Genome-wide association study of prolactin levels in blood plasma and cerebrospinal fluid

Lyndsay A. Staley†, Mark T. W. Ebbert†, Sherady N. Parker, Matthew Bailey, for the Alzheimer’s Disease Neuroimaging Initiative, Perry G. Ridge, Alison M. Goate and John S. K. Kauwe*

From 12th Annual Biotechnology and Bioinformatics Symposium (BIOT-2015)
Provo, UT, USA. 10-11 December 2015

Background
Prolactin, a hormone mostly secreted from the lactotroph cells within the anterior pituitary gland [1] and expressed by the PRL gene, plays an important role in milk lactation for pregnant women [1], helps regulate the menstrual cycle, and also affects reproduction, metabolism, homeostasis, tissue growth, osmoregulation, immunoregulation, and behavior [2, 3]. Prolactin levels are regulated in a short-loop feedback mechanism by prolactin inhibitory factors (PIF), dopamine being an important example [4]. This feedback system changes during pregnancy, and prolactinomas, hypothyroidism, medications, stress, exercise, herbs, and certain foods can also affect prolactin levels [5, 6]. Prolactin has also been shown to suppress apoptosis, and increase survival and function of cells, including T-lymphocytes [7].

Cerebrospinal fluid (CSF) and plasma separated by the blood–brain barrier and levels of expression in these biological fluids are often independent, suggesting the genes are regulated independently across tissues on either side of the blood–brain barrier [8]. Currently, little is known about genetic markers that affect prolactin expression in plasma or CSF. In this study we conducted a genome-wide association study of prolactin levels in the CSF and in the plasma of individuals from two datasets, looking for SNPs that are associated with prolactin levels in both CSF and plasma. Further research of the variants we identified will help researchers further understand how prolactin is regulated across multiple tissues in the human body and how it affects human health.

* Correspondence: Kauwe@byu.edu
†Equal contributors
1Department of Biology, Brigham Young University, Provo, UT 84602, USA
Full list of author information is available at the end of the article

© 2016 The Author(s). Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Methods

Subjects and data description

CSF and plasma samples were collected from the Knight-Alzheimer’s Disease Research Center at Washington University School of Medicine (Knight ADRC) and from the Alzheimer’s Disease Neuroimaging Initiative (ADNI). In this study, we used 297 CSF and 347 plasma samples from ADNI, and 246 CSF and 240 plasma samples from Knight ADRC. The majority of the samples were controls, although 7% of Knight ADRC samples were Alzheimer’s disease cases, and 15% of ADNI samples were AD cases. Levels for 190 biomarkers were measured for each sample using the Human DiscoveryMAP Panel v1.0 and a Luminex 100 platform [9] and the samples were genotyped using the Illumina 610 or the Omniexpress chip. A description of the collection methods and the Knight ADRC samples has been previously published [10, 11] and the ADNI samples were collected as part of the ADNI biomarker study [12], and were obtained from the ADNI database (adni.loni.usc.edu).

All samples were of European descent, and varied in age from 58 to 91 years, with an average age of 76 years, for the ADNI samples, and varied in age from 49 to 91 years, with an average age of 73 years, for the Knight ADRC samples. All individuals whose data were included in this study were explicitly consented, following appropriate Institutional Review Board policies.

SNP imputation

SNPs were imputed as previously described [13]. Beagle was used to impute SNPs from the data from the 1000 Genomes Project (June 2012 release). Imputed SNPs with the following criteria were removed: (1) an $r^2$ of 0.3 or lower, (2) a minor allele frequency (MAF) lower than 0.05, then used RegulomeDB [21] and functional annotations explicitly consented, following appropriate Institutional Review Board policies.

