Genotype-Phenotype feasibility studies on khat abuse, traumatic experiences and psychosis in Ethiopia

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Introduction

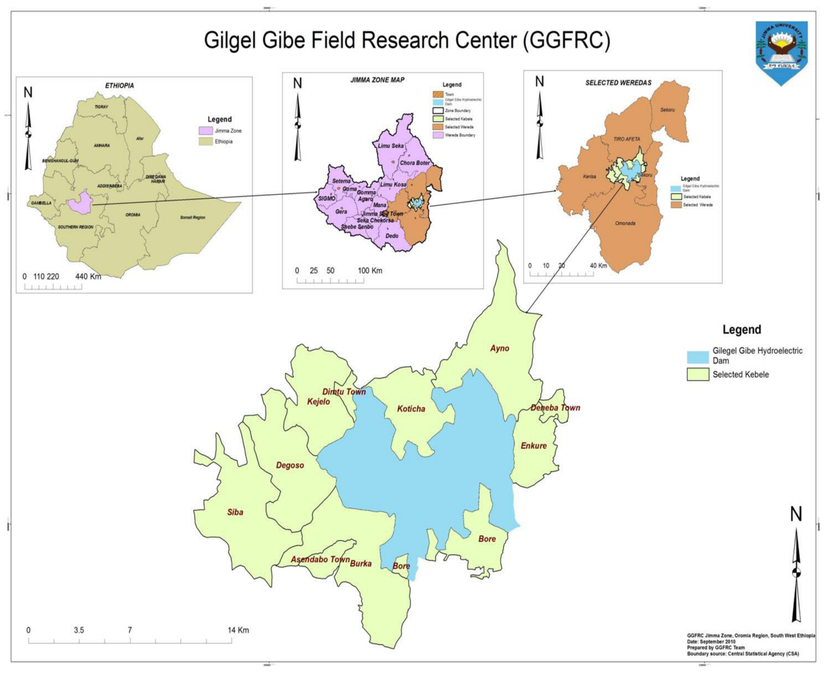
Khat trees are native to East Africa and the Arabian Peninsula; their leaves contain amphetamine-like alkaloids such as cathinone, cathine, and norephedrine and are chewed for their stimulating and euphorigenic effects. Chewing khat has a long tradition among specific ethnic groups but due to increased crop cultivation it has spread further among the male population. Khat use varies by season: in the dry season, there is limited availability and market prices are high; in the rainy season, the opposite is true. Typical patterns of consumption range from moderate to problematic. Excessive use is associated with dependence and khat-induced psychosis.

Pilot Study

Environmental factors are considered important factors in the etipathogenesis of psychosis. In a pilot study we reported on the environmental factors khat use and traumatic experiences and their association with psychotic symptoms in the general population of Southwestern Ethiopia.

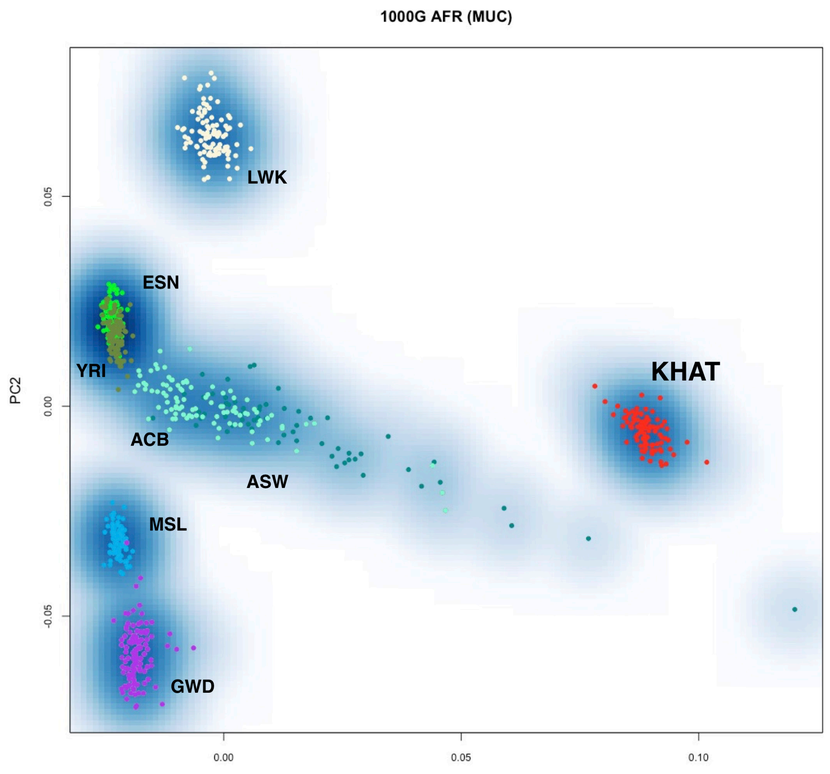
(Adorjan K […] Schulze TG: Khat use and occurrence of psychotic symptoms in the general male population in Southwestern Ethiopia: evidence for sensitization by traumatic experiences, World Psychiatry, October 2017).

