the male and female trisomies (3 copies of the sex chromosomes): Klinefelter syndrome/47,XXY, Jacob's syndrome/47,XYY and Triple X syndrome/47,XXX due to their low genetic detection (fewer than 25 % are detected[4]) and the latter two due to the paucity of research[2]. Turner syndrome (females with a single X chromosome), the last of the four most prevalent SCAs, will be included as a comparison group when data permits.

1.1. Needs description

New knowledge for the health services and potential for improvement: Understanding the genetic underpinnings of mental health is crucial for the future development of mental health care services. SCA carriers live 5-10 years shorter than population means[21-23]; likely an effect of low socioeconomic status and somatic diseases[24]. This stark statistic underscores the potential for substantial healthcare improvement.

The full natural course for those with SCAs is not clear[25]. There are no longitudinal Norwegian studies on 47,XXX and 47,XYY. Internationally, early trajectory mapping of SCA children has also been inherently missing due to the late genetic diagnosis (typically beyond early teens)[4]. This gap underscores the critical need for enhanced clinical understanding, especially with the rise in early diagnosed individuals with the recent introduction of noninvasive prenatal screening[26].

Population studies suggest the same level of psychiatric diagnoses in those without knowledge of their SCA[3,6]. Further evidence is needed to evaluate these findings and, not least, whether timely genetic detection allows for optimized care, better health and quality of life. By leveraging the longitudinal pregnancy-cohort MoBa with questionnaire data, we mitigate the survivor's bias observed in older population studies[27], allow the direct comparison with age-matched psychiatric disease groups AND offer comprehensive data on undetected SCA carriers through detailed information beyond registry-obtainable variables.

Beyond the advanced genetic-clinical mapping, we have added an intervention component to the proposal. Translating and adapting the Dutch SMT program for Norwegian health care responds to a large unmet need for patients and health care providers. The uncertainty about best care practices is

evidenced by the lack of international guidelines for clinicians to provide best quality care for Klinefelter, 47,XYY and 47,XXX. This causes disparities in treatment and variable outcomes. While guidelines exist for Turner and those for Klinefelter are under way (work led by Dr. Gravholt), development for 47,XXX and 47,XYY was deemed premature due to insufficient data[25]. By evaluating SMT delivered online, we respond to the regional health authorities calls for personalized, decentralized, and digital services. We and others have shown that men with Klinefelter experience a high degree of negative psychosocial effects on everyday functioning[19]. Through the SMT, TRICXY-MINDS will offer actionable insights into improving clinical assessments and tailoring interventions to meet the specific needs of SCA carriers.

TRICXY-MINDS results will be incorporated into the work on Klinefelter guidelines through our bi-annual meetings with Dr. Gravholt, our appointed advisor. These guidelines are essential for existing services to provide customized health care for already identified SCA carriers and may, in the future, provide useful indicators for whom to test for the presence of a SCA.

Societal benefits: Overall, SCAs are associated with several risk factors for lower functioning which means reduced quality of life. For service providers and society at large, this translates to significant welfare costs due to reduced social competence, increase in somatic diseases, and diminished educational and workforce participation[24]. If our digital SMT intervention enhances executive function and social skills, its implementation might reduce dropout rates from societal participation, thereby curbing welfare expenses and reducing the need for prolonged follow-up.

Opportunities for generalization and broad application of knowledge: Our SMT program has the potential to shift treatment paradigms towards integrating psychosocial therapies with medical interventions. Although the planned SMT program is targeted for men with Klinefelter, the learning outcome may benefit other disease groups with similar symptomatic profiles incl. 47,XXX and 47,XYY or autism. This latter translational potential is a key for the K.G. Jebsen Centre, which this project is affiliated with.

Likewise, understanding rare genetic neurodevelopmental disorders with distinct etiologies and known high effects sizes may inform general disease mechanisms and psychiatric treatments. For instance, hormone replacement therapy (HRT) is common in Klinefelter syndrome for treating physical symptoms[28]. Low testosterone is associated with more depressive symptoms[29] and testosterone treatment is already in use for treatment against depression although with mixed effects[29]. Consequently, detailed research on individuals where some routinely use HRT may pave new avenues for psychiatric treatments.

TRICXY-MINDS will inform and thus refine health services through improved SCA mapping and a pilot SMT study, potentially influencing new treatment avenues.