| SNP      | Chr | Base Pair Position | Major Allele | Minor Allele | MAF  | Predicted Function | Gene       | RegulomeDB score | Meta-analysis p-value |
|----------|-----|--------------------|--------------|--------------|------|--------------------|------------|------------------|-----------------------|
| rs12548348 | 8   | 70430077           | A            | G            | 0.1222 | Intronic           | SULF1     | No Data          | Plasma 6.288e-11 CF5 9.841e-26 |
| rs13408093 | 2   | 62251682           | A            | T            | 0.0699 | Intronic           | TRBP2     | 5                | Plasma 6.881e-10 CF5 2.119e-25 |
| rs1150703  | 6   | 28184260           | G            | T            | 0.0919 | ncRNA_exonic       | TOB2P1    | 5                | Plasma 3.276e-09 CF5 7.011e-26 |
| rs988083   | 6   | 28177588           | C            | T            | 0.1220 | Intergenic         | ZNF192P1,TOB2P1 | 5            | Plasma 3.276e-09 CF5 7.011e-26 |
| rs988084   | 6   | 28177492           | C            | T            | 0.1218 | Intergenic         | ZNF192P1,TOB2P1 | 6            | Plasma 3.276e-09 CF5 7.011e-26 |
| rs37326888 | 7   | 15021811           | T            | C            | 0.0893 | UTR3               | GIMAP7     | 6                | Plasma 4.209e-09 CF5 6.438e-24 |
| rs8073041  | 17  | 47498253           | T            | A            | 0.0731 | Intergenic         | PHBLOC101927207 | 6            | Plasma 7.878e-09 CF5 2.169e-10 |
| rs1150701  | 6   | 28183886           | A            | C            | 0.1410 | ncRNA_exonic       | TOB2P1    | 6                | Plasma 1.184e-08 CF5 1.443e-26 |
| rs1150702  | 6   | 28184097           | A            | T            | 0.1410 | ncRNA_exonic       | TOB2P1    | 5                | Plasma 1.184e-08 CF5 1.443e-26 |
| rs1233712  | 6   | 28193131           | G            | A            | 0.1406 | UTR5               | ZSCAN9     | 4                | Plasma 1.184e-08 CF5 1.443e-26 |
| rs79268972 | 17  | 47531241           | T            | G            | 0.0749 | Intergenic         | PHBLOC101927207 | 5            | Plasma 2.547e-08 CF5 8.169e-12 |
| rs77482998 | 7   | 47532356           | T            | C            | 0.0665 | Intronic           | TN53      | 5                | Plasma 4.608e-08 CF5 7.983e-12 |
from wAnnovar [22, 23] to identify SNPs that are biologically likely to modify gene function or expression. RegulomeDB scores range from “1a” to “6”. Lower scores indicate stronger evidence that the SNP affects gene regulation based on both empirical data, such as ChIP-seq, and whether the SNP is within a known transcription factor binding motif. We generated regional association plots using SNAP [24] for regions of interest and explored

Fig. 1 PathwayCommons output showing the gene that codes for prolactin along with the major players SULF1 and TRIB2. Our significant SNPs, rs12548348 and rs13408093, fall in SULF1 and TRIB2, respectively. This means that although none of the SNPs fall directly in or near PRL, they could still be affecting the prolactin pathway because they are regulated by some of the same transcription factors.
whether any genes of interest are part of the same pathway or regulatory network using PathwayCommons [25]. For SNPs where linkage disequilibrium data is unknown in SNAP, we modified the SNAP source code to plot all SNPs in the region regardless of linkage disequilibrium status and omit $r^2$ values. By default, SNAP only plots SNPs with a known $r^2$ greater than 0. We also generated q-q plots in R to check for evidence of inflation of $p$-values.

