Genetic validation of neurokinin 3 receptor antagonists for ischemic heart disease prevention in men – A one-sample Mendelian randomization study

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Summary

Background Ischemic heart disease (IHD) is a leading cause of mortality, particularly for men. Few interventions have focused on protecting specifically men. Emerging evidence may implicate testosterone. Neurokinin 3 receptor (NK3R) antagonists, an existing class of drugs being considered as treatments for reproductive conditions in women, affect testosterone; this study addresses genetic validation of their use to prevent IHD in men.

Methods A one-sample Mendelian randomization (MR) study using the UK Biobank cohort study, based on independent (r^2 < 0.005) genetic variants predicting testosterone in men (n = 157738) at genome wide significance in the target gene for NK3R antagonists (*TACR3*), was used to assess associations with IHD (cases=15056, non-cases=151964) and positive control outcomes (relative age voice broke, children fathered, hypertension) in men and a negative control outcome (IHD) in women using summary statistics. A two-sample MR study using the PRACTICAL consortium was used for the positive control outcome of prostate cancer.

Findings Two relevant *TACR3* genetic variants (rs116646027 and rs1351623) were identified in men. Genetically mimicked NK3R antagonists were inversely associated with IHD (odds ratio 0.54 per standard deviation lower testosterone, 95% confidence interval 0.31, 0.94) and with control outcomes (older relative age voice broke, fewer children and lower risk of hypertension and prostate cancer) as expected in men and in women (unrelated to IHD).

Interpretation Genetic validation of a role of NK3R antagonists in IHD suggests their consideration as a new means of preventing IHD in men. Whether they protect against prostate cancer might bear further consideration.

Keywords: NK3R; IHD; Sex; Evolutionary biology; Men

Ischemic heart disease (IHD) is the leading cause of death globally with higher mortality rates in men than women.[1] Development of new means of preventing and treating cardiovascular disease has stalled recently.[2] To address this gap chronic diseases, similar to infectious diseases, are increasingly seen within an evolutionary biology framework,[3] where drivers of growth and reproduction may trade-off against long-term health. Correspondingly, interventions, such as calorie restriction, targeting the drivers of growth, have long been investigated as potential interventions.[4] One of the newer drugs for type 2 diabetes, particularly recommended for those at higher risk of cardiovascular disease,[5] is thought to be a calorie restriction mimetic.[6] Interventions targeting sex hormones are widely used to treat hormone-related cancers.[7,8] Emerging quasi-experimental evidence suggests that androgens may be involved in cardiovascular disease,[9–11] and one of its key risk factors, hypertension.[12] Correspondingly, one of the most effective drugs for preventing and treating cardiovascular disease, statins, reduces testosterone[13,14] and operates partially by that pathway in men.[14] Many recommendations concerning a healthy lifestyle likely reduce testosterone in men,[15] few interventions for IHD have explicitly addressed this oppurtunity.[15]

An existing drug class, originally developed as antipsychotics, currently being considered as a treatment for reproductive conditions in women,[16] i.e., neurokinin

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Research in context

Evidence before this study

Cardiovascular disease rates are higher in men than women and contribute to shorter lives in men than women. Few interventions designed to address this disparity exist, although recent research suggests testosterone may be a contributing factor. A widely developed class of drugs, neurokinin 3 receptor (NK3R) antagonists, originally developed as anti-psychotics, are now being tested as a treatment for reproductive conditions in women. NK3R antagonists are known to reduce testosterone and have been proposed as a treatment for cardiovascular disease, which might be particularly relevant in men. No study has assessed cardiovascular effects of NK3R antagonists, according to this search in PubMed ("neurokinin 3 receptor cardiovascular human")

Added value of this study

This quasi-experimental study provides genetic validation suggesting that NK3R antagonists may protect men against ischemic heart disease, as well as having expected associations for several control outcomes (higher relative age voice broke, fewer children fathered, and lower risk of hypertension and prostate cancer).

Implications of all the available evidence

As well as NK3R antagonists being considered as treatment for reproductive conditions in women, they should also be considered as a means of addressing sexual disparities in health and lifespan.