In collaboration with the Gilgel Gibe Field Research Center of Jimma University, we studied khat use and khat-induced psychotic symptoms in a survey of 1,100 men aged 18 to 40 years (M 28.4; SD 6.6), selected from the center's population registry by stratified (rural, urban) cluster random sampling. Initial participation rate was 79.1%. Total population is 60,000, an estimated 63% of men use khat.



Results - Phenotype

Our findings that either high lifetime trauma or recent trauma in presence of low lifetime trauma are associated with elevated presence of khat-induced psychotic symptoms fit with the hypothesis that individuals with traumatic experiences are sensitized to the psychotomimetic effects of khat. The sensitization model of psychosis suggests that repeated administration of amphetamines or exposure to stress can cause sensitization of dopamine neurons and consequently a higher dopamine release. Sensitization of presynaptic striatal dopaminergic activity is thought to be a mechanism in developing positive symptoms and psychotic episodes. We found that the sensitization by traumatic experiences for the psychotomimetic effects of substances might not just be limited to childhood experiences but might involve experiences across the whole lifetime.

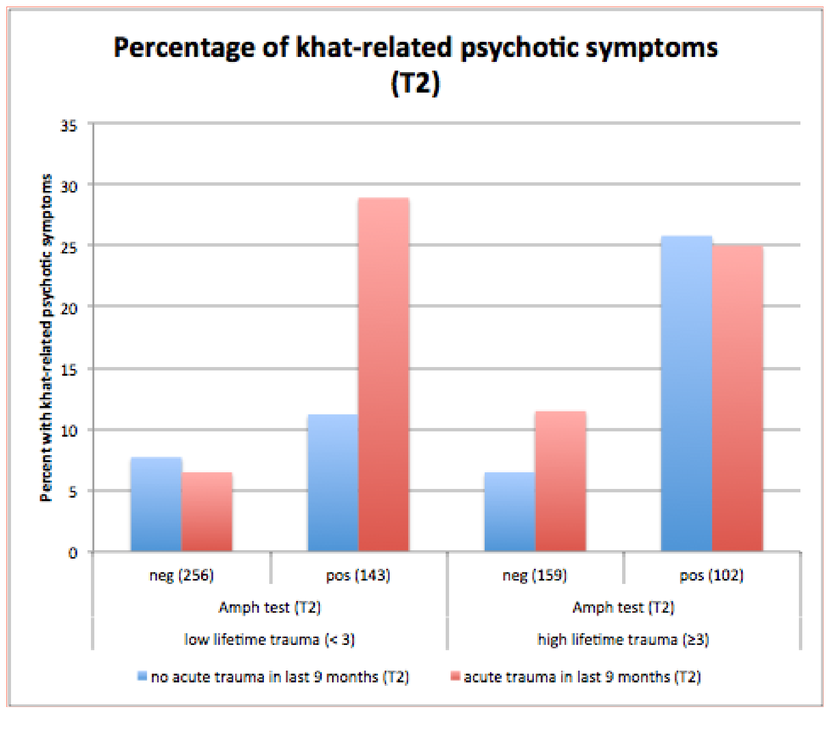


Figures 1. Legend to Figure 1: ACB: African Caribbean in Barbados (n=96); ASW: Americans of African Ancestry in SW USA (n=61); ESN: Esan in Nigeria (n=99); GWD: Gambian in Western Divisions in the Gambia (n=113); LWK: Luhya in Webuye, Kenya (n=99); MSL: Mende in Sierra Leone (n=85); YRI: Yoruba in Ibadan, Nigeria (n=108); KHAT: Gilgel Gibe Field Research Center (GGFRC), Southwestern Ethiopia, located 55 km northeast of Jimma City (n=96). PC1: principle component 1; PC2: principle component 2.