Results
We identified 37 SNPs associated with prolactin levels in plasma and 666 SNPs associated with prolactin levels in CSF (Additional files 1 and 2), none of which are located in or around the \textit{PRL} gene. Significant SNPs were spread across 21 chromosomes for the CSF results and across 10 different chromosomes for the plasma results. There are several hits on chromosome 6, but all are more than 5 million base pairs away from where the \textit{PRL} gene is located. There were 12 SNPs in common between the plasma and CSF results (Table 1), 6 of which were on chromosome 6, approximately 6 million base pairs away from the \textit{PRL} gene. RegulomeDB scores for the 12 SNPs ranged from 4 to 6 and MAFs ranged from 0.06 to 0.14. None of the 12 SNPs were found in the NHGRI catalog of published genome-wide association studies. The q-q plots demonstrated no evidence of inflation (genomic inflation factor = 1.0; Additional files 3 and 4). According to PathwayCommons, \textit{PRL}, \textit{SULF1}, and \textit{TRIB2} are all regulated by some of the same transcription factors (Fig. 1) including \textit{PBX1}, \textit{XBP1}, \textit{TCF3}, \textit{LEF1}, \textit{VSX1}, \textit{PITX2}, and \textit{LHX3}. There were no other known relationships among the genes identified in this study.

Discussion
Twelve SNPs were significantly associated with prolactin levels in both plasma and CSF, 6 are located on chromosome 6 and the remaining 6 SNPs are scattered across chromosomes 2, 7, 8, and 17. The 6 SNPs on chromosome 6 cluster in and around ZSCAN9, TOB2P1, and ZNF192P1, according to Annovar, though visualizing the SNPs’ locations in the NCBI viewer shows that 3 of the 6 SNPs fall within a ZSCAN9 intron for one specific transcript (XM_011514877.1) as well as within TOB2P1—a pseudogene that falls within the same intronic region of...
ZSCAN9. SNP rs1233712 is in the 5′UTR region of ZSCAN9. SNPs rs988083 and rs988084 are between ZNF192P1 and TOB2P1, according to Annovar. ZNF192P1 is also a pseudogene that is proximal to ZSCAN8. In short, all 6 SNPs on chromosome 6 are located in or around ZSCAN8 and ZSCAN9, both of which are protein-coding genes, while 3 of the 6 fall directly within a pseudogene (TOB2P1). Of the significant SNPs on chromosome 6, rs1150703 was most significantly correlated with prolactin levels in plasma (Fig. 2) while rs1150701 was most significantly correlated with prolactin levels in CSF (Fig. 3).

The remaining 6 SNPs are located on chromosomes 2, 7, 8, and 17, where 2 of the SNPs are intergenic, 3 are intronic, and one is located in a 3′UTR region (Table 1). SNP rs12548348 is an intronic SNP within the SULF1 gene on chromosome 8 and was most significantly associated with prolactin levels in plasma out of the 12 found in common between the two fluids. It was also one of most significantly associated with prolactin levels in CSF. SNPs rs13408093 and rs77482998 are intronic SNPs within the TRIB2 (chromosome 2) and TNS3 (chromosome 7) genes, respectively. SNPs rs8073041 and rs79268972 are intergenic SNPs that are both located on chromosome 17 between the gene PHB and a non-coding RNA LOC101927207. The next closest protein-coding gene is NGFR. SNP rs73726888 is located in the 3′UTR region of GIMAP7 on chromosome 7. While rs77482998 (TNS3) and rs73726888 (GIMAP7) are both located on chromosome 7, they are distant from each other on opposite arms of the chromosome, suggesting their associations with prolactin levels are independent of each other.

While there is no direct evidence that any of these markers directly impact prolactin expression, it appears that PRL, SULF1, and TRIB2 in that they are all regulated by common transcription factors, including PBX1, XBP1, TCF3, LEF1, VSX1, PITX2, and LHX3. It is possible that these genes and variants are involved in PRL regulation through more complex biological relationships. This may be significant because genes regulated by the same transcription factor are often active in the same tissues at the same time [26, 27].

**Conclusions**

In summary, we have identified significant and replicable association between several genetic variants in both...
plasma and CSF levels of prolactin. These results provide a foundation for a better understanding of prolactin regulation, and in turn the host of phenotypes in which prolactin plays a role, including lactation, immunoregulation, apoptosis and T-lymphocyte function [1–3, 7]. Future work on these associated markers will provide meaningful insights into these phenotypes.