2. Hypotheses, aims and objectives

TRICXY-MINDS' **long-term goal** is to improve medical care for SCA carriers. To reach this, we will document SCA health profiles in population & clinical samples, and initiate a targeted intervention building a knowledge basis for novel medical therapies.

Short term aims:

1) Identify young SCA health profiles through mapping of early phenotype trajectories in genetically detected and undetected SCA carriers. We hypothesize that detected SCA carriers have better health profiles than those undetected.

2) Identify potentially distinguishing patterns of the health and symptomatic profile in adult individuals with SCAs including genetic architecture (risk and protective factors). We hypothesize that SCA carriers may have similar or worse health profiles to that of psychiatric patients overall.

3) Test the efficacy of social management training to improve mental health in males with Klinefelter. We hypothesize that Social Management Training (SMT) will significantly improve mental health in males with Klinefelter syndrome.

In the short-term, we expect to provide comprehensive knowledge on the comparative trajectories of individuals with SCAs in Norway and develop, pilot and document SMT efficacy as a treatment to

improve social functioning in males with Klinefelter. In the long-term, we aim to contribute to establishment of guidelines for treatment and provide early intervention programs for SCA carriers.

3. Project methodology

TRICXY-MINDS is organized in three work packages with corresponding activities and milestones including planned dissemination (Fig. 2).

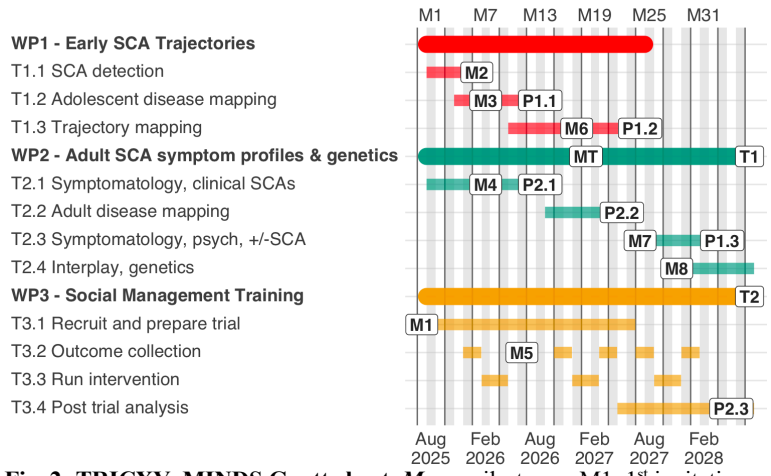

Fig. 2: TRICXY_MINDS Gantt chart. ***M*** are milestones: M1: 1st invitation letters sent, M2: SCA screen of all in-house samples done; M3: QC of diagnosis data done, M4: Clinical data QC done, M5: First trial done, M6: QC of questionnaire data done, M7: Psych symptomatology QC done, M8: PRS calculated. ***Ps, MTs*** & ***Ts*** represent the Papers, Midterm Evaluation and Theses of PhD1 & PhD2. Refer to the online activity plan for paper topics and details.

Participants: This grant will recruit two PhD students: PhD1 (supervisor: the PI) and PhD2 (supervisor: Dr. Fjermestad) and a part-time trial coordinator. The entire team (the two PhDs, their supervisors and the coordinator) will have weekly meetings to ensure timely progress. TRICXY-MINDS is based at the Depart. of Medical Genetics (DMG), Oslo University Hospital (OUS) and affiliated with the K.G. Jebsen Centre for Neurodevelopmental disorders, the Department of Psychology, University of Oslo (UiO) and Frambu. Frambu is part of the specialist health services and develops knowledge on rare diagnoses and disseminate this to patients and the professional environment. Frambu has previously supported interventions headed by Prof. Fjermestad, e.g. the intervention SIBS-RCT[17] and Frambu has agreed to assist with personnel for conducting this SMT. Prof. Fjermestad will recruit research assistants (e.g. psychology master students) as he has done previously from his main UiO position.

Overview of plans: Based at the K.G. Jebsen centre, the two PhD students will immediately initiate the analysis of disease and trajectories (WP1-2) supported by the PI and the K.G. Jebsen biostatistical team. PhD1, focusing on big data in WP1 & 2, will implement the SCA screen with collaborator Andrés Ingason, an expert on rare disorders. PhD2 will focus on clinical data and adult diagnoses data (WP2) initially and then play a central role in the SMT at Frambu, supervised by Prof. Fjermestad. Prior to start and during the project, our trial coordinator at Frambu will recruit participants for the intervention (WP3), coordinate the distribution of questionnaires and help PhD2 and Prof. Fjermestad coordinate with the group leaders for the SMT session (health care providers within psychology and special education from Frambu). The intervention team will be further assisted by psychology master students and interns to enhance feasibility. Dr. Sophie van Rijn's team (initiator, the original SMT) has agreed to help design and operationalize the trial. For knowledge transfer, the entire intervention team will hold a three-day session with the team of Dr. Rijn at the beginning of the project.

