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Introduction

The current issue of Chapman University's *E-Research* samples the breadth of research conducted by Schmid College of Science undergraduate majors and general education students. Student researchers studied the efficacy of the naturally occurring phytochemicals in pomegranate juice extract in promoting cell adhesion in pancreatic cancer cells. Others used satellite data to determine changes in ocean acidification by detecting fluctuations in oceanic chlorophyll content or computational techniques to track demographic trends. Finally, other student researchers applied mathematical analyses to problems posed by molecular genetics or to better understand the efficacy of combining psychopharmaceuticals and therapy to treat cocaine addiction.

These papers present the work of undergraduate, not graduate, students. Undergraduate research is a hallmark of a Chapman University science education. Such research, whether conducted by science majors or general education students, allows students to learn science by doing science. Doing science engages students in the quest for new knowledge and in the application of classroom learning to solving real world problems. Doing science also exposes students to working in teams whose members contribute different disciplinary expertise to answering scientific questions. We believe you will be astounded by the quality of these papers.

Janeen Hill, Guest Editor
Professor of Biological Sciences and Senior Associate Dean, Schmid College of Science

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On the ranking of the disease susceptibility locus in family-based candidate gene studies: a simulation-based analysis

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Abstract

The ranking of the p-value of the true causal single nucleotide polymorphism in the ordered list of individual SNP p-values is an important factor for achieving success in the ultimate objective of association studies - identifying deleterious genetic variants. Thus, we undertake a study to assess the implications of complex, multimarker correlation structure, sample size and disease models on the ranking of the causal SNP. We carry out an extensive family-based candidate gene simulation study to analyze the position of the disease susceptibility locus in the complete list of individual SNP p-values ordered according to their statistical significance. We simulate data based on the haplotype distributions of ten randomly selected genes extracted from the HapMap database, various sample sizes (600,1000 and 2000) that current association studies employ, and disease models that mimic the characteristics of complex human disorders.

We conclude that the average ranking of the causal SNP for sample sizes 600, 100 and 200 of 10.97, 9.65, and 8.34 are dramatically distant from the most significant and intuitively appropriate top position. This result is even more pronounced for genes with high average correlation and large number of common SNPs. Moreover, the gain of the DSL ranking when comparing sample sizes 600 to 1000 and 1000 to 2000, averaged over disease models, causal SNPs and genes, was approximately 1.3. These outcomes both reveal the importance of the sample size and quantify the magnitude required to unequivocally determine the identity of the DSL in family-based candidate gene studies.

Our results show the overwhelming importance of large sample sizes in the localization of deleterious SNPs even under simple disease models. These conclusions possess pronounced importance for the design and result interpretation of candidate gene, next generation high-density genome-wide association studies, as well as for the construction and implementation of association tests based on the distribution of the most significant (minimum p-value) test statistics.

Keywords: Fine Mapping, FBAT, Causation

Background

There has been a continual effort in finding the best analytical methods for identifying disease causal mutations in the human genome. The chronological progression of the research methodology involves linkage analysis [Chioza, et al. 2009; King, et al. 2000], candidate gene studies [Mollaki, et al. 2009; Murphy, et al. 2009; Rakovski, et al. 2007b] and currently, genome-wide association scans based on hundreds of thousands of single nucleotide polymorphisms (SNPs) with case-control [Barrett, et al. 2009; Beecham, et al. 2009; Check Hayden 2009; Himes, et al. 2009; Palmer, et al. 2009; van de Mortel, et al. 2000] and family-based data [Malarstig, et al. 2009; Van Steen and Lange 2005]. The ultimate objective of detecting and assessing the effects of the disease susceptibility loci (DSL) is predicated not only on the existence of significant corresponding test statistics p-values but also on their ranking in the sorted list of all (commonly SNP-specific) p-values. This is especially true with genome-wide

association scans due to the severity of the multiple testing problems which is generally handled by significance level adjustment methods that fail to reorder the test statistics. Thus, the emergence of the true DSL at the top of the significance list is pivotal for the success and replicability of genome-wide association scans.

In current large-scale studies, the physical locations of the genotyped SNPs are usually far apart and linkage disequilibrium (LD) is not an issue. On the other hand, in family-based and case-control candidate gene association studies we come across high level of LD between the pairs of SNPs in candidate regions containing a putative deleterious allele which causes multiple significant p-values to arise from association testing. In fact, we generalize a related question about the induced test statistics correlation for pairs of SNPs in LD [Pritchard and Przeworski 2001]. The problem of the position of the DSL in the significance ranking in candidate gene studies arises both from multiple testing and complex multimarker correlation structure represented by the region's haplotype distribution. Finally, the analysis of next generation genome-sequencing data [Check Hayden 2009; Pennisi 2009] will include a combination of both challenges, a dramatic increase of the magnitude of the multiple testing problem and an additional complexity of high correlations between closely positioned SNPs. In general, it is intuitive to expect that the most significant p-value will originate from the true causal SNP, but as with genome-wide association studies, this may not always be true. Lastly, there is a class of powerful testing strategies that are based on the distribution of the most significant (minimum p-value) individual marker test statistic that make the implicit assumption that the statistic with the minimum p-value corresponds to the causal SNP [Kimmel, et al. 2007; Rakovski, et al. 2007a]. We have undertaken a family-based simulation study to explore the validity of this assumption.

Methods

We simulated and analyzed family data consisting of family trios with complete data and discordant sib pairs with missing parental data. The results for both family designs were practically identical with respect to the question of interest; thus, in the subsequent presentation we report results for discordant sib-pairs data analyses only. With respect to data analyses approaches, we used the two natural tools for detecting difference in allele frequencies at a particular locus between related cases and controls, conditional logistic regression and the classical family-based test for association [van de Mortel, et al. 2000]. Since the results obtained under the implementation of both methods were extremely similar, we only report the outcomes of our work for conditional logistic regression. As customary, we employ additive coding of the marker genotypes by assigning values of 0, 1, and 2 to AA, Aa, and aa, respectively. In the conditional logistic regression model, we treated each family as a stratum containing the affected-unaffected pair of offspring and fitted all single covariate models to assess the unadjusted effect sizes of all markers in the candidate region.

Simulation

We randomly selected 10 genes from autosomal chromosomes of the human genome, extracted the resequenced unphased genotypes of the CEPH families from the publicly accessible ENCODE data from the HapMap database [Tanaka 2009] and calculated the corresponding haplotype distributions using the EM algorithm [Dempster, et al. 1977] via its implementation in the FBAT software package [Girirajan, et al. 2009]. We used R version 2.9.2 [Thauvin-Robinet, et al. 2009] for both the simulation of the genotype data and for the analysis of these data via FBAT and conditional logistic regression. Summary statistics of the ten genes are shown in Table 1.

Table 1. Summary characteristics of the 10 genes.

Gene	Common SNPs*	Mean MAF	Mean $\mathbf{r}^2$	Size**	Common Haplotypes***
1	18	0.14	0.38	21	4
2	20	0.18	0.24	13	6
3	30	0.30	0.42	11	5
4	41	0.22	0.24	22	6

5	14	0.15	0.25	10	4
6	61	0.24	0.39	19	6
7	96	0.23	0.32	60	4
8	29	0.30	0.37	34	4
9	46	0.25	0.89	38	5
10	36	0.18	0.37	17	5

*Number of SNPs with minor allele frequency (MAF)> 0.05.
**Measured in kilobase pairs (kb).
***Number of haplotypes with frequency >0.05.

We randomly paired haplotypes to create each of the parents and simulated pairs of offspring through Mendilian transmissions of haplotypes. Further, in consecutive simulation steps, we used six different disease models that mimic the small effect sizes of complex human disorders [Rakovski, et al. 2007b] to assign disease status to each offspring. We implemented two additive, two dominant and two recessive models defined through triplets of penetrance function with details shown in Table 2. For each of the ten genes and under each disease model, we simulated populations of families with pairs of offspring and created datasets of sizes 600, 1000 and 2000 by ascertainment of families with discordant offspring disease status. In each of the 1000 simulated dataset the causal SNP was randomly chosen and the parental genotypes were removed from the data and subsequent analyses. Summary statistics of the ten genes are shown in Table 2.

Table 2. Disease models used in the simulation study.

	Penetrance functions		
	f_0	f_1	f_2
Model 1	0.005	0.01	0.015
Model 2	0.01	0.02	0.03
Model 3	0.005	0.01	0.01
Model 4	0.01	0.02	0.02
Model 5	0.005	0.005	0.01
Model 6	0.01	0.01	0.02

Results

Our results show the importance of the three analyzed factors: gene, disease model and sample size on the ranking of the DSL. Moreover, we present the outcome of our simulation study that describes the three-way interaction of these factors. The details on the ranking of the DSL p-value for each sample size, gene and disease model combinations are displayed in Tables 3, 4 and 5.

Table 3. Ranking* of the DSL averaged over randomly chosen causal SNPs for sample size 600.

	Gene									
Disease Model**	1	2	3	4	5	6	7	8	9	10
1	4.35	4.41	5.29	5.80	4.05	11.25	16.09	4.73	20.34	7.48
2	3.97	4.06	5.71	7.38	3.50	13.73	18.03	4.94	20.10	8.23
3	2.88	3.12	4.71	4.70	2.82	10.95	13.22	3.56	19.24	6.49
4	3.07	3.48	4.72	4.95	2.72	11.02	13.71	3.44	17.87	6.51
5	8.77	8.55	13.69	17.13	6.88	25.00	40.23	11.45	22.15	16.08
6	9.20	8.70	13.88	17.96	7.02	26.79	38.89	12.23	23.11	14.14

*Ties among ranked p-values were resolved by assigning averages.
** These six disease models are described in the Simulation Design section.

Table 4. Ranking* of the DSL averaged over the randomly chosen causal SNPs for sample size 1000.

	Gene									
Disease Model**	1	2	3	4	5	6	7	8	9	10
1	2.53	3.64	4.39	5.05	3.26	7.86	14.59	3.12	18.37	6.92
2	3.26	3.34	4.08	4.56	2.92	8.99	14.79	2.63	18.07	7.07
3	1.93	2.83	4.04	3.66	2.09	8	8.72	3.23	17.39	5.35
4	2.09	2.54	3.91	3.40	2.05	6.81	10.24	3.09	17.44	5.16
5	9.04	8.65	10.34	15.26	7.04	22.97	40.44	8.98	22.26	16.19
6	8.45	7.6	10.74	17.18	6.43	25.28	43.37	10.04	22.14	13.26

*Ties among ranked p-values were resolved by assigning averages.

** These six disease models are described in the Simulation Design section.

Table 5. Ranking* of the DSL averaged over the randomly chosen causal SNPs for sample size 2000.

	Gene									
Disease Model**	1	2	3	4	5	6	7	8	9	10
1	2.18	3.21	3.66	3.63	1.77	7.53	9.02	2.47	18.54	5.78
2	1.73	2.62	3.79	3.61	1.96	6.89	9.64	3.03	17.36	5.51
3	1.64	2.49	3.70	3.48	1.67	5.90	7.45	2.60	17.15	4.79
4	1.56	2.00	3.48	3.44	1.74	7.25	7.30	2.92	16.99	5.09
5	7.96	7.26	9.54	16.41	6.06	20.73	29.68	8.98	21.50	12.73
6	8.93	8.64	9.17	13.77	5.98	18.30	34.54	7.31	22.18	14.29

*Ties among ranked p-values were resolved by assigning averages.

