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Application R436-2023-1175

Name Kasper Munch
Position Lektor

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Institute Bioinformatics Research Centre
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Budget subtotals & total

Budget line	Year 1	Year 2	Total
Løn - postdoc	582.996,00 kr.	600.486,00 kr.	1.183.482,00 kr.
Rejser & konferencer	25.000,00 kr.	25.000,00 kr.	50.000,00 kr.
Drift	150.000,00 kr.	150.000,00 kr.	300.000,00 kr.
Andet	0,00 kr.	30.000,00 kr.	30.000,00 kr.
Udstyr	25.000,00 kr.	0,00 kr.	25.000,00 kr.
Projektrelaterede indirekte omkostninger	78.300,00 kr.	80.548,00 kr.	158.848,00 kr.
Total	861.296,00 kr.	886.034,00 kr.	1.747.330,00 kr.

Project title

Moonlighting brain genes: Does gene expression in spermatogenesis shape the genetic basis of autism?

Summary of application

The proportion of the Danish population diagnosed with autism has risen quickly in the past years. It will most likely reach at least 3%, emphasizing its societal impact. Understanding its genetic basis is crucial for improved diagnosis, support, and possibly future medical help for children with autism. Past studies identifying autism genes have searched only the 22 chromosomes shared by males and females and neglected the more complicated analysis of the X chromosome that only females carry in pairs. However, sex differences in autism and recent research on brain anatomy suggest that the X chromosome plays a unique role in

autism. This project will quantify the X chromosome's genetic contribution and explore the hypothesis that selfish genes, which only operate on sex chromosomes, increase this contribution. In spermatogenesis, selfish genes on the X chromosome promote their natural selection by killing sperm cells that carry a Y chromosome rather than the X chromosome. This phenomenon, called meiotic drive, operates across vertebrates, insects, fungi, and plants, and from a recent report, most likely also in humans. The selfish advantage in spermatogenesis easily trumps any modest harmful effect the selfish gene variant may impose on brain development. Since one in four sperm genes is also crucial to brain function, selfish genes on the X chromosome may continuously contribute new autism gene variants compromising normal brain development. Preliminary results produced for this application suggest this hypothesis is true. The project will affirm this and identify the properties and functions of the responsible genes. The project combines methods from research on sex chromosome evolution and individuals' shared ancestry with proven techniques for associating gene variants with autism. Through collaboration with the Danish iPSYCH consortium, the project has privileged access to the genetic variation in large cohorts of Danes with and without autism.

Project start date	2024-01-01
Project end date	2025-12-31

Personal information

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The project

Project title

Moonlighting brain genes: Does gene expression in spermatogenesis shape the genetic basis of autism?

Project

A: Project title

Moonlighting brain genes: Does gene expression in spermatogenesis shape the genetic basis of autism?

B: In a nutshell:

Despite recent work showing that the X chromosome is particularly important to some aspects of autism-related brain folding, efforts to link genes to autism rarely consider genes on the X chromosome because the different numbers of X chromosomes in males and females complicate such work. This exploratory project aims to show that the X chromosome is important to autism more generally because a large proportion of its neuron genes are also active in male sperm cells. These genes may be frequently and strongly promoted by natural selection for their roles in spermatogenesis, even if the same gene variants are mildly deleterious to neuron function and brain development. The project exploits theory and methodology from basic research in genome evolution to identify genes subject to such selection and to associate genes with autism risk more effectively. If successful, the project will identify new classes of risk genes and show how selfish genes sustain a higher polygenic autism risk on the X chromosome.

C: Research idea and context:

A recent study shows that the X chromosome explains 20% of neuro-anatomical variation relevant to autism spectrum disorder (ASD), although it represents only 5% of the genome [1]. Despite this apparent privileged role in human neurodevelopment, most genome-wide association studies (GWAS) of brain-developmental disorders have excluded the X chromosome partly due to the specialized analyis required to account for different penetrance and dosage associated with different numbers of chromosomes in males and females.

The recurrent bursts of strong natural selection observed on human and primate X chromosomes propel a selected variant and its chromosomal surroundings to high frequency, most recently 45-55,000 BP, where strong selection at fourteen different loci dramatically affected allele frequencies for 10% of the chromosome [2]. The scale of this phenomenon is exclusive to the X chromosome, which strongly suggests that such selection acts on gene function in male spermatogenesis. Considering that 24% of human neuron genes are also expressed in male spermatids [3], it raises the question of whether the unique evolution and the recurrent allele frequency changes also shape the genetic variation relevant to neuro-development and ASD. In a pilot study conducted for this application using MAGMA on an iPSYCH ASD cohort, I found that the subset of neuron genes also expressed in spermatids are, in fact, significantly enriched for ASD association in males.

