

RESEARCH ARTICLE

Shared Genetic Factors of Anxiety and Depression Symptoms in a Brazilian Family-Based Cohort, the Baependi Heart Study

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Abstract

To investigate the phenotypic and genetic overlap between anxiety and depression symptoms in an admixed population from extended family pedigrees. Participants ($n = 1,375$) were recruited from a cohort of 93 families (mean age \pm SD 42 \pm 16.3, 57% female) in the rural town of Baependi, Brazil. The Hospital Anxiety and Depression Scale (HADS) was used to assess depression and anxiety symptoms. Heritability estimates were obtained by an adjusted variance component model. Bivariate analyses were performed to obtain the partition of the covariance of anxiety and depression into genetic and environmental components, and to calculate the genetic contribution modulating both sets of symptoms. Anxiety and depression scores were 7.49 \pm 4.01 and 5.70 \pm 3.82, respectively. Mean scores were affected by age and were significantly higher in women. Heritability for depression and anxiety, corrected for age and sex, were 0.30 and 0.32, respectively. Significant genetic correlations ($\rho_g = 0.81$) were found between anxiety and depression scores; thus, nearly 66% of the total genetic variance in one set of symptoms was shared with the other set. Our results provided strong evidence for a genetic overlap between anxiety and depression symptoms, which has relevance for our understanding of the biological basis of these constructs and could be exploited in genome-wide association studies.

Introduction

The National Comorbidity Study in the US estimated that depressive disorder has a lifetime prevalence of approximately 16%, making it one of the most frequently diagnosed psychiatric disorders [1]. By 2020, major depressive disorder is projected to be the second leading cause of disability worldwide [2]. Anxiety disorders have a lifetime prevalence estimated to be as

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high as 18%; thus, they are also one of the most frequent psychiatric diseases in the general population [1].

Depressive and anxiety disorders have a complex etiology, involving both genetic and environmental factors. Heritability estimates range between 31% to 42% for major depressive disorder and between 20% to 40% for anxiety disorder [3, 4]. An important issue in the search for risk factors of anxiety and depressive disorders is the frequent co-morbidity between those phenotypes [5]. Depression and anxiety symptomatology are strongly linked [1, 6, 7], and the co-morbidity between those disorders results in lower social competence, in addition to more severe symptoms than those patients diagnosed with a single disorder [8].

One way to uncover additional information about the relationship between anxiety and depression is to examine the extent of shared genetic factors that influence these traits. Studies investigating the heritability of combined depression and anxiety disorders published to date have been based on twin samples mostly of European ancestry and, have shown a high genetic correlation between anxiety disorders and major depression [5]. An alternative approach that can aid in the identification of genetic susceptibility factors in those disorders is phenotypic variation. Depressive and anxiety disorders can be conceptualized as extreme diagnostic entities in a continuum of varying severity. Therefore, instead of relying on categorical phenotypes only, quantitative analysis of depressive and anxiety symptomatology can also improve the detection of meaningful genetic risk markers [9]. Indeed, heritability estimates were independently obtained from twin studies in the Netherlands and Australia showing that a common genetic factor is responsible for most of the variance in the phenotypic presentation of combined anxiety and depressive symptoms in the general population [10, 11].

The current study used the Hospital Anxiety Depression Scale (HADS) [12] to examine the heritability and shared genetic factors between symptoms of anxiety and depression from large extended families of a population that is highly admixed, mainly along the axis between European and African ancestry. The population chosen, the Baependi Heart Study cohort [13], is uniquely suited to our study purpose. Located in a rural town in Brazil (18,307 inhabitants recorded in the 2010 census), the study population is remarkably homogeneous in terms of their lifestyle, which remains very traditional. Thus, the ethnic background, living conditions, and family-based study design representing large and complex pedigrees, offer potential novel insights into the biology and genetic basis of depression and anxiety as well as other behavior traits [14].