3 receptor (NK3R) antagonists, also modulates testosterone.[16] As such, NK3R antagonists represent an existing drug class that might provide a new means of combating cardiovascular disease,[17] particularly in men. However, no relevant experimental evidence exists. Mendelian randomization provides an alternative, by leveraging the random allocation of genetic material at conception to obtain estimates largely free of the confounding that can bias observational studies.[18] Mendelian randomization studies can clarify mechanistic pathways[19] and likely drug effects, and have foreshadowed results of clinical trials.[20] This study assessed genetic validation, or otherwise, of NK3R antagonists as preventing IHD in men. Androgens or their precursors would be expected to affect fertility and have been associated with hypertension[12] and prostate cancer[12,21] so these were all considered as positive control outcomes. Finally, testosterone is much lower in women than men,[22] so associations with IHD in women were considered as a negative control outcome.

Methods

This Mendelian randomization study, using two-sample methods, takes advantage of the largest relevant publicly available sex-specific genetic summary statistics, i.e., from the UK Biobank (http://www.nealelab.is/uk-biobank), for the exposure, i.e., genetic mimics of NK3R antagonists (in standardized units of testosterone), and for the main outcome, i.e., IHD. UK Biobank genetic summary statistics were also used for the positive control outcomes (fertility (relative age voice broke and children fathered) and hypertension), the negative control outcome (IHD in women) and any multivariable Mendelian randomization analysis. The PRACTICAL consortium[23] was used for the positive control outcome of prostate cancer. Finally, Biobank Japan (BBJ)[24–26] was used for replication because it has sex-specific genetic associations with IHD. As such, this study largely used a one-sample Mendelian randomization study design, with a two-sample design used for prostate cancer and replication for IHD in BBJ.

The UK Biobank is a large cohort study of half a million people, intended to be aged 40 to 69 years at recruitment in Great Britain from 2006 to 2010, and thereafter followed-up via record linkage to hospital records and death (https://www.ukbiobank.ac.uk).[27] Summary sex-specific UK Biobank quality controlled genetic associations for 13.7 million variants, used here, pertain to men (n < = 167020) or women (n < = 194,174) of white British descent adjusted for age, age^2 and 20 principal components. PRACTICAL is a consortium of case series, case-control and cohort studies of prostate cancer mainly in men (cases mean age 65.7 years, controls mean age 61.6 years) of European descent[23]; quality-controlled summary genetic associations, used here, were adjusted for principal components and study relevant covariates stratified by country and study.[23] BBJ is a hospital-based registry study of about 200,000 people (mean age 62 years, 53.1% male), followed-up via medical records and national death registration,[24,25] and supplemented by 34,793 controls from existing Japanese population based cohort studies.[26] Summary sex-specific BBJ quality controlled genetic associations with IHD for 8.9 million variants, pertain to 109,347 men of East Asian descent adjusted for age and the top 5 principal components.

Genetically mimicked NK3R antagonists

Genetic mimics of NK3R antagonists were obtained from UK Biobank summary statistics (http://www.nealelab.is/uk-biobank) for testosterone in 157,738 men, as all genome wide significant ($p < 5 \times 10^{-8}$) independent ($r^2 < 0.005$) variants with minor allele frequency (MAF) >0.01 from the relevant target gene (+-50 kbp), *TACR3*,[28] predicting testosterone with *F*-statistic>10. To assess possible pleiotropic effects of the selected

genetic variants via other biasing pathways, their associations with other phenotypes were checked using two curated genotype to phenotype cross-references (http://www.phenoscanner.medschl.cam.ac.uk and https://gwas.mrcieu.ac.uk/phewas/).

Ischemic heart disease in men and women

Summary genetic associations with IHD from the UK Biobank, defined using a wide definition, international classification of diseases 10 codes I20−5 of events, comprised 15,056 cases and 151,964 non-cases in men, and 5801 cases and 188,373 non-cases in women.