This project explores the hypothesis that the strong selection acts on selfish X-linked gene variants, promoting their preferential transmission into gametes by escaping meiotic sex chromosome inactivation to negatively affect the fidelity of meiosis and integrity of Y-bearing sperm cells. This phenomenon is called meiotic drive and is observed across vertebrates, insects, fungi, and plants [4]. The transmission advantage of a selfish gene variant can easily offset a modest deleterious effect of the same variant on neuronal function. The idea behind this project is that such selfish spermatid genes, also expressed in neurons, fuel a conflict between the two tissues, increasing ASD-relevant variation and X chromosome heritability.

The societal impact of autism spectrum disorder will keep increasing in the foreseeable future. Extrapolating the rate of newly diagnosed Danes below age 18, the diagnosed proportion may easily reach 3% within this decade. The prevalence of ASD highlights the importance of distinguishing and separating neuro-developmental conditions spanned by the new ICD-11 ASD diagnosis, understanding the relevance of sex, and predicting, treating, and supporting neuro-divergent children effectively.

D: Proposed method of solution or concept:

In this project, I will combine state-of-the-art evolutionary modeling with standard statistical association. This unique approach increases the statistical power of both association and heritability estimation and allows me to quantify natural selection on identified causal variants.

Male meiotic sex-chromosome inactivation (MSCI), which limits the scope for meiotic drive in spermatogenesis, and somatic X chromosome inactivation (XCI), which inactivates the extra X in females, most likely evolved from the same highly conserved mechanism of un-synapsed chromatin silencing [5] and thus overlap in their control and chromatin organization. I hypothesize that meiotic drivers, in addition to other detrimental effects, fuel an inactivation-dosage-conflict between the sexes: If genes that escape MSCI in males also escape XCI in females, the deleterious excess female dosage must be resolved by lower gene expression, in turn leading to dosage deficiency in males (Figure 1). In my pilot for this application, I not only confirm the expectation that ASD association in males is significantly elevated among genes escaping female XCI but also that female ASD association is stronger among candidate meiotic driver genes with a homolog on the Y chromosome.

I will embed the standard linear mixed models for GWAS association in population genomic models of cohort ancesty [6]. Directly modeling ancestry in the iPSYCH cohorts (4822 female cases, 14948 male cases, 18917 female controls, and 19310 male controls) along the chromosome increases power over standard imputation methods, especially for rare variants, which my hypothesis predicts play an outsized role on the X chromosome. It also lets me infer any recent selection on meiotic drivers escaping MSCI. To test individual predictions of my dosage-conflict hypothesis, I will estimate ASD heritability and polygenic risk scores (PGS) in pairs of nested gene sets, each exposing one characteristic of the spermatid/neuron interaction. To test the additional hypothesis that ASD risk-variants "hitchhike" to a higher frequency with genes under strong selection or sperm function (Figure 2), I will use multivariate multivariable PGS regression on variant frequency. I will use the same models to detect whether the relevant gene sets also show differential load on ICD-10 autism subtypes.

E: Major gain and obstacles:

I expect to provide evidence that the X chromosome carries an elevated ASD load on genes linked to neuronal migration and functions controlling neurodevelopment and that this can be attributed to strong

natural selection acting on the spermatid function of these genes.

As the expanded iPSYCH X chromosome data has yet to be thoroughly analyzed, the crucial base calling, curation, and quality control steps are incomplete. However, I have made a plan with the iPSYCH team to overcome this obstacle before the proposed project begins.

I will consider the project successful if I accumulate enough evidence for the proposed hypotheses to initiate a new research field on the relationship between sex chromosome evolution and neurodevelopmental disorders. Such evidence would include identifying responsible gene functions and pathways shared with spermatids. Given the novelty of the idea and the large cohorts at my disposal, I find it realistic to publish one high-impact topical paper in a journal like Nature Genetics.

A natural next step beyond this project would be to pool iPSYCH data with the existing but unexploited X chromosome variant summary statistics from the ASD cohorts of PGC (~6500 affected and 8100 unaffected) and SPARK (7800 trios and 16000 affected singletons) and repeat the heritability and polygenetic risk analyses amendable this more extensive data set. A more ambitious future project would be to test whether meiotic drivers on both the X and Y chromosomes contribute to ASD load by assigning cohort individuals to Y haplogroups and including them as variables in the regression models described.

F: Anticipated outcomes:

I will develop new population genomic methods sensitive to rare X-linked variants and recalibrate existing ones for association and heritability analysis of X-linked markers and polygenic scoring.

I will quantify the X-linked variation associated with ASD.

I will test the hypothesis that a neuron/spermatid dosage conflict contributes to an elevated ASD load on the X chromosome.

I will test the hypothesis that ASD risk-variants near strongly selected spermatid genes "hitchhike" to a higher frequency, increasing their population prevalence.