Materials and Methods

Participants

This study included 1,375 individuals (57% female) belonging to extended pedigrees (95 families) aged 18–98 (mean \pm SD = 42.52 \pm 16.28) from the Baependi Heart Study, a genetic epidemiological study with a longitudinal design, who provided valid data to this study. The pedigrees had mean size of 24.15 \pm 31.79 members and were from 3 to 4 generations in their majority (63%). There were 640 sibships with mean size of 2.45 \pm 1.87 and the following numbers of main pairs of relatives were: parent/offspring– 3138; sibling/sibling– 2253; grandparent/grandchild– 2590; avuncular– 4418; half siblings– 40; and cousins: 3743. Further characteristics of the population and the sampling methodology have been described previously [13]. 6.4% ($n = 88$) of the participants were using antidepressant medication at the time of the interview. Subjects with cognitive impairments were not included. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was approved by Ethics committee of the

Hospital das Clínicas, University of São Paulo, Brazil. Participation in the study was voluntary, and each volunteer provided informed written consent before participation. For illiterate participants or for those without the capacity to sign, the consent term was completely read by a researcher in the presence of an authorized representative of the volunteer, usually a relative, who signed the authorization according to the participant decision. Data were collected between December 2005 and January 2006.

Instruments

Symptoms of depression and anxiety were assessed using a Portuguese translation of the HADS questionnaire made and validated for use in Brazil [15]. The scale consists of 14 self-administered items, with seven questions relating to anxiety and the other seven to depression [16]. The questions are based on a four-point Likert scale with scores ranging from 0 to 3. The Portuguese version of HADS has previously been shown to be easy to understand, and to have a high sensitivity and specificity [17]. The questionnaire was completed by the participants in the permanent field station of the Baependi project, and was read in its entirety by the researcher acting as a scribe to participants with an insufficient level of literacy.

Statistical analysis

Comparisons among general characteristics were performed with SPSS 20 for Windows (IBM, Armonk, NY). Continuous variables like HADS scores were tested using ANOVA, and for dichotomous variables such as sex and age category differences, the chi-square test was used. The variance component model is a well-known tool for heritability estimates in family studies and was used to calculate polygenic heritability estimates [18]. In the most narrow sense, the heritability of a trait represents the proportion of the phenotypic variance attributable to additive genetic effects and is given by $h^2 = \sigma_a^2 / \sigma_p^2$, where σ_a^2 is the variance due to the additive effects of genes, and σ_p^2 is the phenotypic variance. The overall phenotypic variance was estimated from the observed distribution of trait values in the sample, and was partitioned into genetic and environmental components using the observed covariance among family members, as $\Omega = 2\phi\sigma_a^2 + I\sigma_e^2$, where Ω is an “n x n” matrix of the “n” individuals in the data set, 2ϕ is the structuring matrix of the coefficient of relationship, and “I” is an identity matrix that represents the structuring matrix for σ_e^2 , the variance due to residual environmental factors. Estimates of the mean and variance components were obtained using maximum likelihood methods [19, 20].

Joint analysis of multiple related phenotypes can be used to answer questions about the nature of the relationship between the traits and to increase power to localize genes influencing the traits [21, 22]. In this context, in the bivariate model, the genetic correlations (ρ_g) and environmental correlations (ρ_e) represent the additive polygenic effect of shared genes or common genetic effects on the two traits (pleiotropy) and environmental factors (non-genetic) in the phenotypic variance of each trait, respectively. The overall phenotypic correlation (ρ_p) between two traits can be broken down into a genetic and environmental component:

$$\rho_p = \rho_g \sqrt{h_1^2} \sqrt{h_2^2} + \rho_e \sqrt{1 - h_1^2} \sqrt{1 - h_2^2} \quad (1)$$

In this correlation, h_1^2 and h_2^2 are the heritabilities in trait 1 and trait 2. Using this nomenclature, the proportion of the total genetic variance that is due to shared genetic effects is estimated by the square of the genetic correlation (ρ_g). Likelihood ratio tests were used to separately test the hypotheses that the two traits share no common genetic basis ($H_0: \rho_g = 0$) and that the two traits have the identical genetic basis ($H_0: |\rho_g| = 1$).

In this study, the heritability of HADS-A and HADS-D as well as bivariate genetic correlations were conducted using the maximum likelihood estimate based variance components approach implemented in the statistical genetics software package, SOLAR [21]. Four models were fitted to the data: (1) unadjusted, (2) adjusted by age, (3) adjusted by gender and (4) adjusted by age and gender. In each adjusted model, the estimates of the parameters in question were calculated.