Box 1: Description, in-house population samples

The MoBa sample[1] (284k -114k children, 95k mothers, 75k fathers): Prospective pregnancy cohort (Norway, 41% participation rate, born 1999-2009). 84% genotyped[5] on Illumina arrays (only these can be targeted for SCA identification), see box 2. Access: Dr. Andreassen.

ALSPAC (prospective pregnancy cohort, ~11k, Avon Longitudinal Study of Parents And Children, genetics, diagnosis & developmental milestones, Application B3840. Access: Dr Havdahl. https://proposals.epi.bristol.ac.uk/?q=node/130421).

ABCD[7] (10k, ages 10-12, clinical/genetics, http://abcdstudy.org). Access: Dr. Westlye.

HUSK (36k, ages 40-49, 70-74, Hordaland county, husk.w.uib.no). Registry linkage. Extensive questionnaires with focus on chronic diseases (cardiovascular disease, cancer, osteoporosis), anxiety, depression. Access: Dr. Haavik.

3.1. Project arrangements, method selection and analyses

Infrastructure: TRICXY-MINDS has access to the necessary datasets (Table 1, boxes 1 & 3), recruitment of SMT participants (men with Klinefelter already consented for research), personnel (hired through

this project and at Frambu, interns) and computational resources for large-scale storage and analyses at the University of Oslo "Services for Sensitive Research Data" (TSD) (through K.G. Jebsen).

WP1: Early trajectories of SCA carriers to detect differences in standard of care (PhD1, PI)

Background: Clinical SCA carriers display high prevalence rates of mental disorders[30-33], and formal associations of SCAs with any psychiatric disease along with individual disease associations were recently established in a completely population-representative Danish study[3]. Even in the Nordic countries, the majority of SCAs go genetically undetected: Klinefelter (77% undetected), 47,XYY (82%), 47,XXX (93%) and Turner (30%)[4]. This diagnostic gap is even larger in the few other countries with records[34,35]. Along with the low individual frequency of each SCA (Klinefelter: 1 in ~600 males, 47,XYY: 1 in ~1000 males; 47,XXX: 1 in ~1200 females; Turner: 1 in 2000 females[2]), this limits knowledge on this collectively substantial population. Population-scale genotyping now allows for identification of previously undetected SCAs[36,37].

Box 2: Moba trajectories and outcome details
Diagnoses: ICD10 from registries: National Patient Registry (NPR; specialist care data, 2008-) and KUHR (primary care physician's data, 2006-) incl. age of onset of major psychiatric disorders (ADHD, ASD, BP, MDD, SCZ), intellectual disability, epilepsy, relevant somatic diseases (e.g. congenital abnormalities, cardiovascular disease, diabetes, osteoporosis, kidney diseases, infertility). SCAs (Q96, Q97, Q98).
Questionnaires: as reported by (primarily) the mother. *Behavioral and emotional problems:* Symptoms of conduct problems, oppositional defiant problems, hyperactivity and inattention [the Rating Scale for Disruptive Behavior Disorders (RS- DBD)], depressive symptoms [Short Moods and Feelings Questionnaire], anxiety symptoms [Screen for Anxiety Related Disorders], emotional problems [The Child Behavior Checklist (CBCL) at 18 months, 3 years and 5 years & 8 years]. *Temperament and personality measures:* Temperament [Infant Characteristics Questionnaire at 6 months], emotionality, shyness, sociability and activity [Emotionality, Activity and Shyness Temperament Questionnaire at 18 months, 3 years and 5 years], personality [Short Norwegian Hierarchical Personality Inventory, 8 years]. Psychotic like symptoms (CAPE-16, 14-years). *Quality of life:* Satisfaction with life-scale (Q14 & for parents), bullying & friends.

Choice of method: Our approach leverages population-scale genotyping and longitudinal data to provide a comprehensive map of the health trajectories of both detected and undetected SCA carriers to understand their psychiatric and somatic challenges from early in life.