The project will showcase the insight missed when neglecting the analysis of sex chromosomes and how modeling the evolutionary context of associated genes can inform disease etiology

G: Appropriateness:

The proposed project applies evolutionary theory and basic research methodology to a purely health-related problem. Judging both the validity of proposed methods and the health relevance of their application requires a genuinely interdisciplinary board that I will not find among the topical funding schemes of FNU, FSS, and NNF. Despite reassuring results from my pilot, the proposed exploratory project is high-risk/high-gain and fits a funding body valuing such projects.

G: Probable objections:

Q: If the X chromosome carries more ASD variance, why do you not find any individually significant genes in the X chromosome? A: We do. Although no individual SNPs rise to genome-wide significance, our MAGMA pilot significantly associates the genes TSPAN7 and SLC25A14 linked to

autism but not previously identified in a GWAS.

Q: What if the hypothesis is not true? A: The project will still yield a careful X chromosome GWAS and methodology applicable for analyses of other disorders.

Q: Will there be enough statistical power to analyze the sexes separately? A: Reduced power likely affects our ability to identify individual risk variants. However, the project uses heritability estimation and polygenetic risk scores for nested gene sets, which have enough power for the proposed analyses.

References:

[1] Mallard *et al.*, "X-chromosome influences on neuroanatomical variation in humans," *Nat Neurosci*, pp. 1–9, 2021, doi: 10.1038/s41593-021-00890-w.
[2] Skov et al., "Extraordinary selection on the human X chromosome associated with archaic admixture," *Cell Genom*, p. 100274, 2023, doi: 10.1016/j.xgen.2023.100274.
[3] Matos et al., "Brain and testis: more alike than previously thought?," *Open Biol.*, vol. 11, no. 6, p. 200322, 2021, doi: 10.1098/rsob.200322.
[4] Zanders and Unckless, "Fertility Costs of Meiotic Drivers," *Curr Biol*, vol. 29, no. 11, pp. R512–R520, 2019, doi: 10.1016/j.cub.2019.03.046.
[5] Hornecker et al., "Meiotic sex chromosome inactivation in the marsupial Monodelphis domestica," *genesis*, vol. 45, no. 11, pp. 696–708, 2007, doi: 10.1002/dvg.20345.
[6] Zhang, et al., "Biobank-scale inference of ancestral recombination graphs enables genealogical analysis of complex traits," *Nat. Genet.*, vol. 55, no. 5, pp. 768–776, 2023, doi: 10.1038/s41588-023-01379-x.

Figure 1:

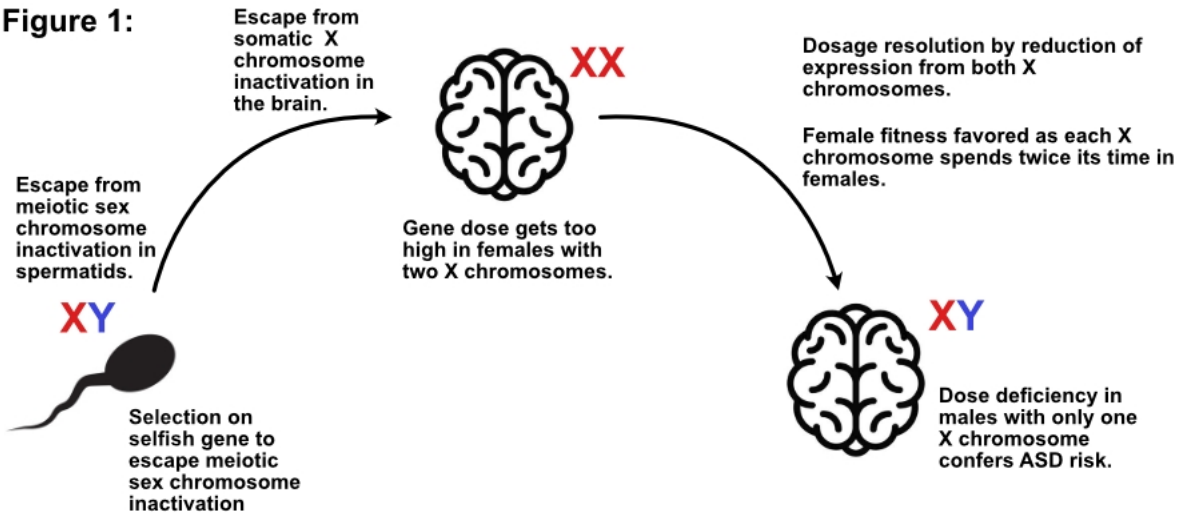
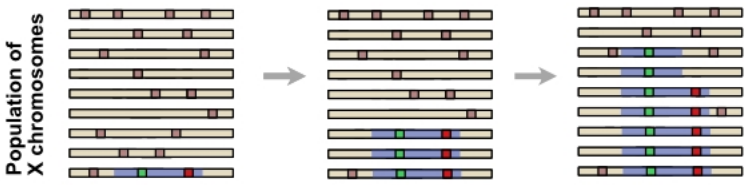


Figure 2:



Mildly deleterious ASD risk variant (red) "hitchhikes" to a higher frequency in the genomic neighborhood (blue) of a strongly selected selfish gene variant (green).