Results

Description of the sample

Socio-demographic characteristics of the sample are given in [Table 1](#). All age categories were represented and there was no gross difference between genders. 62.9% of subjects were married or living with a partner. 41.6% of the sample had four years or less of schooling, and 86.4% of the sample had a monthly household income of less than R\$1,500. No more than 1% of any used variable was missing.

HADS values

The following coefficients of internal consistency (Cronbach's alpha) were obtained: anxiety ($\alpha = 0.74$), depression ($\alpha = 0.73$), and total score ($\alpha = 0.83$). Anxiety and depression were correlated with $r = 0.59$ (Pearson, $p < 0.0001$) and $r = 0.58$ (Spearman's ρ , $p < 0.0001$). Similar significant results were obtained when the sample was divided by gender: For males, $r = 0.54$, and for females, $r = 0.59$.

The mean scores and standard deviations in the HADS-A and HADS-D scale for the total population were 7.49 ± 4.01 and 5.70 ± 3.82 , respectively, with responses ranging from 0 to 21. Women reported higher scores than men for anxiety symptoms ($F = 53.32$ $p = 0.001$). This gender difference persisted for depressive symptoms, but to a lesser degree ($F = 40.57$ $p = 0.001$). Age differences were noted in both anxiety ($F = 4.02$ $p = 0.003$) and depression ($F = 13.19$ $p = 0.001$). HADS-A and HADS-D mean scores increased with age until reaching their peak in the range of 40 to 59 years of age as illustrated in [Figs 1](#) and [2](#), respectively.

Heritability

Heritability estimates for HADS-A and HADS-D are shown in [Table 2](#). Estimates were similar in the various adjusted models and were all statistically significant ($p < 0.0001$). Heritability, without taking covariates into account (unadjusted model), was 0.28 for HADS-D and, 0.30 for HADS-A. When corrected for age and sex, the numbers increased to 0.30 (HADS-D) and 0.32 (HADS-A). The significant effects of age and sex for both phenotypes indicate that there is an increase in precision when these factors are accounted for. Thus, we also divided the sample into an older and a younger age group, the cut-off point being the 50% percentile (41 years), and ran a set of heritability estimates. A marked difference was found between these age groups. The younger age group had higher heritability estimate compared to the older age group for HADS-A (younger/older = 0.32/0.22) and, HADS-D scores (younger/older = 0.31/0.18). The added inclusion of age² and age x gender did not change the results of heritability for any of the scores.

Bivariate analyses

Testing the null hypothesis that the genetic correlation between HADS-A and HADS-D measures was zero (that is, that there is no shared genetic basis between HADS-A and HADS-D) resulted in highly significant P-values ($< 10^{-6}$), and this hypothesis was therefore rejected

Table 1. Socio-demographic characteristics of the sample.

| | <i>Total</i> <i>n</i> | % | <i>Males</i> <i>n</i> | % | <i>Females</i> <i>n</i> | % |
|--------------------------------------|--------------------------|------|--------------------------|------|----------------------------|------|
| Age category | | | | | | |
| 18–29 years | 370 | 26.9 | 158 | 26.9 | 212 | 26.9 |
| 30–39 years | 258 | 18.8 | 109 | 18.6 | 149 | 18.9 |
| 40–49 years | 314 | 22.8 | 129 | 22.0 | 185 | 23.5 |
| 50–59 years | 208 | 15.1 | 88 | 15.0 | 120 | 15.2 |
| ≥60 years | 225 | 16.4 | 103 | 17.5 | 122 | 15.5 |
| Marital status | | | | | | |
| Married or living together | 865 | 62.9 | 375 | 63.9 | 490 | 62.2 |
| Single | 368 | 26.8 | 178 | 30.3 | 190 | 24.1 |
| Divorced | 60 | 4.4 | 17 | 2.9 | 43 | 5.5 |
| Widowed | 73 | 5.3 | 13 | 2.2 | 60 | 7.6 |
| Education | | | | | | |
| ≤ 4years | 571 | 41.6 | 263 | 45.0 | 308 | 39.1 |
| 5–8 years | 309 | 22.5 | 127 | 21.7 | 182 | 23.1 |
| 9–11 years | 360 | 26.2 | 143 | 24.5 | 217 | 27.5 |
| ≥ 12 years | 132 | 9.6 | 51 | 8.7 | 81 | 10.3 |
| Household income ^a | | | | | | |
| Class E (Less than R\$ 300.00) | 249 | 18.7 | 100 | 17.5 | 149 | 19.6 |
| Class D/C (R\$ 300.00-R\$ 1.500.00) | 901 | 67.7 | 373 | 65.3 | 528 | 69.5 |
| Class B (R\$ 1.500.00-R\$6.000.00) | 172 | 12.9 | 93 | 16.3 | 79 | 10.4 |
| Class A (More than R\$ 6.000.00) | 9 | 0.7 | 5 | 0.9 | 4 | 0.5 |