Control outcomes in men

Relative age voice broke, on a comparative scale, i.e., younger than average, about average and older than average, (n = 144,645), number of children fathered (n = 154,888) and hypertension (cases = 49,744, non-cases = 117,244) were self-reported at UK Biobank recruitment. Prostate cancer (cases = 79,148, controls = 61,106) was defined as diagnosis.[23]

Consideration of potential confounders

Statins were considered as potential confounders because of their well-known effects on IHD, including specifically in men,[14] and their potential relevance to NK3R antagonism. The effect of statins in men was mimicked by rs12916 as previously.[14]

Replication in Biobank Japan

IHD in BBJ was defined as myocardial infarction, stable angina, and unstable angina. In men there were 21,611 cases and 87,736, controls.

Co-localization analysis

Co-localization was used to assess the posterior probability of the *TACR3* gene sharing the same one causal variant for testosterone and IHD or for testosterone and each of the four positive control outcomes.[29] The default prior probabilities were used, i.e., 10^{-4} for an association with testosterone only, 10^{-4} for an association with the outcome only and 10^{-5} for an association with both.[29] A posterior probability of more than 0.8 was taken as evidence of colocalization.[29] The prior probabilities for an association with one trait only are well-established, but the choice of prior for an association with both traits needs careful thought,[30] and with a prior of 10^{-6} possibly more suitable for small samples, as here, and a larger prior possibly more suitable for theoretically based associations, as here.[30] So sensitivity analysis was conducted using a prior of 10^{-4} and 10^{-3} for an association with both traits.

Statistical analysis

F-statistics were approximated by squared estimate for genetic variant on testosterone divided by its variance. Inverse variance weighted Mendelian randomization estimates were obtained from a meta-analysis of genetic variant specific Wald estimates (genetic variant on outcome divided by genetic variant on exposure) using fixed or multiplicative random effects as appropriate. For multivariable Mendelian randomization instruments for exposure and potential confounder were combined, correlated instruments ($r^2 < 0.005$) dropped and the conditional *F*-statistics calculated.[31]

Statistical analysis was conducted using R 4.0.2.[32] The MR-Base[33] R package ld_clump was used to obtain independent variants, the Mendelianrandomization package to obtain estimates, metafor to obtain forest plots, MVMR to obtain the conditional *F*-statistics and coloc for the co-localization analysis. An established approximation was used to convert genetic associations reported as probabilities of events to logodds.[34] This study only uses genetic summary statistics previously created from information and materials previously collected with informed consent. The data extracted to conduct the MR analyses is given in Supplementary Tables 1, 3 and 5.

Role of the funding source

This study had no funding

Results

Genetic instruments mimicking NK3R antagonists

Of the 936 genetic variants from *TACR3* +/- 50 kbp with MAF > 0.01 two independent genetic variants (rs116646027 and rs1351623) (*TACR3*) predicted testosterone at genome wide significance in men, *F*-statistics 88.2 and 58.2, respectively (Supplementary Table 1). Other associations at genome wide significance for these variants were almost entirely with testosterone related attributes, i.e., body composition, height, pubertal characteristics and bone density (Supplementary Table 2).

Associations of genetically mimicking of NK3R antagonists with IHD and the control outcomes

Genetically mimicking NK3R antagonists was associated with lower IHD risk in men (Table 1). Genetically mimicking NK3R antagonists was also associated with all the control outcomes as expected, i.e., had no association with IHD in women, but was associated with older relative age voice broke, fewer children and lower risk of hypertension and prostate cancer in men (Table 1). Associations tended to be driven by rs116646027, which was associated in men with IHD and all the positive control outcomes except hypertension (Figure 1).

Outcome	sex	type	estimate Odds ratio	95% confidence interval	*p*-value	Q statistic (*p*-value)
Ischemic heart disease	men	main	0.54	0.31 to 0.94	0.030	0.67 (0.41)
	women	control	0.79	0.33 to 1.87	0.591	0.01 (0.92)
			beta			
Relative age voice broke	men	control	0.16	0.10 to 0.21	6.2e-9	0.89 (0.34)
Number of children	men	control	-0.15	-0.28 to -0.02	0.021	1.07 (0.30)
			Odds ratio			
Hypertension	men	control	0.60	0.43 to 0.84	0.003	0.47 (0.49)
Prostate cancer	men	control	0.60	0.39 to 0.91	0.016	0.70 (0.40)

***Table 1*: Mendelian randomization estimates for associations of genetically mimicking NK3R antagonists (based on rs116646027 and rs1351623, *TACR3*) with ischemic heart disease in men and the control outcomes (ischemic heart disease in women, relative age voice broke, number of children fathered, self-reported hypertension and prostate cancer in men) per standard deviation lower testosterone in men from the UK Biobank (http://www.nealelab.is/uk-biobank) and the PRACTICAL consortium[23] (prostate cancer).**
The data underlying this table is given in Supplementary Table 3.