** These six disease models are described in the Simulation Design section.

Table 3 shows the average ranking of the DSL p-value for sample size of 600. The three consistently least favorable results are associated with genes 6, 7 and 9 with rankings averaged over all disease models and causal SNPs (both unknown quantities) of 16.45, 23.36 and 20.47 respectively. These results are foreseeable as the above-mentioned genes possess the three largest numbers of common SNPs, 61, 96 and 46 and three of the top four biggest average correlations. In particular, the average correlation of the ten genes is 0.33 while the average correlation within gene 9 is 0.89. In contrast, the lowest rank of 2.72 was obtained for the combination of disease model 4 and gene 5; this is the smallest candidate region with very low average correlation. On the other hand, disease models 3 and 4 attained the highest rankings, averaged over all DSLs and genes, of 7.17 and 7.15. Models 1 and 2 performance followed closely with ranks of 8.38 and 8.96 while models 5 and 6 could only achieve average scores of 16.99 and 17.19. However, these differences are expected due to the differences in the underlying distinctions in the effect sizes. Further, looking within disease models of the same type, the average increase taken over models 1, and 2, 3 and 4, and 5 and 6 is only 0.3. Lastly, the ranking of the DSL for sample size 600 averaged over all simulation setting parameters is 10.97.

Table 4 shows the average ranking of the DSL p-value for sample size of 1000. Again, genes 6, 7 and 9 attain the largest average rankings of 13.32, 22.02 and 19.28 respectively. The lowest rank of 1.93 was obtained for the combination of disease model 3 and gene 1; this is the second smallest candidate region which also possesses low average correlation. Moreover, as anticipated the order for all disease models is constant across sample sizes, models 3 and 4 attained rankings of 5.72 and 5.67, models 1 and 2 followed with 8.38 and 8.96 while models 5 and 6 average achieve 16.99 and 17.19. Further, looking within disease models of the same type, the average increase

taken over models 1, and 2, 3 and 4, and 5 and 6 is only 0.1. Lastly, the ranking of the DSL for sample size 1000 averaged over all simulation setting parameters is 9.65.

Table 5 shows the average ranking of the DSL p-value for sample size of 2000. Similarly to the previous sample sizes, genes 6, 7 and 9 attain the largest average rankings of 11.10, 16.30 and 18.95 respectively. The lowest rank of 1.55 was obtained for the combination of disease model 4 and gene 1. Models 3 and 4 attained rankings of 5.10 and 5.17, models 1 and 2 followed with 5.78 and 5.61 while models 5 and 6 average achieve 14.08 and 14.31. Further, looking within disease models of the same type, the average increase taken over models 1, and 2, 3 and 4, and 5 and 6 is only 0.05. Lastly, the ranking of the DSL for sample size 1000 averaged over all simulation setting parameters is 8.34.

Interestingly, the ascendancy of the DSL across the ranking list as sample size increases is much slower than anticipated. We observe that the average ranking improves by only 1.3 as we increase the sample sizes from 600 to 1000 and from 1000 to 2000. On the other hand, expectedly, based on their inherent characteristics, genes 6, 7, and 9 attain the lowest ranking regardless of sample size. Even sample size of 2000 is not sufficient to overcome the detrimental effect of large number of SNPs and high average correlation present within these genes. Further, for all simulated settings there are no observed DSL rankings of less than 2 for sample size 600 and there is only one such ranking (1.925) for sample size 1000. In contrast, for sample size 2000, we see multiple (7) average rankings of less than 2. Clearly, however, these conclusions are not particularly inspiring or helpful for the localization and identification of the causal SNP.

Discussion

We implemented a simulation-based study to explore the behavior of the ranking of the p-value of the DSL in family-based candidate gene association studies. We used classical FBAT as well as the Wald test arising from conditional univariate (SNP-specific) logistic regression models to carry out the statistical analyses. We did not use tagging SNPs because the deleterious variant might be absent from the set and that diverts the focus of the study in a different direction.

There are several important conclusions with far-reaching consequences produced by our study. For all of the studied scenarios, the overall ranking of the DSL is dramatically distant from the intuitively anticipated top position. The departure is especially remarkable with smaller sample sizes and for genes with high average correlation and large number of common SNPs. This is a somewhat surprising result that contradicts the intuitive expectation that the DSL will likely yield the most significant p-value regardless of the correlation structure, gene span and sample size. The gain in the DSL ranking when comparing sample sizes 600 to 1000 and 1000 to 2000, averaged over disease models and genes, is approximately 1.3 .This reveals the magnitude of the sample sizes needed to precisely determine the location and identity of the DSL in family-based candidate gene studies. This problem could be further exacerbated if we are testing multiple genes matching a linkage peak, performing a second stage genome-wide scan or next generation genome sequence analysis.

Lastly, minimum p-value-based association methods make the implicit assumption that the most significant test statistic is driven by and associated with the true causal SNP. We have shown that it is rarely the case and in the vast majority of the cases the most significant test corresponds to a SNP that is in LD with the causal SNP. This means that common practice to report the most significant results would potentially omit the DSL and make the findings unlikely to replicate in subsequent studies, especially in populations with different LD structure.

Conclusions

Our results underline the necessity of large sample sizes in the localization of deleterious SNPs even under simple disease models. These conclusions possess pronounced importance for the design and result interpretation of

candidate gene, next generation high-density genome-wide association studies, as well as for the construction and implementation of association tests based on the distribution of the most significant (minimum p-value) test statistics.

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Computational Methods for Historical Research on Wikipedia's Archives

Jonathan Cohen

Abstract

This paper presents a novel study of geographic information implicit in the English Wikipedia archive. This project demonstrates a method to extract data from the archive with data mining, map the global distribution of Wikipedia editors through geocoding in GIS, and proceed with a spatial analysis of Wikipedia use in metropolitan cities.

Keywords: Wikipedia Archive, Data Mining, Geocoding, Spatial Data Analysis

Introduction

Wikipedia is one of the most powerful sources of information on the Internet. The site is ranked number 5 among all Internet websites, far ahead of the New York Times, which is the highest-ranked newspaper at number 97 and Encyclopedia Britannica, which is the highest-ranked encyclopedia at number 2,566.[i] The site received over 300 million unique visitors in December 2009 alone.[ii] Wikipedia's content is widely read and cited, and for many, their primary source of online information.[iii]

Wikipedia's popularity and prominence has made it an emerging issue in technology and education. Founded in 2001 as a free online encyclopedia, the defining feature of Wikipedia is that each user can change any page. Each article displays a link which reads "edit this page" to allow any user to change the article's content. This means that when a user makes a change it will be visible to the next user who visits the page. Anecdotal reports suggest that Wikipedia has become immensely popular among students and the bane of teachers in higher education.[iv]

Previous Scholarship

Wikipedia's accuracy and reliability as a source of information has been called into question by the media and academic researchers.[v] Much of this criticism has focused on issues of accuracy connected to the fact that

Wikipedia has no formal peer-review process. However, this criticism mostly faded once the journal *Nature* published a study that found the accuracy of Wikipedia comparable to that of Encyclopedia Britannica.[vi] The criticism of systemic bias on Wikipedia has proved more dogged. Wikipedia editors tend to contribute information that is important and correct to them, but not important and not correct to users in other countries.[vii] This self-focus bias leads Wikipedia toward the problematic representation of Western knowledge defined as all human knowledge. The purpose of this study is to contribute to existing lines of research on systemic bias inherent in Wikipedia.

Prior research has aimed to develop a greater understanding of the population and demographics of the Wikipedia community of editors. To date the most prominent and comprehensive study of the Wikipedia community of editors was presented in Dr. Felipe Ortega's doctoral thesis "Wikipedia: A quantitative analysis."[viii] Ortega traced the evolution of the Wikipedia community in a comparative study of the top ten language versions of Wikipedia. Ortega demonstrated dynamic population trends among this community. The most heavily publicized findings in this study suggested that the population of Wikipedia editors declined by 4,900 in the first three months of 2008 and by 49,000 in the first three months of 2009.[ix] These population trends deserve further study.

One possible means of explaining demographic trends is to break down the data in terms of location to explore precisely *where* Wikipedia editors are leaving the project. Previous scholarship has explored a number of methods to map the location of Wikipedia editors. Some projects made use of a method known as spatial data mining to show that it was possible to link individual Wikipedia editors to a general geographic region. Lieberman demonstrated that users' edits often contained an implicit geographic focus that provided an indication of that users' general location.[x] For example, edits to the Wikipedia articles for the New York Stock Exchange, Central Park, 5th Avenue, and the United Nations would provide a strong indication that the user was born in New York city or had lived in the area. This type of spatial data mining allows researchers to identify Wikipedia editors with a general geographic region to gain insight into user population trends.

Other scholarship refined this spatial data mining methodology to more accurately identify the geographic locations of Wikipedia users. These projects demonstrated a method to link individual Wikipedia editors to a specific geographic location. Hardy demonstrated that the location of anonymous Wikipedia editors could be identified through the IP addresses associated with each of their edits.[xi] Hardy used IP-based geolocation to estimate editor location based on the street address of their Internet service providers.[xii] IP-based geocoding allows for a significantly more focused analysis of population data.

IP-based geolocation can illuminate earlier studies on the social networks of the Wikipedia community of editors. These studies lacked the necessary geographic information to locate social networks of Wikipedia editors and instead represented these networks with abstract diagrams. Zlatic et. al., for example, studied network characteristics among several language versions of Wikipedia and illustrated the results with abstract web graphs.[xiii] This study clearly demonstrated that the Wikipedia community of editors can be understood and

analyzed as a social network. With the incorporation of geographic information to this analysis, social networks can be derived from users who contribute to articles from similar geographic locations. This allows researchers can analyze and illustrate the evolution of the Wikipedia community of editors through their activity in geographically-situated social networks.

Methods/Data

This project was based upon a sample from the edit histories in the digital archive of Wikipedia. [xiv] This digital archive represents a complete history of change on the site. It logs the nature of each edit along with a timestamp, a user name, an IP address, and a comment from the user. A computer program was written in the Python programming language to download a sample of 23,741 anonymous edits for processing and analysis.[xv]

Each of the 23,741 anonymous edits in the sample were linked to implicit geographic information.[xvi] The IP address of an anonymous Wikipedia edit can be traced to the street address of an Internet Service Provider. The IP addresses in the sample were converted into a database of latitude and longitude coordinates and represented on a map using Geographic Information Systems software. The resulting map clearly represented the spatial distribution of Wikipedia edits.

The most significant uncertainty inherent in this methodology is connected to the fact that the *wiki_tracker.py* program only collects data about anonymous Wikipedia users. Wiki software masks the IP addresses of registered users, so geographic information is only easily available for anonymous users. Research to date suggests that the editing patterns of anonymous users are comparable to those of registered users.[xvii] However, the Wikimedia Foundation will need to provide a list of IP addresses of registered users for a more comprehensive study.

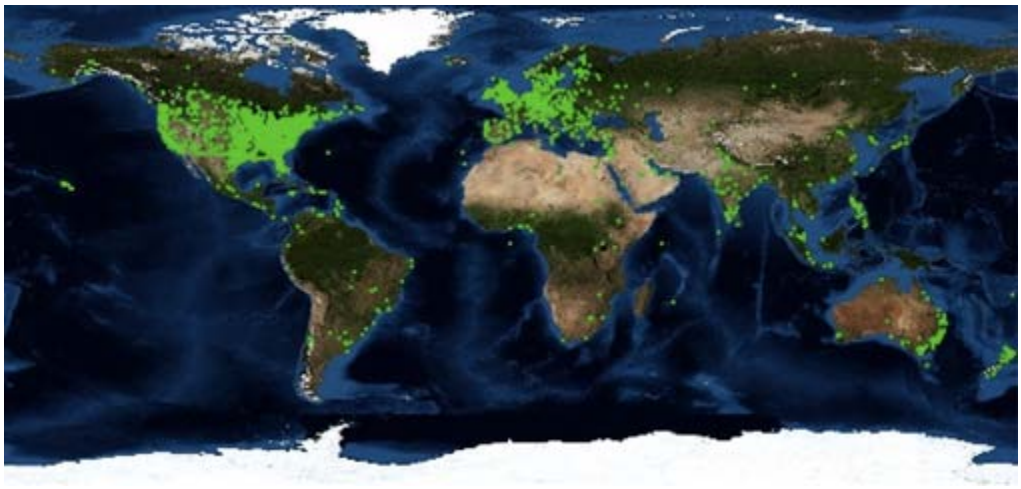

Fig.1 Map of edits to Wikipedia (2001-2009)

Analysis/Discussion

The spatial distribution of Wikipedia editors is clearly represented on the map after geocoding the IP address information. Edit activity is concentrated in North America, Western Europe, and Australia. A small number of countries can account for a majority of the edit activity. About 80% of the edits in the sample originated from the United States (59.5%), United Kingdom (11.8%), Canada (6.6%) and Australia (5.4%). Also, edit activity originating from South America, Africa, and Asia is scarce even when the measures were normalized to account for the relatively lower prevalence of Internet-access in those areas. This suggests that spatial factors do play a role in the population mechanics among the Wikipedia community of users.