Preliminary results

We have screened ¾ of the MoBa sample and have identified 161 SCA carriers. This initial screen indicates frequencies similar to population estimates for the children while significantly lower in the parents (as expected due to ascertainment bias caused by e.g infertility and volunteer bias). In line with previous indications of higher mental load, MoBa children with SCA trisomies display twice as many mental (ICD10 F-chapter) diagnoses as their peers without SCAs (Table 2).

Table 2: MoBa children with SCAs display significantly higher frequency of mental (ICD10-F chapter) diagnoses than children without SCAs.

	no diagnosis	F diagnosis	frequency diagnosis	Pvalue
Boys	25,331	7,292	0.22	
Klinefelter	20	17	0.46	0.001*
47,XYY	16	13	0.45	0.007*
Girls	23,267	7356	0.24	
47,XXX	14	16	0.53	0.0004*

Question: Do early life SCA health trajectories and standard of care differ between those with and without a genetic diagnosis?

Tasks: ***T1.1 SCA screen*** *(M1-6).* ***T1.2 Disease mapping*** *(M4-13).* In our adolescent samples, we will map the somatic and psychiatric disease trajectories (see Box 2 for specific diagnoses) and *compare outcome* to the general population as well as relevant disease groups (e.g. autism, ADHD) and between detected (based on registry records) and those non-detected (our SCA screen). ***T1.3 Trajectory mapping in MoBa children*** (Fig. 3) (M10-24): We will discover *age-dependent health indicators from pregnancy to adolescence* by comparing SCAs to the groups mentioned above. Based on questionnaire data (Box 2), we will map (longitudinally when data allows): *a) Early psychiatric risk (childhood and youth):* Developmental milestones, child behavioral, emotional and temperament and personality measures (Box 2) and sleep, *b) quality of life* and *c) anthropometrics trajectories* (height, weight and age at menarche).

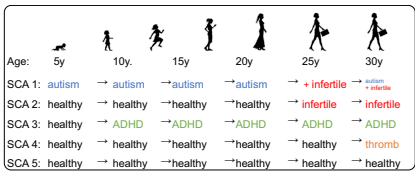

Fig. 3: Suggestive diagnosis trajectories of SCA carriers with sequence analysis.

WP2: Symptomatic health profiles of SCA adults and their genetics (PhD1 + PhD2, PI, Fjermestad)

Question: What are the health and symptomatic profiles of adult SCA carriers in Norway?

Choice of method: The unique combination of clinical symptomatic and population disease mapping incl. genetic analysis (PRS) positions this study to uncover novel insights into adult SCA health profiles.

Preliminary results: Since early 2024, we have collected consent forms from 134 SCA carriers (~1/4 of the 537 SCA carriers known to Frambu) (Table 3). More than half (72) have returned the questionnaire packages (Box 3 for details), and we will continue to follow up to increase this number. Our current SRS2-derived data indicates higher levels of autistic symptoms in all three trisomy groups compared to Turner (Table 4). This points towards the potential utility of social management training (see WP3) in all three groups.

Table 3: Current in-house data on SCAs

	Known	Consents	%
Klinefelter	193	53	(27 %)
47,XYY	58	32	(55 %)
47,XXYY	13	4	(31 %)
Turner	241	44	(18 %)
47,XXX	32	7	(22 %)
Total	537	134	(25%)

Table 4: Autistic symptoms, adult clinical SCA carriers.

		frequency		
Diagnosis	n	normal	moderate	severe
Klinefelter	28	0.57	0.36	0.07
47,XYY	9	0.44	0.56	0
Turner	28	0.79	0.21	0
47,XXX	5	0.40	0.60	0