If resubmission of application

This is not a resubmission.

Potential benefit for neuroscience of the proposed project

If successful, the project will not only improve our understanding of autism's etiology and help explain the sex bias of the condition. It will also introduce evolutionary conflicts between tissues as important contributors to the genetic basis of ASD. Other neurodevelopmental disorders such as ADHD and psychiatric disorders such as anorexia nervosa, schizophrenia, and major depression have large genetic overlaps with autism and have sex biases that make them relevant for a similar analysis. More broadly, the association analysis methods developed and used in the project will assist the inclusion of chromosome X in GWAS of any trait and disorder and the generation of polygenic scores based on X chromosomes, which are currently absent. Systematic inclusion of X-linked markers in GWAS and polygenic scores will greatly improve our understanding of the X chromosome's contribution in general. Among the neuron/sperm genes to be investigated, many have known functions in spermatids related to actin or microtubule transport and organization, functions important in tangential neuronal migration, and other neurodevelopmental processes linked to ASD. Exposing the interface between neuron and spermatid function may thus provide new insights regarding the function, expression, and properties of genes associated with ASD and inspire wet lab researchers specializing in particular candidate genes to generate new testable hypotheses.

Roadmap to impact

I will contact Sohini Ramachandran (Brown) and Benjamin Neale (Broad, MIT), leading methodological developments in GWAS, to embed the developed X chromosome analyses in their widely used research software packages. This way, the X chromosome will be automatically included in future GWAS studies. In my research on the X chromosome's unique and dramatic evolutionary processes, I have advocated for its inclusion and analysis in whole genome projects and contributed this expertise in collaborations. In talks and research visits, I will highlight the importance of considering the X chromosome in neurodevelopmental disorders. I will visit Karen Parker (Stanford), who is working on ASD-like subsociality in macaques, and Prof. Susan McConnel (Stanford), who specializes in neuronal migration and axon guidance and works actively on the microtubule-associated Doublecortin protein, which shows the strongest recent natural selection and is a promising candidate of a neuron/spermatid conflict. Completing this project, I will apply for a grant in either the Exploratory Interdisciplinary Synergy Programme or the Interdisciplinary Synergy Programme from Novo Nordisk Foundation (this agency presently funds another line of my X chromosome research with a project grant). For this future project, I plan an evolutionary approach modeling both human ASD cohorts and macaque cohorts scored for subsociality in collaboration with Karan Parker and Susan McConnel, as well as my current iPSYCH contact.

Comments to the budget

Postdoc salary is based on an average postdoc salary at Aarhus University, including pension and social taxes.

Computing expenses cover running the extensive analyses on the GenomeDK computing cluster. Estimated 12 CPU years per project month.

Equipment covers a high-powered Apple computer for the development and testing of analyses deployed on GenomeDK. An Apple computer is required because of its underlying Linux architecture.

Conferences and travel cover two yearly international conferences to disseminate results and to establish the network required to maximize the impact of the results and the new methodology produced.

Publication costs cover the charges of publishing in top topical journals under Cell, Nature, or Science.

Budget

Year Type	Name	Amount of money	Details
1			
AppliedLF			
	Løn - postdoc	582.996,00 kr.	Postdoc salary
	Rejser & konferencer	25.000,00 kr.	Travel and conference expenses
	Drift	130.000,00 kr.	Computing
	Udstyr	25.000,00 kr.	Powerful desktop computer for development and testing
	Drift	20.000,00 kr.	Licenses, software, small items
	Projektrelaterede indirekte omkostninger	78.300,00 kr.	
		861.296,00 kr.	
2			
AppliedLF			
	Løn - postdoc	600.486,00 kr.	Postdoc salary
	Rejser & konferencer	25.000,00 kr.	Travel and conference expenses
	Drift	130.000,00 kr.	Computing
	Andet	30.000,00 kr.	Publication costs
	Projektrelaterede indirekte omkostninger	80.548,00 kr.	
	Drift	20.000,00 kr.	Licenses, software, small items
		886.034,00 kr.	
		1.747.330,00 kr.	

Appendix

Letter of support:
Host letter_Lundbeck Experiment 2023.pdf
Letter of support from host institution

CV:
CV.docx
CV