Edit activity is concentrated around major metropolitan centers. Major population centers are defined as urban spaces with 100,000 or more Internet users. Examples are New York, Los Angeles, London, Montreal and Melbourne. Spatial analysis of Wikipedia edit activity indicates that 91% of edit activity (21,604 of 23,741 edits) originates within 25km of highly populated urban areas.

There is also a temporal component to the changes in edit activity. Fig. 1 suggests that the population of Wikipedia users grew exponentially from 2002 to 2006. It also suggests that the population of *new* Wikipedia editors started to decline after 2006.[xviii] These data show similar population patterns across the top four countries where Wikipedia is used, suggesting that non-geographic factors are involved in the dynamics of these Wikipedia social networks.

	United States	United Kingdom	Canada	Australia
2002	94	17	16	4
2003	220	41	29	21
2004	619	109	77	49
2005	2924	558	375	236
2006	5122	1197	559	479
2007	2622	449	243	253
2008	1536	225	148	180
2009	964	140	118	71

Fig.2 Yearly edits by anonymous users for top four countries (2002-2009)

Geographic information helps provide a better understanding of how the demographics and population of the Wikipedia community of authors changes over time. Existing lines of research can benefit from this method to map the geographic location of Wikipedia users and to analyze spatial relationships between them.

Conclusion

This paper has demonstrated a method for data mining, geocoding, and spatial analysis of data from the English Wikipedia archive. While earlier Wikipedia research into the population and demographics of Wikipedia editors

has focused on the activity of individual authors, data mining and geocoding can generate new information about the Wikipedia community of authors. This paper uses those methods for spatial analysis to discover how geographic location factors into the population and demographic changes of Wikipedia editors embedded in metropolitan social networks.

The data suggests that geographic is a significant factor in the population and demographic patterns of Wikipedia editors. Most Wikipedia editors originate from a small number of English-speaking countries with high degrees of Internet access. Furthermore, the vast majority of Wikipedia editors live in close proximity to a major metropolitan center. Future research will explore the unique contributions that each metropolitan social network makes to Wikipedia.

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[iv] This issue is discussed in most news reports on the topic of Wikipedia. For a visual representation of Wikipedia's popularity among students see Google Trends: http://www.google.com/trends?q=wikipedia. From 2005 to present, Wikipedia use peaks during the times of year when schools and universities are in session and plummets during periods of summer and winter vacation.

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[xi] Darren Hardy. "Discovering behavioral patterns in collective authorship of place-based information," in *9th International Conference of the Association of Internet Researchers (IR9: Rethinking community, Rethinking Place)*, Copenhagen, Denmark, October 15-18, 2008.

[xii] Geographic information obtained via this method has weaknesses. Users who access the Internet via a proxy connection would be mis-placed on the map. Also, IP address information is collected only for anonymous users, not registered users.

[xiii] V. Zlatic, M. Bozicevic, H. Stefancic, and M. Domazet. "Wikipedias: Collaborative web-based encyclopedias as complex networks." *Physical Review E: Statistical, Nonlinear, and Soft Matter Physics* 74 (2006). Specifically, this study traced degree distributions, growth, topology, reciprocity, clustering, assortativity, path lengths, and triad significance profiles.

[xiv] Wikipedia XML database dumps are regularly made available at http://download.wikipedia.org/enwiki/.

[xv] See Appendix A for complete program: *wiki_tracker.py*.

[xvi] Sample derived from the top-level pages and taken to be representative of activity on the site as a whole. Top-level pages used were "Main Page," "Arts," "Biography," "Geography," "History," "Mathematics," "Science," "Society," and "Technology."

[xvii] Darren Hardy. "Discovering behavioral patterns in collective authorship of place-based information," in *9th International Conference of the Association of Internet Researchers (IR9: Rethinking community, Rethinking Place)*, Copenhagen, Denmark, October 15-18, 2008. 7, 10.

[xviii] Data in Fig. 2 is best interpreted as an indicator of the amount of new users arriving to the site because this data is not comprehensive. Fig. 1 only includes edits from anonymous Wikipedia editors. Users tend to register and operate under a single user name after some period of anonymous activity.

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Haplotype Variety Analysis of Human Populations: an Application to HapMap Data

Michelle Creek, Cyril Rakovski

Abstract

We undertake a study to investigate the haplotype variety of distinct human populations. We use a natural measure of haplotype variety, the total number of haplotypes (TNH) present that reflects the number of haplotypes with nonzero frequencies estimated from the data at hand for each selection of multiple loci. For the analysis of real human populations, we use the haplotype data of the Denver Chinese, Tuscan Italians, Luhya Kenyans, and Gujarati Indians from release III of the HapMap database. Moreover, we show that the TNH statistic is biased in small sample data scenarios such as the HapMap and implement a nested simulation study to estimate and remove such bias. We perform a preliminary analysis of means and variances of the population allele frequencies in the four populations. Lastly, we implement a generalized linear model to detect and quantify the differences in haplotype structures of these populations. Our results show that all populations possess significantly different adjusted average TNH values. Our findings extend previous results based on alternative statistical approaches and demonstrate the existence of pronounced differences in the haplotype variety of the analyzed populations even after controlling for haplotype span as well as all allele frequencies and their two-way interactions.

Keywords: Multimarker Disequilibrium Analysis, Generalized Linear Models, Single Nucleotide Polymorphism

Introduction

Differences in the genetic structure of human populations have been studied and assessed from various viewpoints [Gu, et al. 2008; Joubert, et al. ; Lundmark, et al. 2008; Marvelle, et al. 2007; Sved, et al. 2008]. In this work we analyze the haplotype variety in distinct human populations via a naturally arising measure, the total number of haplotypes present that reflects the number of haplotypes with nonzero frequencies estimated from the data at hand for each selection of multiple loci. Thus, we define a counting measure, as an alternative to existing approaches such as the haplotype entropy [Nothnagel, et al. 2002] and haplotype diversity [Clayton 2001], that can be inherently modeled by generalized linear models or generalized estimating equations. We employ the TNH statistic to perform analysis of real human populations using haplotype data of the Denver Chinese (CHD), Tuscan Italians (TSI), Luhya Kenyans (LWK), and Gujarati Indians (GIH) from release III of the HapMap database [Tanaka 2009]. It is well known that the HapMap data possesses intrinsic limitations [Biswas, et al. 2007; Check

2007; Zhang and Dolan 2008]. Further, we show that the TNH statistic is biased in small sample data scenarios such as the HapMap and implement a nested simulation study to estimate and remove such bias. We perform a preliminary analysis of means and variances of the population allele frequencies in the four populations. Further, we determine the differences in haplotype structures of these populations via an appropriate generalized linear model. As a secondary note, we estimate the increase of haplotype variety as a function of haplotype span which can be viewed as a multivariate generalization of previous results on the decay of linkage disequilibrium (LD) as a function of physical distance between pairs of single nucleotide polymorphisms (SNPs) [Bosch, et al. 2009].

Methods

Assume that we consider n biallelic markers $M_1, M_2, \dots, M_n$ with alleles at each locus coded as 1 or 2. Let $i_1 i_2 \dots i_n$ be a haplotype based on these markers and $\mathbf{H}^n = \{i_1 i_2 \dots i_n \mid i_k \in \{1,2\}\}$ denote the set of all 2^n such haplotypes with 2 representing the rarer allele. A natural measure of haplotype variety is the total number of haplotypes present (TNH) defined in the following fashion:

$$TNH(M_1, M_2, \dots, M_n) = \sum_{i_1 i_2 \dots i_n \in H^n} I\{P(i_1 i_2 \dots i_n) > 0\}, \quad (1)$$

where $P(i_1 i_2 \dots i_n)$ denote the population frequency of haplotype $i_1 i_2 \dots i_n$.

Clearly, the values of the TNH counting measure are bounded by one and 2^n with one representing the scenarios with the fewest number of haplotypes and all possible haplotypes present respectively. We are interested in comparing the haplotype variety of different populations through the THN statistic after adjusting for potential confounders. However, the true population haplotype frequencies that appear in definition are unknown and we need to replace them with their maximum likelihood estimates (MLE) based on the data at hand,

$$\hat{TNH}(M_1, M_2, \dots, M_n) = \sum_{i_1 i_2 \dots i_n \in H^n} I\{\hat{P}(i_1 i_2 \dots i_n) > 0\}. \quad (2)$$

Clearly, by using equation we add bias to the TNH measure of haplotype variety. For instance, under independent allelic transmissions (i.e. by ignoring the reduction of haplotype variety due to complex multilocus structure), all haplotypes should be present for each selection of loci. For instance, in the simple case of haplotypes based on four markers with minor allele frequencies 0.05 (the lowest considered in this work), the population frequency of the rarest haplotype will be $P(2222) = 0.05^4 = 10^{-6}$ (6.25) and therefore highly unlikely to be observed in small sample data such as the HapMap.

For each selection of markers $M_1, M_2, \dots M_n$ from a particular population and chromosome of the HapMap data, we can estimate the allele frequencies

$$\hat{P}(i_1), \hat{P}(i_2), \dots, \hat{P}(i_n)$$

through the sample proportions which are the MLEs. We can estimate the above-mentioned bias analytically by noticing that under independent allelic transmissions, the joint distribution of all haplotypes is multinomial,

$$P(h_1, h_2, \dots, h_{2^n}) \sim Mult\left[\prod_{k=1}^{n} \hat{P}(i_k^1), \prod_{k=1}^{n} \hat{P}(i_k^2), \dots, \prod_{k=1}^{n} \hat{P}(i_k^{2^n})\right], \quad (3)$$

where $\boldsymbol{h_k = i_1^k i_2^k \ldots i_n^k}$, $\boldsymbol{k = 1, 2, \ldots 2^n}$ denote all elements of $\boldsymbol{H^n}$. However, we proceed with an empirical approach by undertaking a nested simulation study to estimate and consequently remove this bias from the TNH statistic. Similarly, for each selection of selection of markers $\boldsymbol{M_1, M_2, \ldots M_n}$ we estimate the allele frequencies $\hat{P}(i_1), \hat{P}(i_2), \ldots, \hat{P}(i_n)$ and simulate $\boldsymbol{K}$ datasets, $\boldsymbol{D_1, D_2, \ldots D_K}$ consisting of 170 haplotypes (this is the number of independent haplotypes for the selected populations in the HapMap data) under the condition of independent allelic transmissions i.e. $\hat{P}(i_1 i_2 \ldots i_n) = \prod_{j=1}^{n} \hat{P}(i_j)$.

Then, the bias can be empirically estimated by the mean of the sample-specific bias values,

$$\hat{B}ias[T\hat{N}H(M_1, M_2, \ldots, M_n)] = 2^n - \sum_{j=1}^{K} \left\{ \sum_{0 \le i_1 \le \ldots \le i_n \le 4^n} I\{\hat{P}_j(i_1 i_2 \ldots i_n) > 0\} \right\} / K, \quad \textbf{(4)}$$

where $\hat{P}_j(i_1 i_2 \ldots i_n)$ denotes the estimated population frequency of haplotype $\boldsymbol{i_1 i_2 \ldots i_n}$ based on simulated dataset $\boldsymbol{D_j}$.