Multivariable associations of genetically mimicking NK3R antagonists with IHD in men

The association of genetically mimicking NK3R antagonists with IHD was similar after adjusting for genetically mimicked statins (Supplementary Table 5). Genetically mimicked statins were also inversely associated with IHD.

Replication in Biobank Japan

Only one instrument for NK3R antagonists was available in BBJ, because rs116646027 is monomorphic in East Asians. Using rs1351623 and the association with testosterone from UK Biobank, the magnitude of association with IHD was similar to that in UK Biobank (odds ratio (OR) 0.66, 95% confidence interval (CI) 0.36 to 1.23).

Co-localization analysis

Colocalization analysis of 936 genetic variants from *TACR3* +/- 50 kbp with MAF>0.01 identified rs116646027 as the variant with the largest posterior probability for both testosterone and IHD. The overall posterior probability was 14.3% using a prior of 10^{-5} for both traits, but 94.3% using a prior of 10^{-3}. Colocalization for testosterone and the positive control outcomes, similarly identified rs116646027 (or a complete correlate rs17289915) as the variant with the largest posterior probability. The overall posterior probability using a prior of 10^{-5} for both traits was 98.8% for relative age

Figure 1. Forest plots showing the SNP-specific and overall Mendelian randomization estimates for associations of genetically mimicking NK3R antagonists (based on rs116646027 and rs1351623, *TACR3*) with ischemic heart disease in men and the negative control outcome (ischemic heart disease in women) and the positive control outcomes in men (relative age voice broke, hypertension, prostate cancer and number of children fathered in men) per standard deviation lower testosterone in men from the UK Biobank and the PRACTICAL consortium[23] (prostate cancer).

of voice break, 10.1% for number of children, 9.4% for hypertension and 17.4% for prostate cancer; all were over 90% using a prior of 10^{-3} (Supplementary Table 6).

Discussion

Consistent with expectations from evolutionary biology and previous evidence concerning the role of testosterone in IHD,[9–11] genetically mimicked NK3R antagonism in men appeared to beneficial for IHD, as well as for hypertension and prostate cancer but at the expense of reducing fertility. Genetically mimicked NK3R antagonism in men was not associated with the control outcome of IHD in women.

Physiologically, NK3R centrally regulates steroid precursors, particularly gonadotropin releasing hormone. Correspondingly rare variants in *TACR3* are associated with isolated hypogonadotropic hypogonadism.[28] Pharmacological targeting of *TACR3* has been suggested as a means of treating sex-steroid related diseases, such as breast and prostate cancer.[28] NK3R antagonists are thought likely to be safe in clinical practise.[35] Current investigation of clinical applications of NK3R antagonists is focused on treatment of menopausal symptoms and possibly other benefits of suppression of sex-hormone levels in women with less consideration of corresponding benefits for men.[36] Experiments in spontaneously hypertensive rats have shown that NK3R antagonism normalizes mean arterial pressure.[37,38] Similarly, the means by which testosterone might cause IHD have not been extensively explored but might encompass thrombotic or related processes, such as thromboxane A_2,[39] nitric oxide,[40] the haemostatic system[41] or coronary plaque volume.[42] Neurokinins affect both the brain and the gut, so effects mediated by the gut brain axis[43] are possible but untested.

Despite providing new evidence about a novel way of addressing the specific need for IHD prevention in men with minimal confounding, this study has limitations inherent in the design. Mendelian randomization relies on the instrumental variable analysis assumptions of relevance, independence and exclusion-restriction. To address relevance, the instruments for genetically mimicking NK3R antagonists were only extracted from the target gene (*TACR3*) based on the strength of their associations with testosterone in men because NK3R antagonists target *TACR3* and reduce testosterone.[16] Bioavailable testosterone may be more physiologically relevant; the same instruments and a similar interpretation were obtained using bioavailable testosterone from the UK Biobank (data not shown). The *F*-statistics were also high indicating adequate strength of the instruments. Colocalization did not unequivocally indicate that rs116646027 was the causal variant in *TACR3* underlying the observed Mendelian randomization associations using the default priors, apart from for relative age voice broke. However, a larger prior probability for both traits might be more suitable for a hypothesis driven study,[30] and did suggest colocalization.