^a Monthly Income in Brazilian Real

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(data not shown). A second null hypothesis that the genetic correlation between these traits is one (that is, the genetic bases HADS-A and HADS-D are identical) was also rejected as very unlikely ($P < 10^{-4}$) (data not shown).

The overall phenotypic correlation (ρ_p) between HADS-A and HADS-D measures was broken down into a genetic (ρ_g) and environment (ρ_e) component according to Eq (1) described in the Methods section. The genetic correlation between HADS-A and HADS-D measures ranged from 0.78 to 0.81 through all models: the unadjusted, adjusted for gender, age, gender and age and interaction age \times gender (Table 3). The decomposition of the phenotypic correlation revealed a stronger genetic than environmental correlation contribution to the overlap between depression and anxiety symptoms in all models.

The proportion of the total genetic variance due to shared genetic effects is estimated by the square of the genetic correlation, thus, in the best model, adjusted for gender and age, this proportion is $0.81^2 = 0.66$, suggesting that nearly 66% of the total genetic variance in HADS-A is shared with HADS-D.

Discussion

In this study, we quantified the degree of shared genetic variance between anxiety and depression symptoms, assessed via the HADS-A and HADS-D sections, respectively, and also calculated estimates of heritability for the two sets of symptoms in a large sample of Brazilian families.

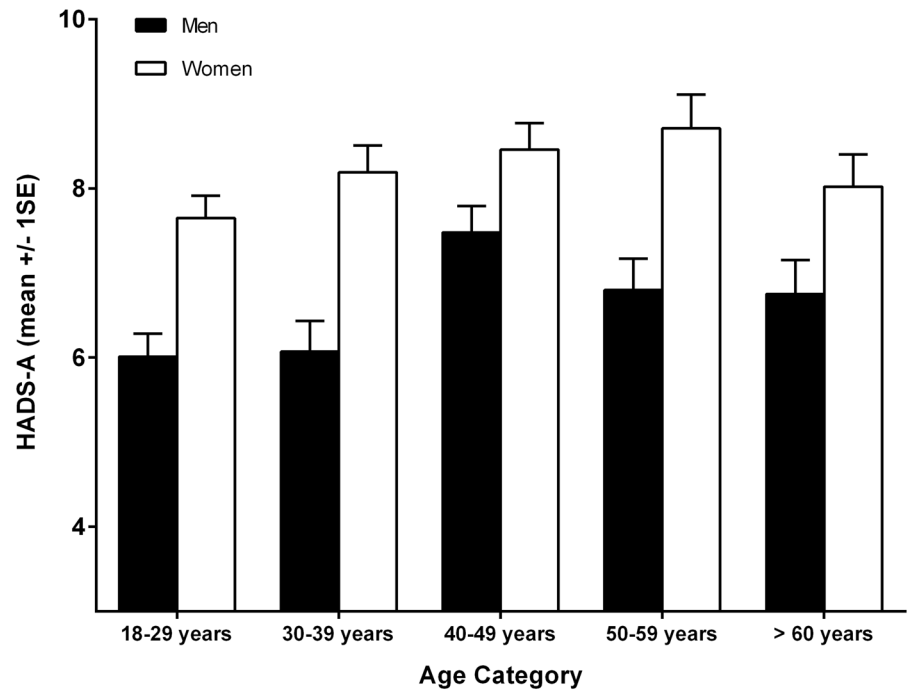


Fig 1. Mean HADS-A scores across age categories.