Next, the comparison of haplotype structures of distinct isolated populations is performed through a linear regression analysis with outcome variable being the bias-corrected TNH. We also investigate the effect of haplotype span, allele frequencies and their two-way interactions,

$$\log[T\hat{N}H_i - \hat{B}ias(T\hat{N}H_i)] = X_i\beta + \varepsilon_i, \ \varepsilon_i \sim N(0, \sigma^2). \quad \textbf{(5)}$$

The logarithmic transformation the TNH measure decreases is a standard approach to attain better fit in count data settings.

Lastly, as a preliminary data analysis step, we compare the variances and means of the allele frequencies of the four populations using classical tools such as F-tests and t-tests. These analytical methods are robust to model misspecification which is likely the case with high density SNP data where the independence assumptions are violated due to presence of linkage disequilibrium (LD).

Data

For the study on isolated populations we chose the Chinese in Denver, Colorado, the Toscani in Italy, the Luhya in Webuye, Kenya, and the Gujarati Indians in Houston, Texas. We use the haplotypes from 22 autosomal chromosomes of the corresponding datasets (CHD, TSI, LWK, and GIH) on unrelated subjects from release III of the HapMap database. Details on these dataset characteristics are shown in Table 1.

Table 1. HapMap datasets characteristics.

Population	Total number of unrelated samples	Total number of haplotypes	Total number of SNPs	Total number of common SNPs*
CHD	85	170	1,387,466	966,507
GIH	88	176	1,387,466	1,071,872
LWK	90	180	1,387,486	1,141,860
TSI	88	176	1,387,486	1,069,127

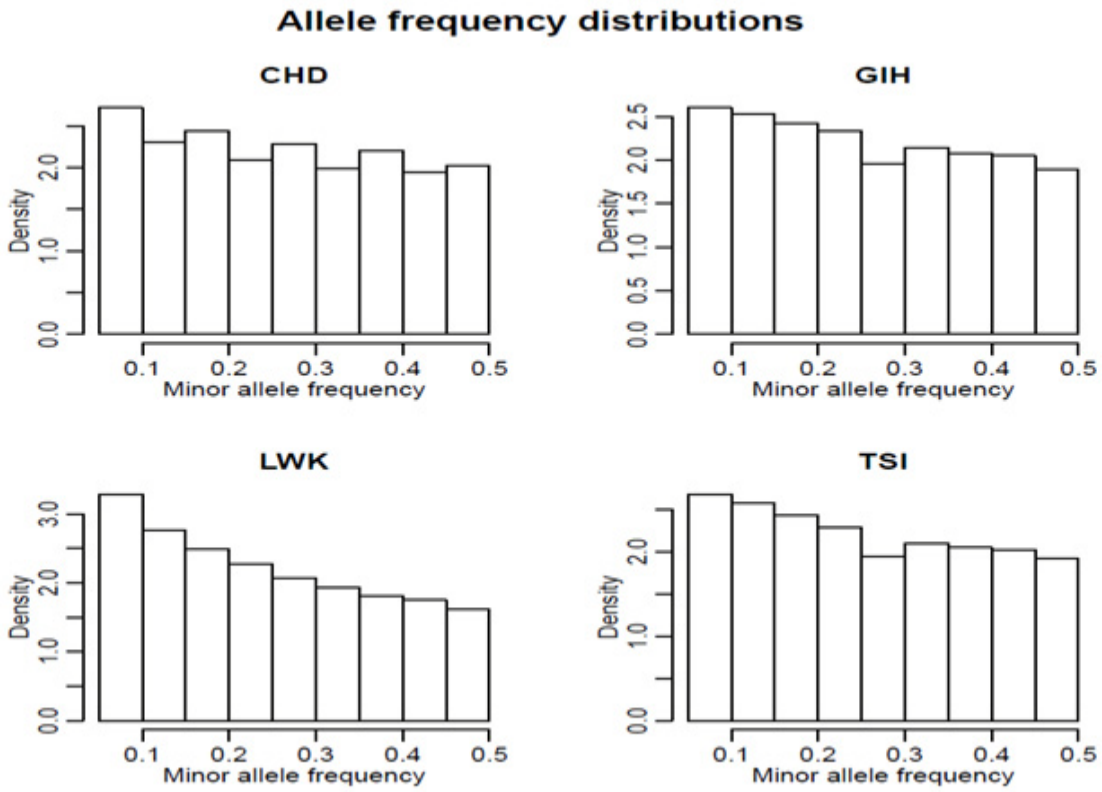
*SNPs with minor allele frequency greater than 0.05.

We removed the SNPs with minor allele frequencies smaller than 0.05 (reduction of 30, 23, 18 and 23% from each dataset respectively). Further, for each population and autosomal chromosome pair we randomly selected 1000 quadruples of loci (a total of 88,000) and in the subsequent analysis, we investigated the diversity of the haplotypes based on these markers via the TNH measure.

Results

We performed the complete analysis for haplotypes based on three, four and five markers. We present results for the case of four-locus haplotypes. The minor allele frequency distributions of the four studied populations reveal noteworthy dissimilarities. The corresponding histograms are shown in Figure 1.

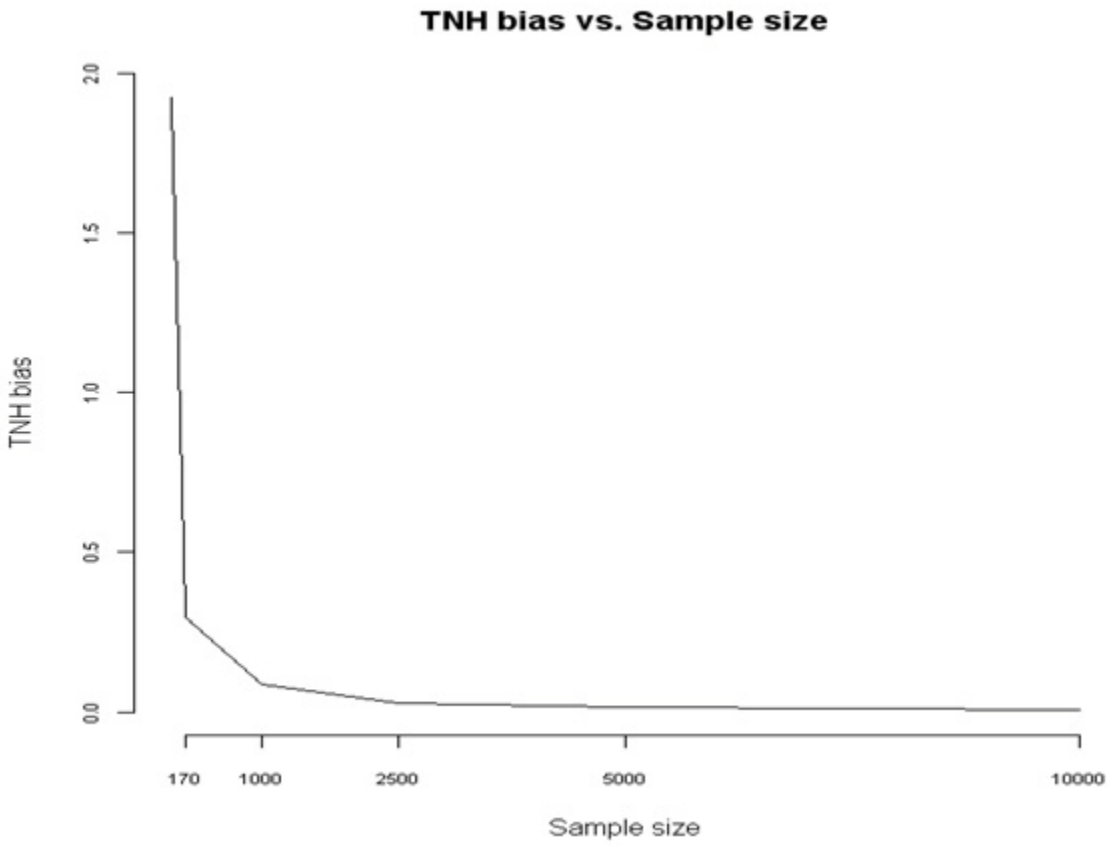
Figure 1: Minor allele frequency distributions for the four populations.



Preliminary analysis via two-sample t-tests confirms that the population average minor allele frequencies differ significantly between all possible pairs of populations with p-values smaller than 3.10^{-14}. Interestingly, the only population variance that differs significantly from the rest is that of the Tuscan Italians with all F-test p-values smaller than 0.002.

Next, we investigate the magnitude of the TNH bias as a function of the sample size. Figure 2 shows the TNH bias averaged over 10,000 randomly selected haplotypes for four different sample sizes, 170, 100, 2500, 5000, and 10000 respectively.

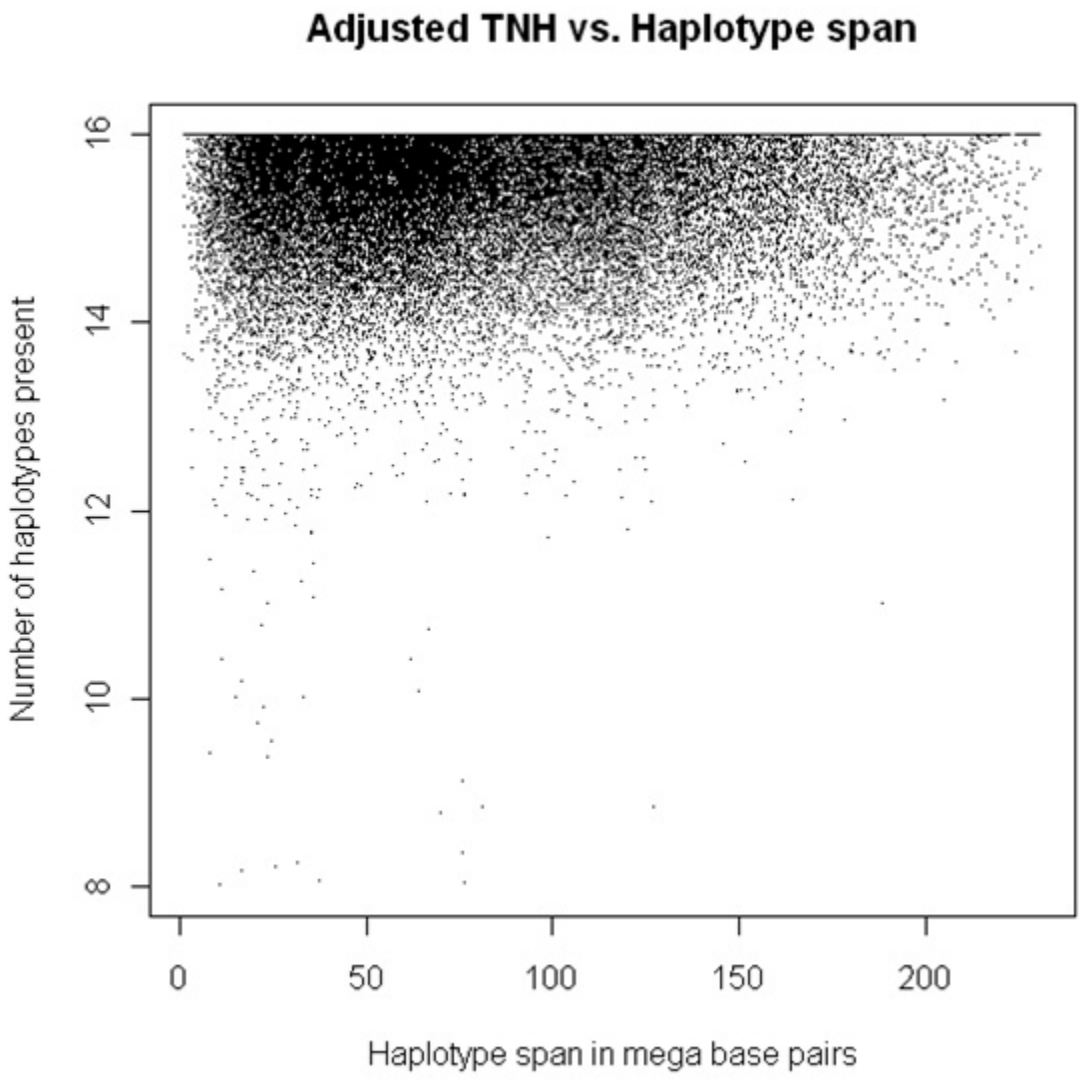
Figure 2: Average TNH bias versus sample size.