**Additional files**

Additional file 1: File contains a table of SNPs significantly associated with prolactin levels in blood plasma by meta-analysis. (DOCX 130 kb)

Additional file 2: File contains a table of SNPs significantly associated with prolactin levels in CSF by meta-analysis. (DOCX 183 kb)

Additional file 3: File contains a Q-Q plot of the plasma data used in this study. (DOCX 74 kb)

Additional file 4: File contains a Q-Q plot of the CSF data used in this study. (DOCX 76 kb)

**Abbreviations**
PIF, prolactin inhibitory factors; ADNI, Alzheimer’s Disease Neuroimaging Initiative; CSF, Cerebrospinal Fluid; eQTL, expression quantitative trait locus; Knight ADRC, Knight-Alzheimer’s Disease Research Center at Washington University School of Medicine; SNP, single nucleotide polymorphism; UTRs, untranslated regions

**Acknowledgements**
The authors acknowledge that many scientists contributed in developing the clinical and genetic resources necessary to collect these data and complete this project. The authors also gratefully acknowledge the efforts of hundreds of individuals who participated as subjects in these studies. The NIH (R01 AG035053, R01 AG042611, P50 AG05681, P01 AG03991, P01 AG026276), the Alzheimer’s Association (MNIRG-11-205368), and the Brigham Young University Gerontology Program provided support for this work. We also acknowledge the Alzheimer’s Disease Genetics Consortium (ADGC) and Genetic and Environmental Risk for Alzheimer’s Disease Consortium (GERAD) for providing genotype data used in this work. GERAD was supported by the Medical Research Council (Grant nu 503480), Alzheimer’s Research UK (Grant nu 503176), the Wellcome Trust (Grant nu 082694/2/07/Z) and German Federal Ministry of Education and Research (BMBF): Competence Network Dementia (CND) grant nu 01GI0102, 01GI0711, 01GI0420. CHARGE was partly supported by the NIH/NIA grant R01 AG033193 and the NIA–AG081220 and AGC contract N01–AG–12100; the NHLBI grant R01 HL105756; the Icelandic Heart Association, and the Erasmus Medical Center and Erasmus University.

ADGC was supported by the NIH/NIA grants: U01 AG032984, U24 AG021886, U01 AG016976, and the Alzheimer’s Association grant ADGC–10–196728. Data collection and sharing for this project was funded by the Alzheimer’s Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer’s Association; Alzheimer’s Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innoventions, N.V.; IQVIA Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC; Johnson & Johnson Pharmaceutical Research & Development, LLC; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC; NeuroRx Research; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer’s Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. Some of the samples used in this study were genotyped by the ADGC and GERAD. ADGC is supported by grants from the NIH (#U01AG032984) and GERAD from the Wellcome Trust (GR082604VA) and the Medical Research Council (G0304029).

Portions of data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report.

The ADNI Executive Committee consists of: Michael Weiner, MD UC San Francisco; Paul Aisen, MD UC San Diego; Ronald Petersen, MD, PhD Mayo Clinic; Rochester; Clifford R. Jack, Jr., MD Mayo Clinic, Rochester, William Jagust, MD UC Berkeley; John Q. Trojanowki, MD, PhD U Pennsylvania; Arthur W. Toga, PhD USC; Laurel Beckett, PhD UC Davis; Robert C. Green, MD, MPH Brigham and Women’s Hospital/Harvard Medical School; Andrew J. Saykin, PsyD Indiana University; John Morris, MD Washington University St. Louis; Leslie M. Shaw University of Pennsylvania. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

**Availability of data and materials**
Data are available to researchers by applying to the respective organizations, ADNI and ADGC consortia. Application is required to protect participant confidentiality. The ADNI data are available at (http://adni.loni.usc.edu/), the Knight ADRC data are available through dbGAP (http://www.ncbi.nlm.nih.gov/gap).

**Authors’ contributions**
LS, SP, MB and JSKK carried out data analysis. LS and ME annotated and analyzed the SNPs for significance and drafted the manuscript. All other authors participated in the conception of the project and obtaining data. All authors read and approved the final manuscript.