Design

Design of the study	T1 Screening	T2 Validation interview	T3 Re-Screening
Performed by:	Local interviewers	Psychiatrist or mental health Specialist	Local interviewers
N: Men 18-30 years of age	N = 852	N = 126	N = 852
Sampling: Multi-stage stratified random sampling	Rural-urban,existing epidemiological cluster, Household, Individual	Randomly selected subgroup of T1; 50 with psychotic symptom presentation, 50 without	Re-assessment of all respondents from T1
Interview-based measures	CIDI	BPRS	CIDI
Biological measures	Urine: Khat alkaloids (amphetamine tests)	Urine: Khat alkaloids (HPLC for NE) Blood: DNA Extraction	Urine: Khat alkaloids (amphetamine tests)
Additional measures	1. Transport conditions, (storage, refrigeration) 2. Time until samples reach laboratory	1. Transport conditions, (storage, refrigeration) 2. Time until samples reach Laboratory	1. Transport conditions, (storage, refrigeration) 2. Time until samples reach laboratory

Khat use - psychosis - trauma



Acknowlegements

This study was supported by the Lisa Oehler Foundation

DNA Testing

We tested the quantity and quality of the DNA extracted in laboratories at JU. For DNA extraction we established a new method on site: from a total of 100 whole venous blood samples DNA was extracted using DNA Blood10k Kits (chemagic, Perkin Elmer). The purity and homogeneity of the samples was tested by spectrophotometric measurements. For photometric determination of the concentration of DNA we used Bio Photometer technology with absorbance measured at 260 nm. The samples were genome-wide genotyped using the Infinium Global Screening Array BeadChip (Illumina). This BeadChip includes a multi-ethnic genome-wide content which was selected at minor allele frequencies of > 1% across all 26 1000 Genomes Project populations including cohorts from Africa.

Results - Genotype

The genotyping of all individuals in the genetic pilot study was successfully completed with high quality. The overall sample call rate after genotyping was 99.21%. We followed standard quality control (QC) protocols to process our data including parameters such as call rate, deviations from HWE, minor allele frequency (MAF) and others. A total of around 2.54% of the SNPs (17,765 SNPs) were removed due to low call rate (98%), 1.67% of SNPs (11,401 SNPs) were removed due to deviations from HWE, and 42.19% (283074 SNPs) showed a MAF < 1%. Please note that a sample size of only 100 individuals doesn't allow for a precise estimation of MAF in this sample cohort. One person needed to be excluded due to low call rate (98%) and an additional 3 individuals were removed to due relatedness within the sample (pi-hat > 0.2 after additional QC and LD pruning following standard protocols). We found no signs of sample contamination (based on inbreeding coefficients).

After QC we subjected our sample to principle component analysis (PCA) to identify potential sample heterogeneity. Furthermore, we were interested to map our cohort in comparison to the African samples that have been part of the 1000 Genomes project. For this purpose we merged our data with the 1000 genomes data for the African populations (661 samples in total). Figure 1 shows the result of this analysis.

As expected, initial GWAS (using a linear regression with PCA covariates 1-4) of the norephedrine concentration in urine in the genotyped markers (n=387,836) did not reveal any reliable association (data not shown). Next steps will include imputation of our study samples using appropriate datasets beyond inclusion of the 1000 genomes from Africa.

Conclusion

Encouraged by the results of our pilot studies we are currently preparing the full study of a sample size of 10,000 to study genetic variation and gene-environment interactions in Ethiopia, especially the relationship of khat abuse and the development of psychotic symptoms and its interactions with genetic factors. The GGFRC offers a unique opportunity to build well characterized collectives of individuals and to perform genetic studies that so far have not yet been undertaken in Ethiopia at this scale and that will supplement other efforts currently underway in Africa. Moreover, we will be in the unique position to study the relationship of khat use, psychosis and trauma. The information obtained by this pilot study on DNA is instrumental for the preparation of a comprehensive genetic study in a developing country.