The average bias for the HapMap dataset sizes is approximately 0.3. In the subsequent analysis, for each random selection of four loci, we estimate and remove this selection-specific bias using expression .

The decrease of linkage disequilibrium as the distance between pairs of markers increase is well-known. Figure 3 shows a multilocus version of these phenomena, the increase of THN as the haplotype span grows.

Figure 3: Bias-adjusted TNH versus Haplotype span in mega base pairs.



As expected, the number of haplotypes approaches the maximum possible number of haplotypes as the physical distance spanned by the flanking markers increases. However, the rate of increase seems much slower than anticipated. The magnitude of the adjusted haplotype span effect is estimated in the subsequent regression analysis.

Lastly, the linear regression results are presented in Table 2. We arrived at this model through standard model fitting techniques such as forward selection and backward elimination of potential predictors as well as residual diagnostic and goodness of fit approaches.

Table 2. Regression analysis results.

Variable	Estimate	SE	t-value	p-value
Intercept	2.773	2.069e-03	1339.883	<2e-16
Haplotype span	1.855e-11	4.271e-12	4.343	1.41e-05
GIH	3.171e-03	5.456e-04	5.811	6.24e-09
LWK	5.646e-03	5.479e-04	10.305	< 2e-16
TSI	3.066e-03	5.457e-04	5.617	1.95e-08
AlFreq1	1.016e-02	5.259e-03	1.932	0.053368
AlFreq2	1.421e-02	5.236e-03	2.714	0.006653
AlFreq3	5.669e-03	5.258e-03	1.078	0.280979
AlFreq4	1.430e-02	5.247e-03	2.725	0.006424
AlFreq1:AlFreq2	2.796e-02	1.121e-02	-2.495	0.012594
AlFreq1:AlFreq3	-1.449e-02	1.121e-02	-1.293	0.196074
AlFreq1:AlFreq4	-2.450e-02	1.120e-02	-2.187	0.028763
AlFreq2:AlFreq3	-1.698e-02	1.122e-02	-1.513	0.130375
AlFreq2:AlFreq4	-3.725e-02	1.124e-02	-3.315	0.000917
AlFreq3:AlFreq4	-2.005e-02	1.124e-02	-1.785	0.074316

The important results from this analysis is that the haplotype varieties differ significantly among the four populations even after adjusting for haplotype span, allele frequencies and their two-way interaction. Controlling for these covariates in the model shows the depth and intricacies of the haplotype structure distinctions. Moreover, based on the adjusted average TNH values, the Luhya Kenyans possess the highest haplotype variety followed by the Gujarati Indians, the Toscani Italians and the Chinese in Denver respectively. The haplotype span is measured kilo base pairs and surprisingly, the adjusted effect of haplotype span is extremely small, 10^{-11} with p-value of 2.10^{-5}.

Discussion

The difference among human populations studied in this work are pronounced and complex. Not only are all allele frequency means and some of the variances significantly different but also the haplotype varieties even after we controlling for span, allele frequencies and their two way interactions. Moreover, the increase of haplotype variety as the distance between the flanking markers increases is dramatically smaller than anticipated given the rate of decay of LD between pairs of SNPs. However, these conclusions are difficult to compare to existing results due to the unique modeling approach.

Having obtained a degree of insight into the intricacies of the haplotypes structures of human populations we can perform a large-scale analysis using more precise measures of haplotype variety. We can explore the joint distributional structure of the all possible haplotypes under independence given in through a Monte Carlo test procedure [Hope 1968] that circumvents the minimum expected count requirements characteristic to classical large-sample chi-square contingency tables goodness-of-fit tests. Alternatively, we can improve the TNH statistic by measuring not only the number of haplotypes present but also the extent of departure of the observed haplotypes frequencies from the expected frequencies under independent allelic transmissions.

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Effects of Ocean Acidification on Chlorophyll Content

C., del Fierro, R., Lloyd, and H. El-Askary

Abstract

Airborne pollutants contribute to ocean acidification and hence to the associated chlorophyll content level. Previous work showed that falling aerosols causing ocean acidification would in turn result in bleaching and productivity loss in coral reef builders. Chlorophyll content has been used as a measure of the concentration of the photosynthetic pigment chlorophyll a (the most common "green" chlorophyll) in the ocean. In our work we have monitored the change in chlorophyll content obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor on board Terra/Aqua satellites from 2000-2009 over selected pilot areas. Moreover, we have used the Goddard Chemistry Aerosol Radiation and Transport (GOCART) NASA chemical model to simulate sulfate, dust, black carbon (BC), organic carbon (OC), and sea-salt aerosols content over the urban centers close to the areas where chlorophyll content is showing a significant decline. These parameters would reflect the natural versus anthropogenic origin of the aerosols falling over ocean waters. We expect to observe an overall decrease in chlorophyll content on the surface of the ocean. Hence, our findings may suggest that the effects of falling air pollutants in the ocean will be so detrimental as to gradually cause a decrease in photosynthetic producing material over several years.

Keywords: Chlorophyll, Black Carbon, Satellite Sensing, GOCART

Introduction

Ocean acidification is an extremely concerning issue today primarily because of it's effects on marine organisms, due to an unprecedented increase in pH level throughout the ocean. Almost a third of the excess carbon dioxide produced by both natural and man-made processes settles into the ocean. As mass amounts of carbon dioxide have been absorbed into the ocean since the industrial revolution, scientists have measured that pH levels have gone down 0.1 units, which actually causes a rise in acidity (as pH levels go down, acidity is increased: for example, going from 0.15 to 0.30 is considered a decrease in pH, and an increase in acidic level). High acidic levels cause calcium carbonate, which is the main skeletal material for many marine organisms as well as the primary reef building material where abundant habitats thrive, to dissolve. Organisms such as shellfish towards the bottom of

the food chain are now going extinct because their shells are literally dissolving, therefore causing large amounts of them to die. Portions of coral reefs are also dissolving, which affects all of the organisms that depend on the reef as a living habitat. As pH levels continue to increase, many food webs will be altered, and humans will see a dramatic decrease in the amount of fish production, which many people around the world depend on. The concern of ocean acidification is one shared by many scientists and policymakers who have observed how rapidly the acidic levels in the ocean have been increasing, and how scary such an occurrence is for the future of our oceans.

Airborne pollutants contribute to ocean acidification and hence to the associated chlorophyll content level. Previous work showed that falling aerosols causing ocean acidification would in turn result in bleaching and productivity loss in coral reef builders. Chlorophyll content has been used as a measure of the concentration of the photosynthetic pigment chlorophyll a (the most common "green" chlorophyll) in the ocean. In our work we have monitored the change in chlorophyll content obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor on board the Aqua satellite from 2000-2009 along the California coastline. Moreover, we have used the Goddard Chemistry Aerosol Radiation and Transport (GOCART) NASA chemical model to simulate sulfate, dust, black carbon (BC), organic carbon (OC), and sea-salt aerosols content over the urban centers close to the areas where we expect chlorophyll content to show a significant decline. These parameters reflect the natural versus anthropogenic origin of the aerosols falling over ocean waters. We expect to observe an overall decrease in chlorophyll content on the surface of the ocean. Hence, our findings may suggest that the effects of falling air pollutants in the ocean will be so detrimental as to gradually cause a decrease in photosynthetic producing material over several years.

We hypothesize that if black carbon aerosols settle into the ocean along the western California coast over time, then chlorophyll content on the surface of the ocean will show an overall decrease. In composing our research, we will observe data over the years of 2003, 2007, and 2009 during the months of January and July. Looking at two regions, the close coastal region and the further out open ocean region, we will compare patterns of chlorophyll growth to that of black carbon content. We expect to see a relationship between the decrease in chlorophyll with the increase of black carbon. The reason why we predict that chlorophyll content will decrease as black carbon increases if because we believe that excess amounts of black carbon, which creates a highly acidic environment in the ocean, will overwhelm photosynthetic production so as to kill off some amount of photosynthetic producing material. In other words, we expect to identify the effects of ocean acidification as extremely harmful to ocean vegetation, due to high pH levels continually increasing as a result of an increased presence of black carbon aerosols settling into the ocean.

Mythology

First we picked a location: the California coastline. Then we browsed data using the Goddard Earth Sciences Data and Information Services Center, through the GIOVANNI-NASA website. On GIOVANNI we decided on the

Moderate Resolution Imaging Spectroradiometer (MODIS) sensor on board the Aqua satellite as our means of collecting data. We then observed chlorophyll content along the California coast in January of 2003, 2005, and 2007. Then we observed chlorophyll content along the California coast in July of 2003, 2005, and 2007. Next we observed data through the Goddard Chemistry Aerosol Radiation and Transport (GOCART) model. On GOCART we observed the content of black carbon along the western California coast in January of 2003, 2005, and 2007. We proceeded to observe the black carbon content in July of 2003, 2005, and 2007. After observing all of the data through the MODIS-Aqua satellite and GOCART model, we then made a chart correlating the months and years being observed, with the chlorophyll content both directly along the coastline of western California, and further off of the coastline of western California in open ocean water. Lastly, we re-evaluated all data, both from the GIOVANNI website, and from the charts and graphs we composed, to make a conclusion of our research.

Analysis of Black Carbon on Chlorophyll

The data we collected throughout our research showed several things. Firstly, in comparing the months of January and July, generally chlorophyll content along the western California coast is more abundant during the summer times, which is fairly predictable given the warmer temperature causing more photosynthetic productivity.The month of July, in 2003, 2005, and 2007, consistently showed more chlorophyll content than January. In addition, there was typically more chlorophyll content closer to the coast then farther out in open water. Secondly, chlorophyll content was more abundant in southern California, but showed a higher concentration in certain areas of northern California. The region from San Francisco to Monterey Bay showed the highest concentration of chlorophyll, which we assume is due to the large amount of kelp beds that are notorious in that region of northern California.