To address independence, potential confounders of genetically mimicked NK3R antagonism on IHD were considered, such as statins, but the estimate for IHD was unchanged. In addition, all the genetic associations used were adjusted for principal components and genetic confounding is uncommon.[44] To address exclusion-restriction, other consequences of the genetic instruments were identified. However most other associations at genome wide significance were plausible consequences of testosterone (Supplementary Table 2). In addition, genetic studies are open to selection bias, particularly for outcomes which cannot be observed because they, or a fatal competing risk, occurred before recruitment.[45] However, the UK Biobank and BBJ participants were not that old, when IHD tends to occur before other cardiovascular conditions, such as stroke,[46] making survival to recruitment less of an issue. UK Biobank participants are healthier than the general population,[47] so the number of cases may be under-estimated. Such under-representation of cases is likely to be independent of genetic make-up and so does not necessarily cause a bias[48] although it may reduce precision. The UK Biobank was used for exposure and most outcomes. Lack of independence between genetic variants on exposure and genetic variants on outcome can bias towards the conventional observational estimates in finite samples. Such, bias is particularly likely when the expected *F*-statistic is small,[49] the sample is relatively small (<100,000),[50] or the MR-Egger estimate is used.[50] Here, the *F*-statistics were quite large, the sample size was ~150,000 and only the inverse variance weighted estimate was used, so substantial bias from conducting the study mainly in one-sample would not be expected. However, the possibility of spurious findings due to type 1 error cannot be excluded, although the consistency across outcomes is somewhat re-assuring. Canalization could also create bias, however expected associations were evident for the control outcomes across the lifespan (Table 1). The samples pertain to people of European descent, however, causes usually act consistently but are not always relevant.[51] Replication was limited by the lack of sex-specific genetic summary statistics for IHD in Europeans, and the lack of comprehensive genetic associations with testosterone for other populations. Meta-analys of the available estimate for IHD in East Asians with the association for both SNPs in UK Biobank gave OR 0.59, 95% CI 0.39 to 0.89. Routine provision of sex-specific GWAS would facilitate the development of sex informed disease prevention, so as to address disparities.

Proposed use of NK3R antagonists in women suggests they have an acceptable safety profile.[52] Given the extensive availability of NK3R antagonists[35] and the lack of new drugs targeting IHD in men further investigation of their use in men to protect against IHD and prostate cancer is warranted. Notably the association of

genetically mimicking NK3R antagonists with lower risk of IHD in men appeared to be independent of the effects of genetically mimicked statins, consistent with the possibility that NK3R antagonists could be acting on IHD in men via an underexploited target. Drugs acting via testosterone may raise the risk of diabetes.[53] However, statins increasing diabetes risk does not outweigh their benefits for cardiovascular disease prevention.[54] Finally, the colocalization analysis suggested, albeit as a *post hoc* hypothesis with a high posterior probability, that one shared genetic variant could be relevant to NK3R antagonism and pubertal timing in boys. As such, NK3R antagonists could also be relevant to modulating pubertal timing in boys. More generally, this study also raises the question as to whether any related neurokinins might relate in men to the same diseases. Notably drugs targeting NK1R (substance *p*) antagonism are a commonly used anti-emetic.[55]

Conclusion

An existing drug class (NK3R antagonists) could be used specifically in men to address IHD risk, possibly with the added benefit of reducing risk of prostate cancer. Further investigation of the role of NK3R antagonists in men is warranted complementary to investigation of their potential use in women to treat reproductive conditions.

Declaration of interests

There are no conflicts of interest.

Contributors

CMS conducted the study. The data used is publicly available and provided in the Supplementary tables. S Li and G Yang very kindly re-checked the analysis.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103901.

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