Tasks: ***T2.1 Mapping of symptomatology in clinical SCA carriers*** *(M1-13, PhD2).* We will map autistic symptomatology (through SCQ and SRS), behavioural and emotional profiles (DBC, SCL-90) in our SCA clinical sample and compare to the profile of individuals in BUPGEN with autism or other rare genetic disease groups with high risk of mental disorders such as 16p11.2 CNV carriers. ***T2.2 Adult SCA disease mapping*** *(M14-22, PhD2 supported by Phd1):* Based on our adult population and clinical SCA samples, we will map diagnoses trajectories (similar to T1.2). ***T2.3 Differences in symptomatic profile in psychiatric patients with and without a SCA*** *(PhD1, M23-33).* We will assess group-wise differences between psychiatric patients with and without an SCA for comorbid disease, age of onset, global functioning, psychotic, affective symptoms, substance use, intelligence (Box 3 for details), socieconomics (education, socioeconomic status (FD-Trygd registry) and treatment resistance (e.g. individuals on clozapine treatment***). T2.4 Interplay between SCAs and common variants*** *(PhD1, M28-36):* One way of estimating genetic disease risk is polygenic risk score (PRS), a single number based on common genetic variants that indicates an individual's level of genomic risk for a particular trait. We will do exploratory interaction analysis for association to specific major psychiatric diagnoses between SCAs and common variants (PRS, as calculated from major psychiatric disorders based on Genome Wide Association Studies (GWAS) summary statistics[38] (GWAS_atlas, http://atlas.ctglab.nl/). Findings will be attempted replicated in Dr Gravholt's SCA and Ingason's iPsych study (see collaborators in section 3.2).

Box 3: In-house clinical samples (all coupled to registry data)

Clinical SCA sample. ~150 consented, still recruiting, 5-60 years. All have journal data, 72 responded to questionnaires where the standard questionnaire package (children) include: Social Communication Questionnaire (SCQ), Social Responsiveness Scale (SRS), Developmental Behaviour Checklist (DBC) and DASS (depression, anxiety and stress scale for next of kin). Adult package: SRS2 & SCL-90 list 90 (SCL-90) - a questionnaire on symptoms of psychiatric disease. PI: Dr. Nærland

Psychiatric TOP general biobank: 6k severe mental disorder (~2k bipolar, 1k major depress, 3k schizophrenia, ongoing recruitment) and 6k controls. Symptom and outcome indicators through clinical assessments: Positive and Negative Syndrome Scale (PANSS), Young Maia Rating Scale (YMRS), the clinician-rated Inventory of Depressive Symptoms (IDS-C), Alcohol Use Disorders Identification Test (AUDIT), the Drug Use Disorders Identification Test (DUDIT), IQ, Global assessment of functioning scale (GAF-symptom and GAF-function). PI: Prof. Andreassen

The neurodevelopmental (NDD) BUPGEN/K.G. Jebsen general biobank: 1470 participants suspected of NDD from all Norwegian health regions (BUP). All participants have DNA genotyping, journal and registry data (NPR, KUHR etc). Depth of journal varies. Most include somatic & and phenotypic data (incl. medical history) and some tests e.g. for ASD symptomatology or IQ scoring. PI: Dr. Nærland.

WP3: Social Management Training program to inform intervention strategies (PhD2, Fjermestad)

Question: Can Social Management Training improve mental health for men with Klinefelter?

Choice of method: We build on the expertise of the Dutch team which was the first to develop and evaluate an intervention program tailored for male SCAs. The SMT program aims to train executive functions of importance for social functioning by improving knowledge on specific topics, practicing new skills, and receiving feedback. The program rests on the knowledge that enhanced working memory and attention increases understanding of social cues and that better planning skills are

associated with enhanced mental health[39]. The original SMT program targets social information processing, attention, inhibition, emotion, flexibility, planning and working memory in a social environment.

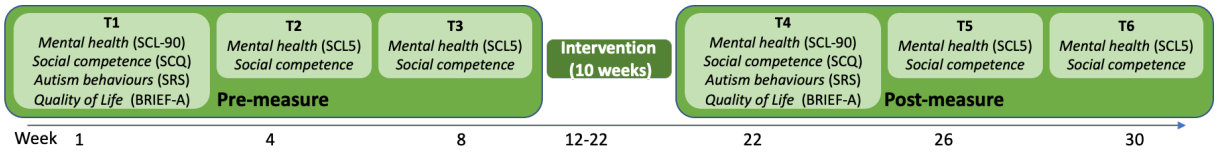

Fig. 4: Outline of the 30 weeks Social Management Training Program for individuals with Klinefelter including outcome measures. The intervention lasts 10 weeks. Prior to the intervention (at 3 time points, T1-T3) and after (at 3 time points, T4-T6), we will collect outcome data from both informants and participants through the use of questionnaires directed at 1) mental health, 2) social competence, 3) autism-like behaviours and 4) quality of life.