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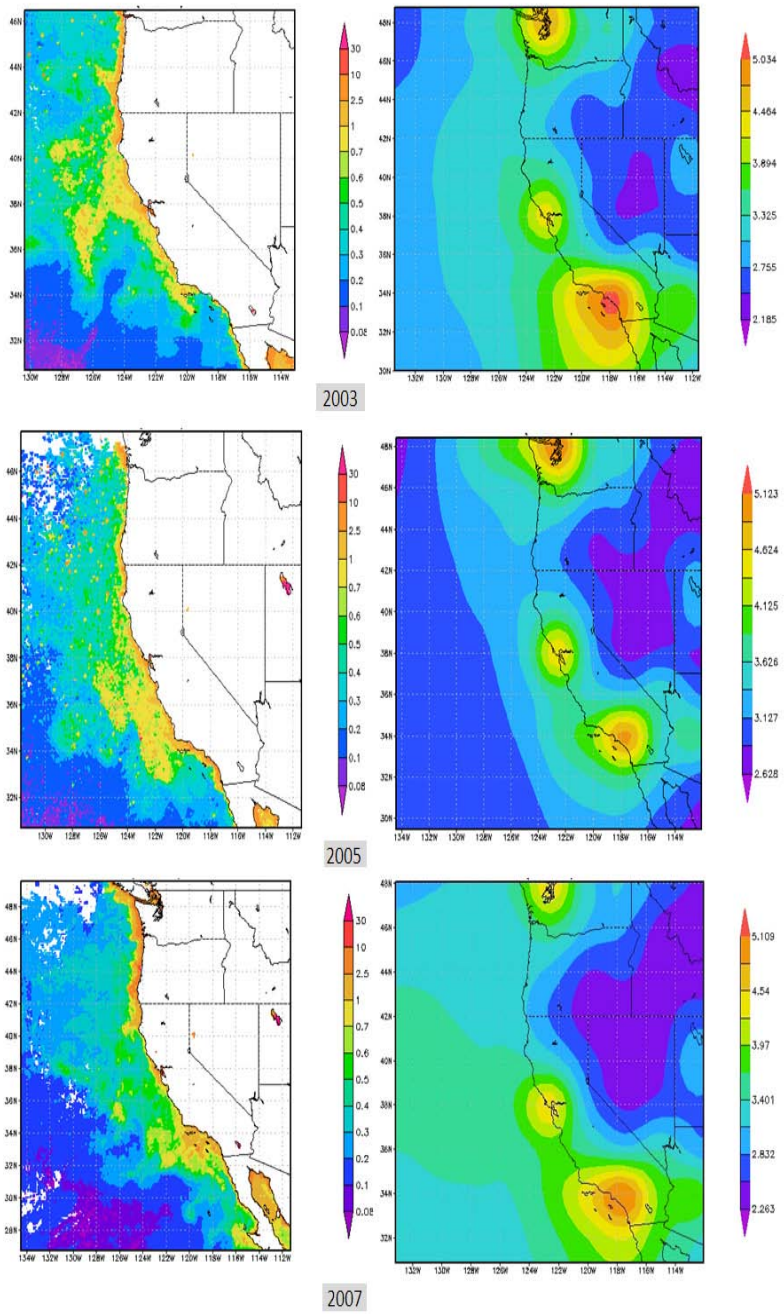

Figure 1: Chlorophyll content (a) versus Black Carbon (b) during January 2003, 2005 and 2007

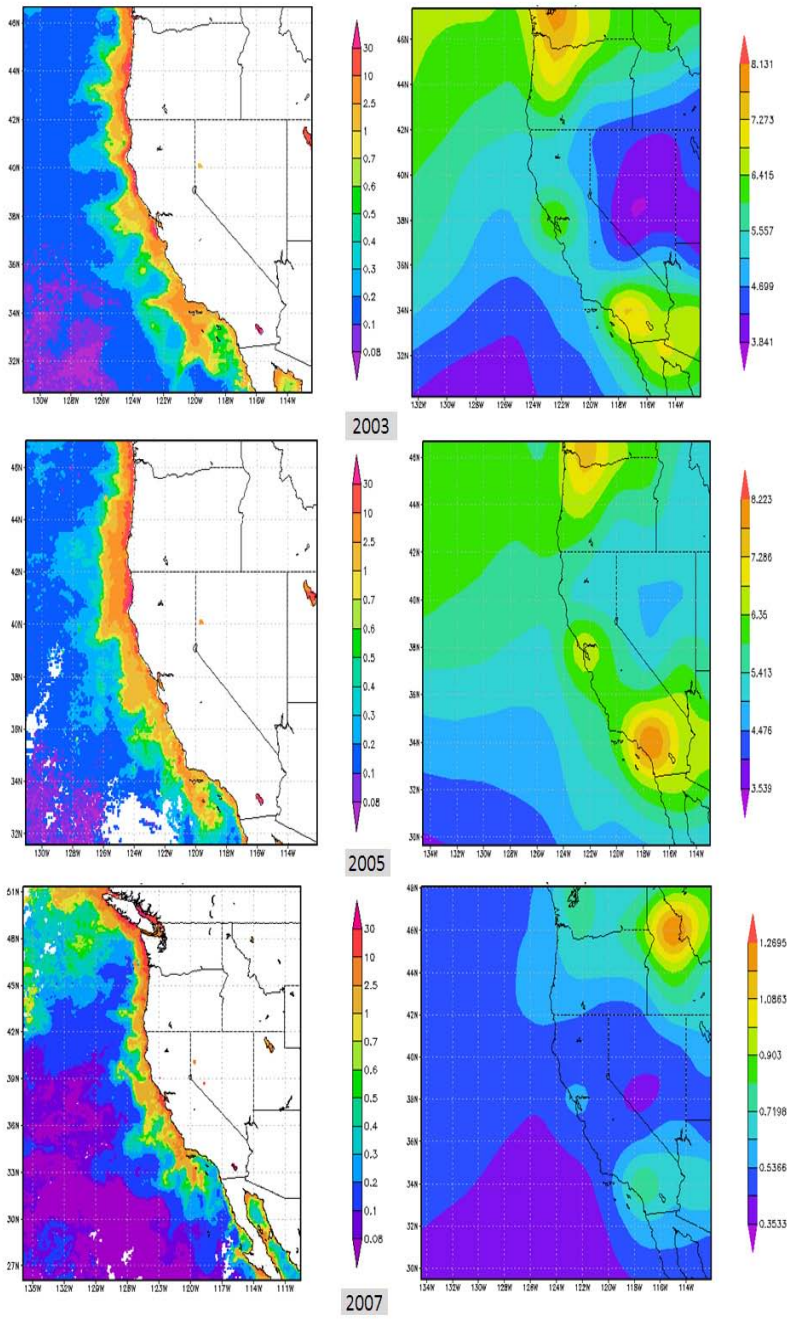

Figure 2: Chlorophyll content (a) versus Black Carbon (b) during July 2003, 2005 and 2007

In our research, we broke up our parameters along the western coast of California into two sections: the close coastal region, and the open ocean region further off of the coast. When comparing the average amounts of chlorophyll content along the close coastal region in January for the years of 2003, 2005, and 2007, to that of July of the same years, a pattern appeared: chlorophyll content was higher in 2003 than in 2005, but lower in 2003 than 2007. Therefore, chlorophyll content went from high to low, to it's highest in 2007. The open ocean coastal region showed almost the same pattern, with some variation in July of 2005 having higher numbers than in 2003,

and 2007, and January of 2007 showing the highest numbers of all three years. Overall, January showed small fluctuation and fairly low chlorophyll content, whereas July proved to show more chlorophyll content along both regions.

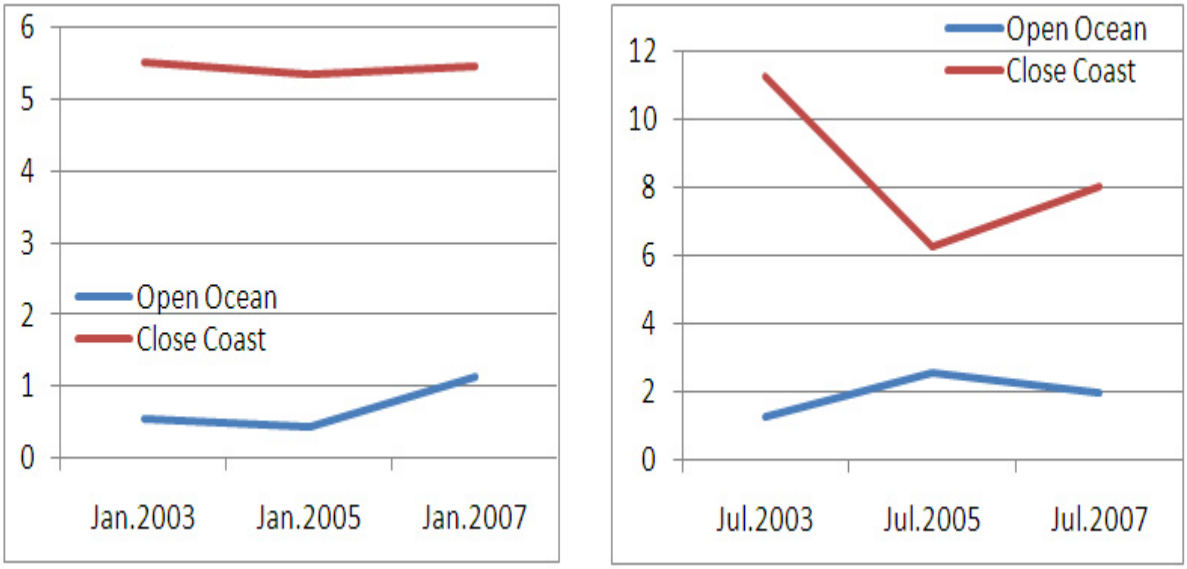

Figure 3: Chlorophyll Content in the open Ocean and along the coast during January and July of 2003, 2005 and 2007

Remarkably, black carbon content showed almost the exact same pattern as chlorophyll content in both regions during January and July. The open ocean region in January of 2003, 2005, and 2007, shows black carbon content going from high to lower, to its highest in 2007, where 2005 shows the least amount. The open ocean region in July of the same years increases in 2005 from 2003, and then drastically drops in 2007. July of 2007 shows the least amount of black carbon from any year during both January and July, along both regions. The close coastal region in January of all three years shows black carbon decreasing, respectively. The close coastal region in July of all three years does not show the same pattern as January: black carbon content is highest in 2005, and lowest in 2007, with 2007 displaying the vast decrease previously noted. Ultimately, black carbon content is always more concentrated along the close coastal region of western California, which is the exact same case with chlorophyll showing its most abundant and concentrated amounts along the same region.

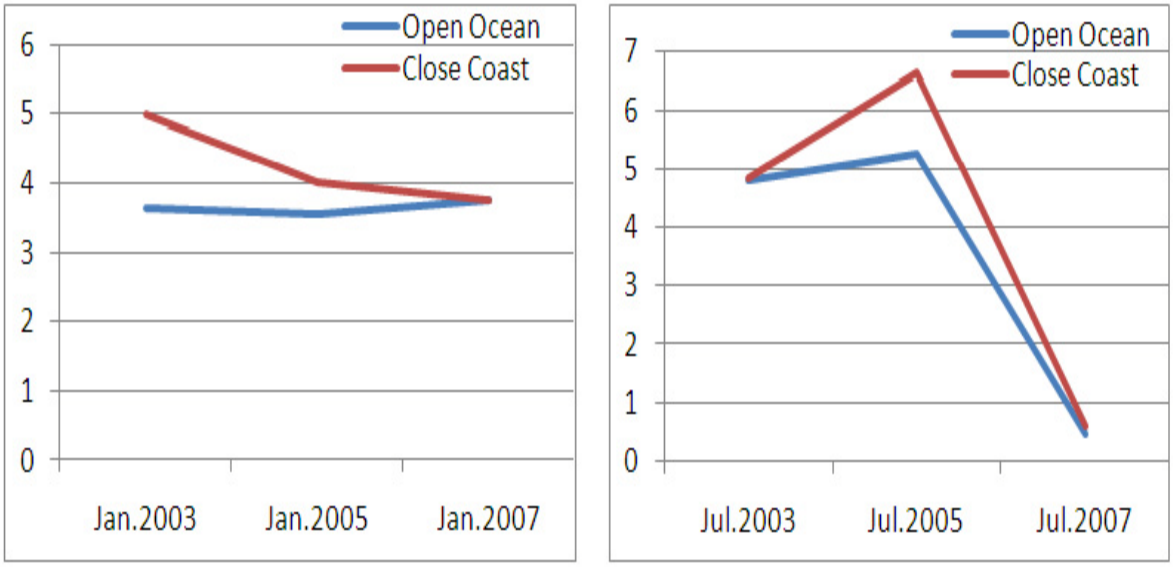

Figure 4: Amount of Black Carbon on Surface of Ocean during January and July of 2003, 2005 and 2007

Conclusions

After extensive research, our hypothesis was ultimately proven wrong: chlorophyll content along the western California coast did not correlate with black carbon content by showing a decrease in chlorophyll content as black carbon amounts increased. Our results were almost consistently the inverse of our hypothesis. However, a very interesting relationship between chlorophyll content and black carbon content was discovered. There appeared to be a direct correlation between the fluctuation of chlorophyll content and black carbon content over the years. Generally, as carbon content increased, so did chlorophyll. In observing the month of January during all three years, for example, the pattern of chlorophyll content growth is exactly the same as black carbon content increase: there was more chlorophyll and black carbon present in 2005 than in 2003, and even more amounts of both in 2007. Although there were slight variations in the patterns of the amounts of both chlorophyll and carbon over the years, the months consistently showed very similar, if not exactly the same, patterns. One of the most interesting things discovered about black carbon content was its dramatic decrease in 2007, far more than any other fluctuation observed over all three years. In relation to our hypothesis, such a discovery was completely unexpected. We expected to see an overall increase of black carbon content from 2003 to 2007, causing a decrease in chlorophyll content. When re-evaluating our data, it actually seems perfectly logical that chlorophyll content would increase as carbon dioxide content increased because of chlorophyll's need for carbon dioxide in photosynthesis. As carbon dioxide is released into the air, and eventually settles into the ocean, vegetation absorbs it in order to continue its photosynthetic production.