**Competing interests**
The authors declare that they have no competing interests.

**Ethics approval and consent to participate**
Data was obtained and analyzed under approval of the Brigham Young University Institutional Review Board.

**Consent for publication**
Not applicable.

**Author details**
1Department of Biology, Brigham Young University, Provo, UT 84602, USA. 2Biology and Biomedical Sciences, Washington University, St. Louis, MO 63110, USA. 3Department of Neuroscience Icahn School of Medicine, New York, NY 10029, USA.

**Published:** 29 June 2016

**References**
1. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiol Rev. 2000;80:1523–631.
2. Ben-Jonathan N, LaPensee CR, LaPensee EW. What can we learn from rodents about prolactin in humans? Endocr Rev. 2008;29:291–41.
3. Grattan DR. 60 years of neuroendocrinology: the hypothalamo-prolactin axis. J Endocrinol. 2015;226:101–22.
4. Peter Fitzgerald TG. Prolactin and dopamine: what is the connection? A review article. J Psychopharmacol. Oxf Engl. 2008:22:12–9.
5. Lassen CM, Grattan DR. Prolactin, neurogenesis, and maternal behaviors. Brain Behav Immun. 2012;26:201–9.
6. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:273–88.

7. Nithya Krishnan OT. Prolactin suppresses glucocorticoid-induced thymocyte apoptosis in vivo. Endocrinology. 2003;144:2102–10.

8. Aluise CD, Sowell RA, Butterfield DA. Peptides and proteins in plasma and cerebrospinal fluid as biomarkers for the prediction, diagnosis, and monitoring of therapeutic efficacy of Alzheimer’s disease. Biochim Biophys Acta. 2008;1782:549–58.

9. John SK, Kauwe MHB. Genome-wide association study of CSF levels of 59 Alzheimer’s disease candidate proteins: significant associations with proteins involved in amyloid processing and inflammation. PLoS Genet. 2014;10, e1004758.

10. Cruchaga C, Kauwe JSDK, Harari O, Jin SC, Cai Y, Karch CM, et al. GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer’s disease. Neuron. 2013;78:256–68.

11. Anne M, Fagan MAM. Inverse relation between in vivo amyloid imaging load and CSF Aβ42 in humans. Ann Neurol. 2006;59:12–9.

12. Trojanowski JQ, Vandeerstichele H, Korecka M, Clark CM, Aisen PS, Petersen RC, et al. Update on the biomarker core of the Alzheimer’s Disease Neuroimaging Initiative subjects. Alzheimers Dement J Alzheimers Assoc. 2010;6:230–8.

13. Kauwe JSDK, Bailey MH, Ridge PG, Perry R, Wadsworth ME, Hoyt KL, et al. Genome-wide association study of CSF levels of 59 Alzheimer’s disease candidate proteins: significant associations with proteins involved in amyloid processing and inflammation. PLoS Genet. 2014;10, e1004758.

14. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559–75.

15. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of hardy-Weinberg equilibrium. Am J Hum Genet. 2005;76:887–93.

16. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.

17. Chang X, Wang K, wANNOVAR: annotating genetic variants for personal genomes via the web. J Med Genet. 2012;49:433–6.

18. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O’Donnell CJ, de Bakker PIW. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. 2008;24:2938–9.

19. Cerami E, Gross BE, Demir E, Rodchenkov I, Babur O, Anwar N, et al. Pathway Commons, a web resource for biological pathway data. Nucleic Acids Res. 2011;39:D685–90.

20. Visel A, Minovitsky S, Dubchak I, Pennacchio LA. VISTA Enhancer Browser—a database of tissue-specific human enhancers. Nucleic Acids Res. 2007;35: D88–92.

21. Kleftogiannis D, Kalnis P, Bajic VB. DEEP: a general computational framework for predicting enhancers. Nucl Acids Res. 2015;43:e6–e6.