Preliminary evidence: The Dutch pilot SMT trial demonstrated pre-post changes in emotional and behavioral problems and social competence with effect sizes (*d*) from 0.50 to 1.07. These are promising findings given the modest sample size of 18 men aged 16 to 56 years. In TRICXY Minds, we build on the pilot with several enhanced design features: a) A doubling of participants (from 18 to 36). Albeit 36 appears modest, it is decent for a rare disorders sample and sufficient to detect effects based on the pilot results. b) Implementation of a controlled multiple baseline design (improving internal validity) in which pre-intervention change is compared to pre-post intervention change. This means the participants will act as their own control group. We considered alternative control conditions, such as treatment-as-usual, but this does not exist for this group. Using waitlist controls often leads to inflated effect estimates and is ethically problematic. Finally, since no tailored interventions except SMT exist, we could not choose an active control condition. c) Adaptation to online SMT delivery for increased feasibility and applicability (this will make it easier for participants across Norwegian regions to participate). The original team will help us operationalize and we will co-design with users. After the trial, we will perform an individual patient data (IPD) meta-analysis with the original SMT for outcome measures overlapping.

Outcome: The SMT pilot used a wide selection of outcome measures. After careful consideration we decided to focus on mental health as the main outcome, while assessing multiple other health-related quality of life items at baseline and final follow-up. We chose mental health as the main outcome as the multiple social cognitive challenges SMT targets are believed to be key to emotional and behavioral problems for men with SCAs. To reduce the burden on participants/informants while securing sufficient data quality, we only use the full outcome set twice (at T1 & T4) while the other measure points (T2, T3, T5 & T6) will use a validated 5-item version of SCL, SCL-5, and SCQ only.

Setup: The SMT consists of 10 group sessions (Table 5) over 10 weeks including 4-8 participants and led by two therapists from Frambu. Importantly, session 7 includes a parent, friend or a partner, depending on the participants' preference. Each SMT session (90 minutes with breaks) will follow the Dutch pilot model: Each session starts with a summary and evaluation of the previous session, an introduction of the new topic, psychoeducation, cognitive-behavioral exercises, and finally instructions for the take home assignment. At the end of the 10 weeks treatment program, there will be one individualized session for each participant with a therapist to discuss the results of the assessment.

Table 5. Overview, the SMT sessions

	Topics
1	Introduction and learning targets
2	Information process in a social context
3	Attention and emotions in a social context
4	Inhibition and emotions in a social context
5	Flexibility and planning in a social context
6	Working memory in a social context
7	Parent/friend/partner session
8	Individual learning targets
9	Individual learning targets
10	Summary/integration

Tasks: ***T3.1 Recruitment and preparation*** (coordinator, M1-M23)**:** The 36 participants (6 groups with 4-8 participants in each) will be recruited widely through our consented BUPGEN clinical sample, Frambu, hospital advertisements and via the Norwegian Klinefelter Association (see below). Inclusion criteria: age > 16 years, IQ > 70, and a genetic Klinefelter diagnosis. We will not exclude participants based on their mental health severity levels or co-morbidities to make delivery as realistic for the group characteristics as possible. *The SMT* is developed at Leiden University, The Netherlands, in collaboration with the department of Clinical Child and Adolescent Studies, Leiden University, and thus needs to be translated from Dutch. ***T3.2 Outcome measures collection*** *(coordinator, M5-M30).*

Through our multiple baseline design, we will assess the outcome variables 3 times before intervention, and 3 times post-intervention (Fig. 4 for details) for each individual. ***T3.3 Running the intervention*** *(PhD2, Frambu personnel, interns, M7-28)***.** We will pilot the trial once in M7 with one group, adapt and incorporate input from users and follow-up with two and three trials respectively run in parallel in the following spring/autumn (see Fig. 2). Online platform for intervention: join/helsenett. ***T3.4. Evaluation of the trial*** *(PhD2, M22-36)***:** The effectiveness of the intervention will be conceptualized as the mean difference in trajectories across the three pre-intervention points and the three post intervention points on the four outcome domains as noted in Fig. 4 legend. Beyond effectiveness, we will assess acceptability and feasibility by examining user satisfaction and attrition modelled by the Dutch pilot trial.

Risk and power assessment across the work packages

Data for WPs1-2 are in-house and preliminary analyses indicate that we have power (Tables 2 & 4). We will attempt to expand the clinical SCA and adult sample through Frambu and inclusion of population cohorts, e.g. Tromsø and HUNT. In the psychiatric sample, we estimate ~30 SCAs (see Table 1), which is likely to make this specific analysis descriptive (as they are probably split on different SCAs). SMT recruitment will be ensured through our regular contact with the Norwegian Klinefelter Association and Frambu users. Mitigation strategies include personalized follow-ups through phone (also for drop-outs), and expanding the intervention to 47,XXX and 47,XYY (who display high levels of autistic symptoms, Table 4). It is risky to base a PhD on an intervention – thus, the first two manuscripts of PhD2 will be based on already collected data.