In reference to ocean acidification, our research showed that measures of ocean acidification effects cannot be obtained by observing chlorophyll and black carbon content. In other words, chlorophyll and black carbon do not have a directly correlating relationship with the effects of ocean acidification. The detrimental effects of ocean acidification are more closely associated with the decrease and dissolving of calcium carbonate in the ocean, and not the growth loss of vegetation. Although creating a highly acidic and polluted environment within the ocean is a horrible result of ocean acidification, vegetation is not as highly affected by ocean acidification as one would expect. Our research does not reveal the terrible effects of ocean acidification, which is primarily due to the fact that remote sensing satellites cannot penetrate deep enough into the ocean to produce results needed to measure such effects.

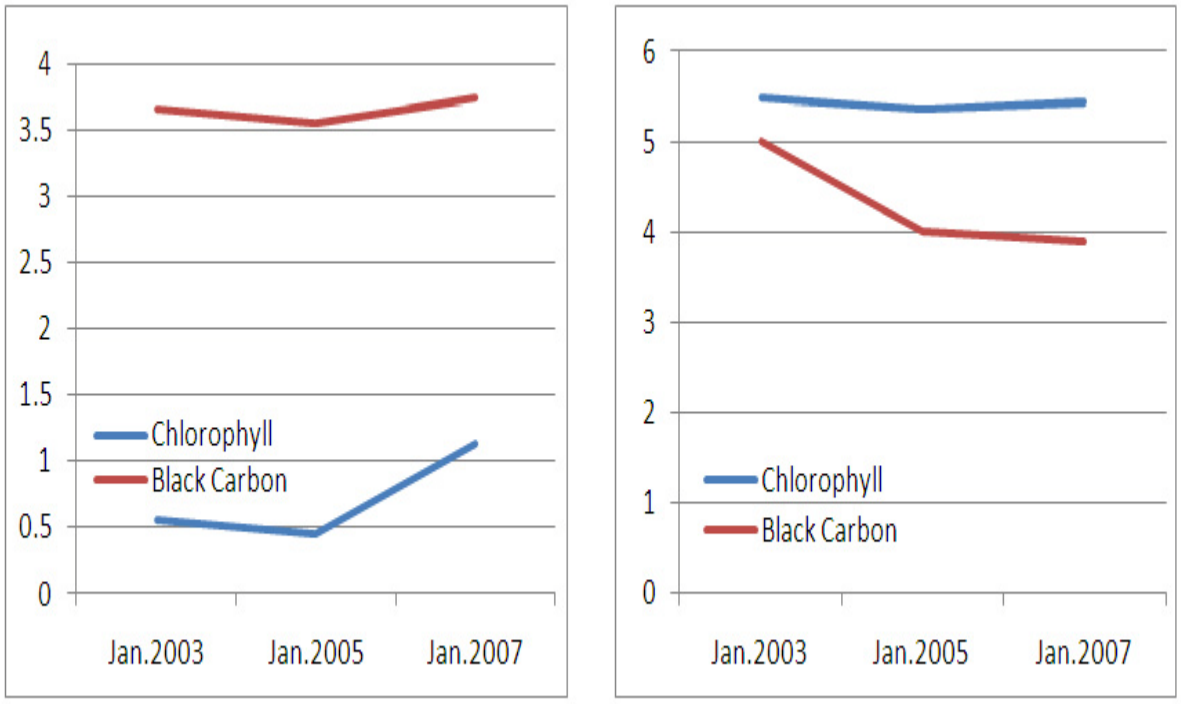

Figure 5: Black Carbon Compared to Chlorophyll in the open Ocean and along the coast during January of 2003, 2005 and 2007

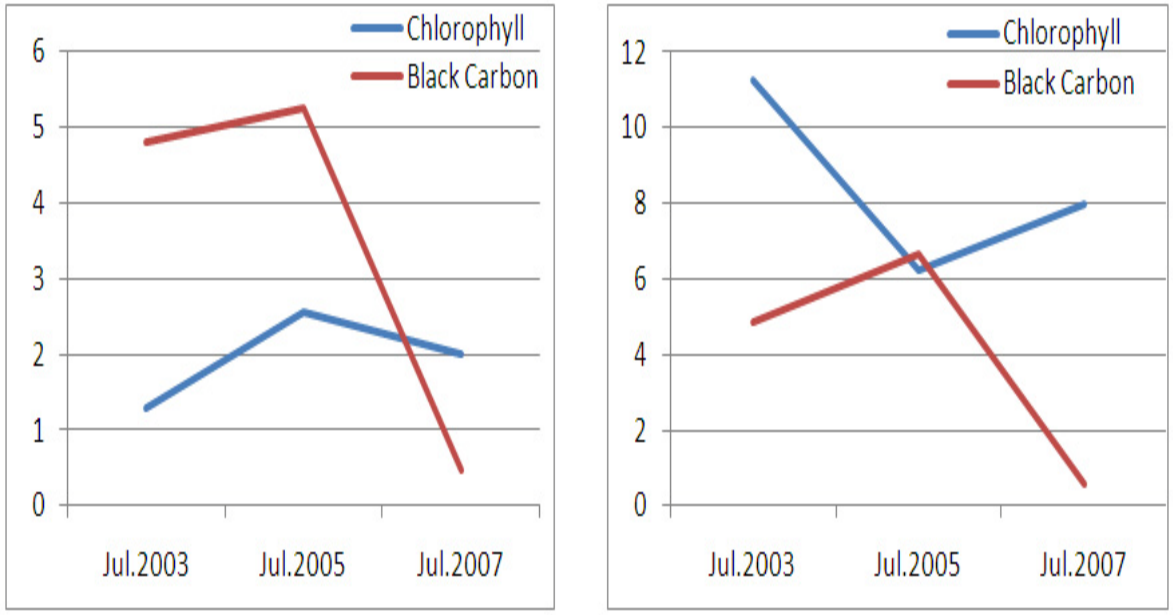

Figure 6: Black Carbon Compared to Chlorophyll in the open Ocean and along the coast during July of 2003, 2005 and 2007

In the future, extensive research that could be done in order to further test the relationship between chlorophyll content and black carbon content could include measuring water and air temperature. In measuring water temperatures, pH levels could be obtained in order to test for any correlation between pH levels and water temperature with chlorophyll content. Since it is an agreed fact by scientist and policy makers around the world that black carbon does in fact increase the pH levels in the ocean, one could perform an experiment that directly measures the pH levels in a specified parameter of the ocean in order to identify the exact percentage relationship between black carbon and pH levels. In addition, using different models, sensors, and satellites could potentially give different results than those obtained by our experiment.

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The effect of pomegranate juice extract on the Hedgehog signaling pathway in pancreatic cancer

Veronica Gomez, Talia Shackelford, Autumn Tocchi, Melissa Rowland-Goldsmith Ph.D.

Abstract

Pancreatic cancer is the fourth leading cause of cancer death in the United States. There have been several reports indicating that phytochemicals in fruits can reduce the risk of cancer due to the anti-oxidant and anti-inflammatory effects of the polyphenols. Our lab has shown that pomegranate juice extract (PJE) has anti-proliferative and pro-apoptotic effects in human pancreatic cancer cells. In the past, we have shown that cells adhere more strongly to the plate when treated with PJE. This observation prompted an investigation of how PJE regulates cell adhesion proteins. Previously, our lab investigated E-cadherin, a cell adhesion protein. Upon activation of the Hedgehog signaling pathway, Gl-1 has been shown to down-regulate E-cadherin. The purpose of this study was to determine if PJE up-regulates ezrin, another cell adhesion protein, by interfering with the Gl-1 transcription factor of the Hedgehog signaling cascade. Through the use of immunoblots, we evaluated Gl-1 and ezrin protein levels after PJE treatment in COLO-357 human pancreatic cancer cells. We showed that pancreatic cancer cells treated with PJE led to decreased expression of Gl-1 and up-regulation of ezrin. This data suggests that PJE can help restore pancreatic cancer cell adhesion by blocking an important signaling pathway, thus serving as a potential suppressor of invasion and metastasis.

Keywords: Pomegranate juice extract, pancreatic cancer

Introduction

Pancreatic ductal adenocarcinoma is a devastating disease in which the overall five year survival rate is approximately 3-5% (1). Non-surgical treatment is generally ineffective due to the resistance of pancreatic cancer cells to chemotherapy and the tumor's ability to metastasize (1).

The demand for dietary alternatives has prompted explorative studies on phytochemicals, which have been shown to be effective in fighting other cancers (2-13). Phenolic acids, flavanoids, and polyphenols are subgroups of phytochemicals, which are found in pomegranate juice extract (PJE) (12). Their ability to act as antioxidants makes them valuable agents in cancer therapies. Other studies have provided evidence for pomegranate juice extract's anti-inflammatory, antioxidant, chemotherapeutic, chemo-preventive, anti-proliferative, and pro-apoptotic properties (2-12). Recently, the complete pancreatic cancer genome sequence has been determined in which 12 signaling pathways involved in promoting the disease were identified (14).

One of these pathways is called the Hedgehog (HH) signaling pathway which has been found to be defective in patients with pancreatic cancer (15-17). Under normal circumstances, as seen in Fig. 1, the Hedgehog pathway

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begins when the HH-protein binds to its receptor Patched (Ptc) (15). When HH is repressed the transmembrane protein Smoothened (Smo) is inhibited (15). When the HH protein binds to its receptor, Smo is activated and transduces a signal, thereby activating Gl-1 transcription factor. Gl-1 then translocates to the nucleus where it regulates gene transcription (15). In many cancers, this pathway is defective, causing an increase in HH protein and an over expression of the Gl-1 (15-17).