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We found that approximately 66% of the genetic variance for HADS-A is shared with HADS-D, and vice versa. These results suggest that the same genetic factors influence the susceptibility to anxiety and depressive scores. A similar methodology was applied by Olvera and colleagues [23], who performed bivariate analyses in a family-based Mexican-American sample

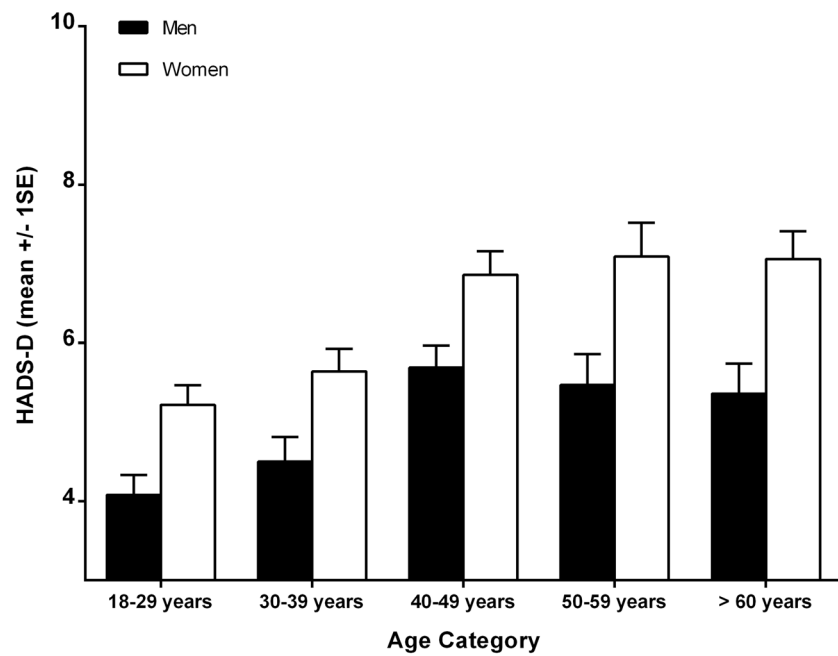


Fig 2. Mean HADS-D scores across age categories.

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Table 2. Heritability estimates.

| Model | h^2_{gA} | p values | h^2_{gD} | p values |
|---------------|------------|------------|------------|------------|
| No adjustment | 0.30±0.06 | <0.0001 | 0.28±0.06 | <0.0001 |
| Gender | 0.31±0.06 | <0.0001 | 0.29±0.06 | <0.0001 |
| Age | 0.34±0.06 | <0.0001 | 0.29±0.05 | <0.0001 |
| Gender, age | 0.35±0.06 | <0.0001 | 0.29±0.60 | <0.0001 |
| Gender*age | 0.33±0.06 | <0.0001 | 0.29±0.60 | <0.0001 |

h^2_g = heritability; A = anxiety scores; D = depression scores. Values are shown with standard deviation.

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of shared genetic background between psychiatric disorders. That analysis found a strong genetic correlation in individuals concomitantly diagnosed with anxiety and depressive disorders ($\rho_p > 0.75$). Shared genetic correlations between anxiety and depressive symptoms have consistently been found in twin studies but those studies have all been based on cohorts of primarily European ancestry [10, 11]. Boomsma et al, 2000, in a study of Dutch adolescent and young adult twins, found a common genetic factor that influenced more than 40% of the variance explained by genetic factors for both depression and anxiety symptoms. Similarly, Kendler et al, 1987, found a common genetic factor that influenced the susceptibility of the concomitant presentation of depression and anxiety symptoms in an Australian adult twin cohort. Thus, our findings are unique in reporting a shared genetic background between the degree of anxiety and depression (as continuous variables) using the HADS scale in an admixed population, and confirmatory with respect to previous reports of a shared genetic background in comorbid symptoms seen in the general population in samples of mostly European ancestry.