Bioinformatic and statistical analyses

SCAs have overlapping characteristics, but also distinct profiles[8]. Thus, each individual SCA will be analyzed separately. All statistical analyses will be performed in R. ***SCA screens*** (T1.1)**:** We will identify SCAs in our population and psychiatric samples through already established protocols[3,36,37] including visual inspection of all Log R Ratio/B Allele Frequency plots of the X/Y chromosomes to rule out false positives. ***Disease association testing*** *(T1.2 & T2.2):* Logistic regression models with SCA vs males/females, respectively, adjusting for relevant covariates such as age, BMI and socioeconomic status. **Trajectory analyses** (T1.3)**:** We will apply advanced longitudinal statistical approaches such as *mixed models* using latent classes and latent processes (LCMM), growth curve modeling, and sequence analysis (Fig. 3) on the chosen variables. We will stratify on the presence of a psychiatric diagnosis where relevant. **Symptomatic analysis** (T2.1 & T2.3): We will use one-way analysis of variance (ANOVA) for continuous normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables and the Chi-Square test to compare categorical data. We will perform sensitivity analysis with matched controls (given the high number of psychiatric patients vs SCA carriers). **Interaction analysis** (T2.4)*:* A nested case-control analysis of values for individuals using standard least squares analysis comparing means for all pairs using Student's *t*-test for specific pairs and Tukey–Kramer HSD for multiple comparisons. **Trial outcome analysis (WP3):** We will apply generalized estimating equations (GEE) to examine the symptom trajectories for participants across the six measurement points while accounting for clustering in groups. GEE is appropriate in designs with few clusters (i.e., 6). We will compare the symptom trajectory across the 3 pre-SMT time points (T1-T3) to the trajectory across the 3 post-SMT time points (T4-T6) as an indicator of SMT effects (multiple baseline design).

3.2. Participants, organisation and collaborations

Dr. Sønderby (PI) heads the K.G. Jebsen rare genetic variant efforts, has several independent grants, a strong publication record in rare genetic variants and project management experience from consortia (see CV and publication list). WP3 leader, Prof. Fjermestad (Frambu & UiO) has extensive knowledge on SCAs and has led several interventions[17,18] in collaboration with Frambu. Both have successfully supervised PhD students (2 & 9, respectively) and they will be the main and co-supervisors for the two hired PhD students. **Teaching environment of PhD students:** The PhD Students will be enrolled at KLINMED, UiO, and integrated in the 'Rare Genetic Variants' group (Sønderby) and the group of Prof. Fjermestad at UiO/Frambu. They will be affiliated with the Department of Psychology, UIO, and the K.G. Jebsen Centre biostatistical group and Frambu to promote knowledge exchange.

Nationally, Frambu (as represented by Kristian E Kristoffersen, director) will assist with personnel for conduction of the intervention (se role in portal). SCA individuals will be recruited with the aid of Dr. Nag, education specialist, Frambu, through the extended BUPGEN clinical network and the Norwegian Klinefelter Association. Kari Velsand leads and represents the Norwegian Klinefelter Association (https://klinefelter.no/) as a reference group member. These players will provide expertise and give access to unique datasets: Dr. Nærland (PhD, head of the K.G. Jebsen Centre): access to BUPGEN and autism expertise. Prof. Andreassen (psychiatrist, director of NORMENT): access to TOP and MoBa and expertise on psychosis with Olav Smeland (PhD, treating psychiatrist). Alexandra Havdahl (PhD, psychologist): access to ALSPAC and expertise on autism. Prof. Haavik (MD, psychiatrist): access to HUSK and expertise on ADHD.

Three **international collaborators** will play **central roles**: *Assoc. Prof. Rijn*, Leiden University, NL, is the main author of the Dutch SMT, and her team has agreed to adapt and further advice the Norwegian trial. *Prof. Gravholt*, MD/PhD, Århus University, Denmark, expert on treatment of and research on SCAs with a long-standing collaboration with the current group has agreed to be advisor on the project with bi-annual meetings. All collaborators will be invited to these meetings for updates. *Dr. Ingason*, Region H, Denmark, an expert on large-scale rare genetic variant analyses, has developed an optimized protocol for SCA detection[3] – he will host PhD1 for a research visit to implement the protocol. Dr. Ingason's access to the iPsych population sample (n=140k) and Dr. Gravholt's SCA sample constitute a possibility to replicate findings (e.g. interaction between SCAs and polygenic risk scores) down the line.