The heritability estimates obtained in the present study were 0.30 HADS-D, and 0.32 for HADS-A. In the literature, heritability estimates for depression range from 0.17 to 0.78 and for anxiety from 0.25 to 0.60 [3, 24]. Heritability measures of anxiety and depression for this study lie on the lower side of the range of studies investigating both disorders [3, 24]. Differences in heritability estimates can be due to sample selection or experimental design. Those differences can be the result of age and/or gender-specific genetic effects that impact on those estimates. For example, we found in our very same cohort that individuals younger than 41 years old had a heritability estimate of 0.32 for anxiety compared to 0.22 in the oldest subset. This could also be the case in different samples with distinct age groups [25–27]. Despite methodological differences between classical twin design and extended pedigree design, rendering distinct heritability estimates, general results indicate that both are able to generate good estimates [28].

In order to discuss the relevance of our genetic findings, it is important to address particularities and possible limitations of the sample and study design. We found a lower frequency of

Table 3. Bivariate analysis.

| Model | ρ_p | p values | ρ_g | p values | ρ_e | p values |
|---------------|----------|------------|-----------|------------|-----------|------------|
| No adjustment | 0.59 | <0.0001 | 0.78±0.07 | <0.0001 | 0.52±0.04 | <0.0001 |
| Gender | 0.58 | <0.0001 | 0.79±0.04 | <0.0001 | 0.50±0.04 | <0.0001 |
| Age | 0.60 | <0.0001 | 0.80±0.07 | <0.0001 | 0.51±0.04 | <0.0001 |
| Gender, age | 0.58 | <0.0001 | 0.81±0.07 | <0.0001 | 0.48±0.04 | <0.0001 |
| Gender*age | 0.50 | <0.0001 | 0.81±0.07 | <0.0001 | 0.50±0.04 | <0.0001 |

ρ_p = phenotypic correlation; ρ_g = genetic correlation; ρ_e = environmental correlation. Values are shown with standard deviation for ρ_g and ρ_e .

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symptoms of anxiety and depression in men compared with women, as reported in previous studies world-wide [29, 30]. Studies performed in Brazil showed the same tendency [15, 17], but were conducted in groups with health problems which tends to increase the HADS scores. It is interesting to note that both the depression and anxiety mean scores found in our population were higher than all the studies referred to above with exception to the Korean general population study where depression results (mean = 6.6) showed higher mean scores than in Baependi [31]. Possible explanations for higher scores in the Baependi population are the low level of education and income in this population (Table 1). These are important covariates to be considered, as low socio-economic status raises the odds for depression and anxiety and decreasing family income over time increased vulnerability to the symptoms of anxiety and depression [32, 33]. Educational level is one of the factors that comprises the socio-economic status index, and seems to exert a protective effect to anxiety and depression disorders. In an 11-year follow up study, people with a low educational level (often resulting in professions involving hard manual work) moved towards higher anxiety and depression scores when compared to others with high level of schooling [34].

The inclusivity of our study, a distinguishing aspect of this study, could also be a reason for the scores being higher than in other general population studies. All participants with valid scores were included, independent of age (participants were aged up to 98), sex, use of medication, socio-economic status, and health condition. The subjectivity of self-assessment scales like HADS, although extensively used in epidemiological studies, can be a potential source of bias as well [26]. Ultimately, given the unique and in many ways advantageous features of this study design (a highly admixed population living in a rural town with a very conservative lifestyle), and some unique complications (e.g. a high level of illiteracy), it becomes difficult to compare it with previous findings.

Conclusions

The finding of a high proportion of shared genetic factors between continuous measures of depression and anxiety shows that the well-known clinical correlation between these systems is reflected on a profound biological level. Furthermore, evidence for shared genetic effects has promising implications for future molecular genetics studies, because they may increase power to localize genes influencing these traits in genome-wide association studies [35].

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Author Contributions

Conceived and designed the experiments: ACP JEK CMO ABN. Performed the experiments: ACP JEK CMO. Analyzed the data: TPT ACP HV MvS NED ARVRH ABN. Contributed reagents/materials/analysis tools: ACP NED ARVRH. Wrote the paper: TPT ACP HV MvS ROA NED ARVRH ABN.

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