3.3. Budget

The proposal will recruit two PhD students and a 15 % trial coordinator (2 x 1,363,000 NOK and 154,500 NOK per year). We estimate running costs for translation of the SMT protocol, publications, registry data and travel at 300,000 NOK. The total cost is thus: 8,941,500 NOK. In kind: Frambu will provide personnel for the intervention and the KG. Jebsen centre will cover computational access fees.

3.4. Plan for activities, visibility and dissemination

Please see Fig. 2 and the online form for milestones and activities including tentative topics of the six intended peer-reviewed (Open Access) publications aiming for high-impact journals. Both the PI, the PhD students and Dr Fjermestad will present the results at conferences including at the international workshop on sex aneuploidies[40]. As in the past (see CVs), the team will perform educational activities towards clinicians (genetic councillors, psychiatrists, Frambu educational courses), psychology and medical students (also by including students in the project) and user organizations (e.g. Norwegian Klinefelter Association, user meetings at Frambu). The latter also allows for user feedback. Results will be disseminated through social media, X (twitter) and traditional communication channels (press releases, Frambu website), ensuring that new knowledge reaches the greater public in a format relevant and understandable to them.

3.5. Plan for implementation

Overall, we aim to improve prognostic information for individuals with SCAs. Our international advisor, Claus H. Gravholt, chaired the new international guidelines on Turner syndrome[17] and is involved in developing those for Klinefelter[40]– he will be regularly updated on the project progress and can include our results in this important work. The project's embedding in a diagnostics department (DMG, OUS) and Frambu Competence Centre for Rare Diagnosis counseling individuals with genetic diagnosis directly helps implementation. To reach clinicians broadly, we will describe our results in 'Tidsskrift for den norske legeforening' and 'Dagens medisin' (medical journals for health care personnel) and be present at 'Psykiatriveka' (Norwegian Psychiatric Association Annual Congress) to emphasize what may enhance treatment of SCAs carriers in psychiatry. We will provide educational activities to clinicians through the extensive psychiatric clinicians' network at the K.G. Jebsen and NORMENT research centres. Beyond the project period, we will disseminate results and offer training to primary and specialist health services if the results are promising. This way, we prevent health services from wasting resources on ineffective interventions, and we save project costs.

4. User involvement

Clinical relevance and patient impact are key factors contributing to the success of the project. We will communicate scientific results beyond academia and integrate perspectives from patients and their families. Our reference group member, Kari Velsand (the Norwegian Klinefelter Association), reviewed the current proposal and commented 'There is a lot of discussion about mental health, social exclusion, suicide, and social problems these days. To take this seriously and be able to address it, it is important to have the facts that a project like this provides'. TRICXY_MINDS' pilot analyses[9] were discussed at the Norwegian Klinefelter Association annual meeting 2023 at workshops (led by Fjermestad). We will consult Kari Velsand to ensure sensitive communication of results to the public regarding a vulnerable patient group. Alternative communication channels with patients and families include presentations at Frambu and user organizations (the Klinefelter Organization), and the established K.G. Jebsen user-board with the Autism Association and rare disorder representatives.

5. Ethical considerations

Due to the sensitive nature of the data, including genetics and disease information, we have obtained ethical approvals: MoBa Genetics: REK 2016/1226. Please note that MoBa is not allowed to deliver genetic information back to its participants – likely, the most severely affected SCAs carriers have been identified. BUPGEN can enroll participants based on genetic status including all SCAs (REK 2009/932). The TOP project was approved by IRB (REK 2009/2485 & 2016/1226) and the Norwegian Data Protection Authority to collect and store sensitive information until 2050. The intervention work package follows the XXY Nordic Study (REK 2018/1061). Small changes required for the details of the Social Management Training will be submitted in autumn 2024. All research activities will be performed according to these approvals to ensure participants' personal integrity and health. All data will be collected, stored and analysed on Services for Sensitive data (TSD). The project will be carried out in accordance with the Helsinki declaration, and we will carefully implement the Responsible Research and Innovation (RRI) framework developed by the European Commission.

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