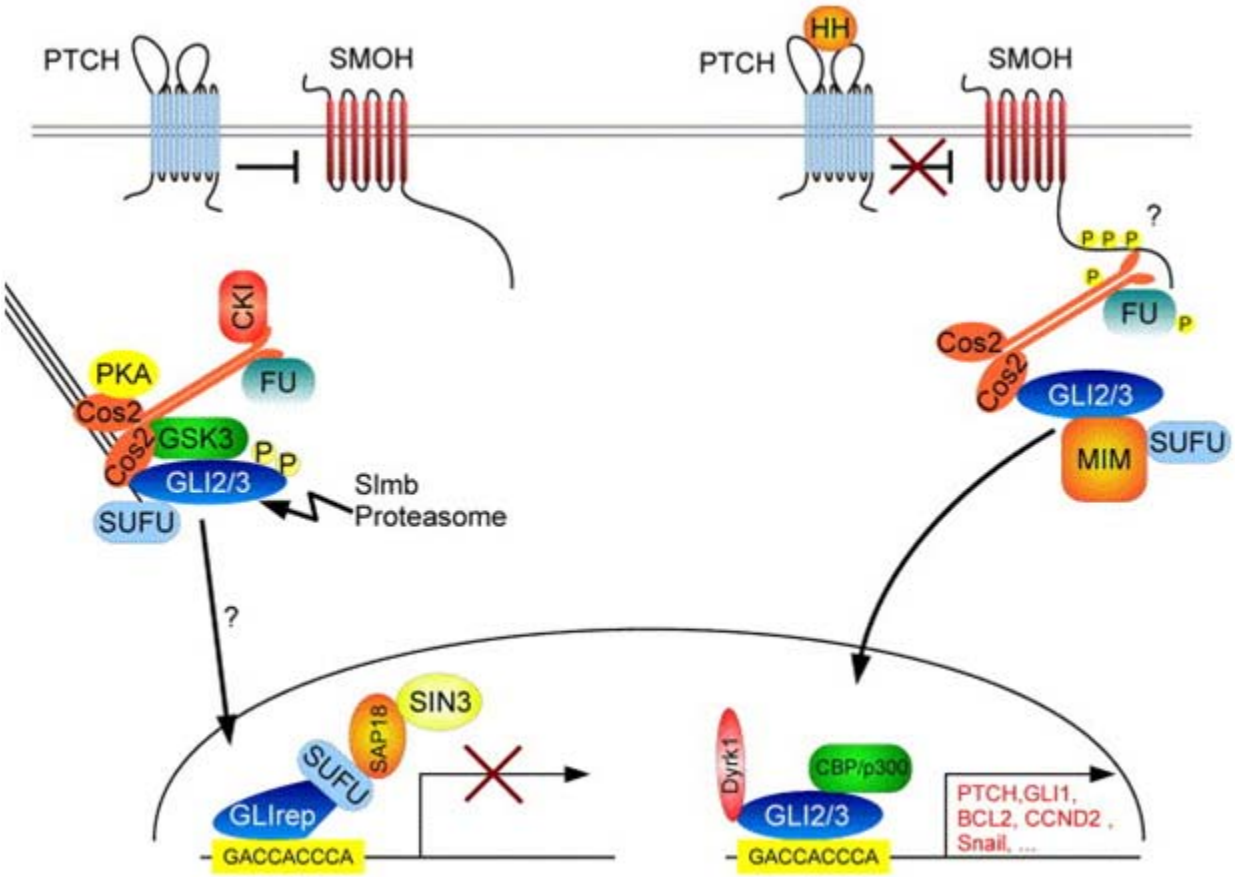

Figure 1: Proposed mechanism of the Hedgehog Signaling pathway showing activation through HH protein binding (15).

In order for tumors to become metastatic, they must exhibit reduced cell-cell adhesion, alteration of tumor-extracellular matrix interaction, and invasion of surrounding tissue (18). Low expression of E-cadherin correlates with increased pancreatic cancer proliferation and metastasis (19). Previous studies suggest that restoring these protein levels in pancreatic cancer cells promotes cell adhesion, leading to an increased rate in apoptosis (19). E-cadherin and ezrin are two cell adhesion proteins that are involved in pancreatic cancer (16,19-22). In pancreatic cancer, E-cadherin is down-regulated by Gl-1 (16). Our laboratory has previously shown that PJE up-regulates E-cadherin levels (data not shown). The present study was conducted to determine if pancreatic cancer cells treated with PJE up-regulates ezrin and whether it does so by interfering with the Gl-1 transcription factor.

Methods

Cell Culture: COLO-357 human pancreatic cancer cells (Dartmouth University) were grown in Dulbecco's Modified Eagle's (DME) complete media (CellGro) containing 10% fetal bovine serum (Irvine Scientific), 0.25 µg/ml fungazone (Omega Scientific), 100 µg/ml penicillin and 100 µg/ml streptomycin (Biowhitaker). Cells were

maintained in a humidified 5% CO_2 and 95% air atmosphere at 37 degrees C. Confluent cells were removed from the plate using trypsin-EDTA (CellGro).

Preparation of Cells for Experiments: Confluent cells were removed from the plate using trypsin-EDTA. $8x10^5$ cells per well were seeded into 6 well plates and grown overnight in DME complete media. Plates were then grown overnight in DME serum free media containing 0.25 µg/ml fungazone, 100 µg/ml penicillin, 100 µg/ml streptomycin, and 1X ITS (insulin, transferrin, selenium) (Biowhitaker) before treatment. Cells were then treated with various concentrations of POM Wonderful PJE (48 hours) in triplicates: no treatment (NT), 10ug/ml PJE, 25ug/ml PJE, and 50 ug/ml PJE.

Immunoblotting: After incubation, protein lysates were collected and the protein concentrations were determined using a BCA assay (Thermo Scientific). The samples were run, along with a pre-stained BenchMark molecular weight marker (Invitrogen), on a 7.5% SDS-PAGE gel (BioRAD), electrotransferred to an Immobilin P membrane (Millipore), and blotted with a goat anti-rabbit ezrin (Santa Cruz) 1:500 dilution or goat anti-rabbit Gl-1 (Santa Cruz) 1:200 dilution. Goat anti-rabbit ERK2 (Santa Cruz) was used at a 1:1,330 dilution as a loading control.

Images: Images of immunoblots were taken using Fotodyne 60-0300 (Fotodyne Inc). Volumetric analysis was performed on images using the Total Lab Software (Nonlinear Dynamics Ltd).

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Results

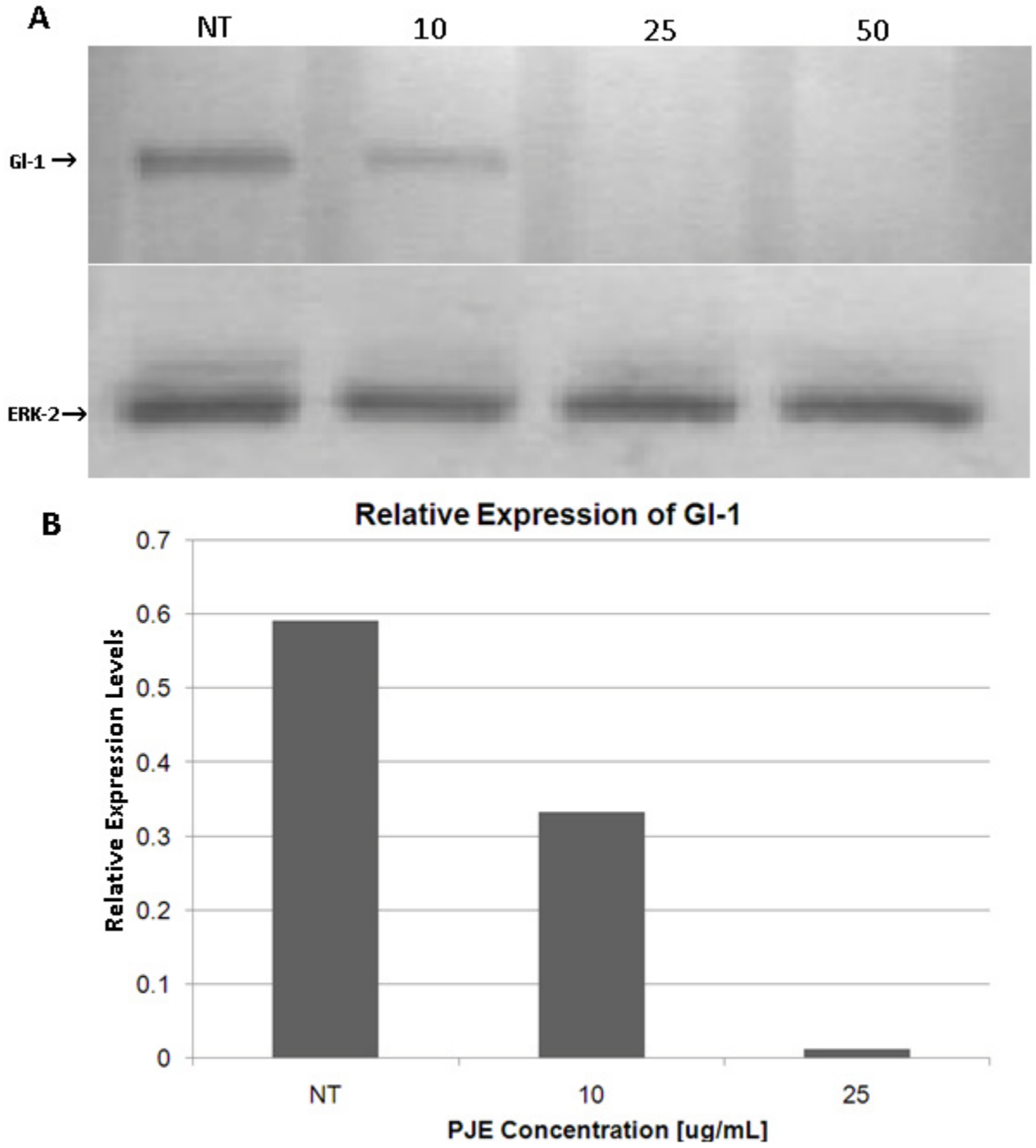
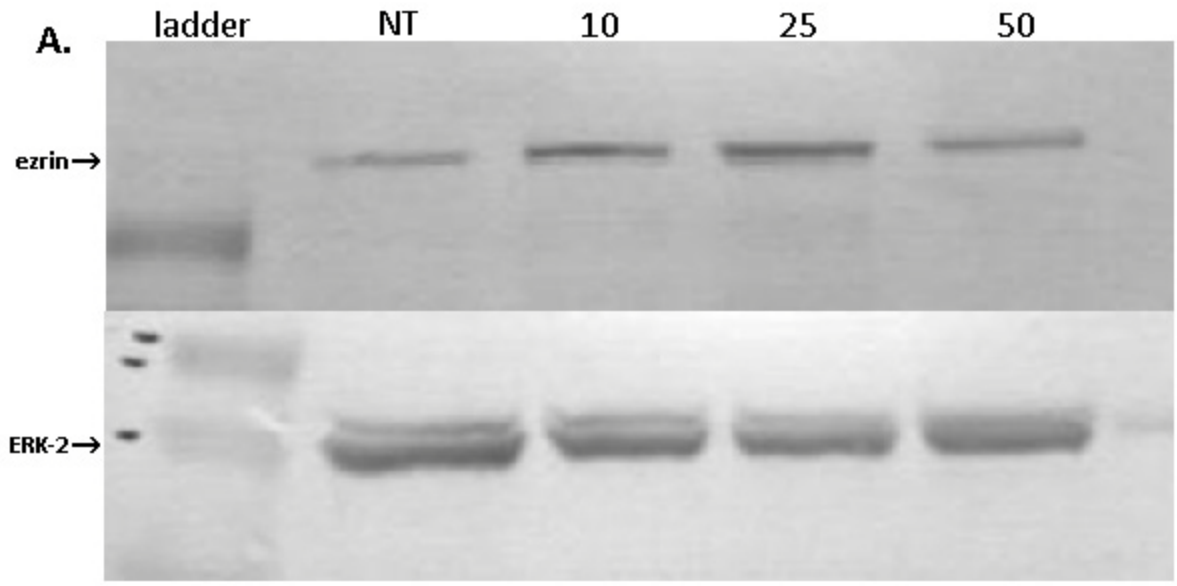


Figure 2: Gl-1 protein expression was decreased by PJE treatment in pancreatic cancer cells. 2a. Pancreatic cancer cell lysates (20ug) were prepared from cells alone (NT); 10 ug/mL PJE; 25 ug/mL PJE; and 50 ug/mL PJE for 48 hrs. They were then subjected to 7.5% SDS-PAGE, electrotransferred to a membrane, and blotted with the anti-Gl-1 antibody (1:200 dilution) or anti ERK-2 antibody (1:1,330 dilution) which was used as a loading control. The western blot was detected using the Western Breeze kit. 2b. Expression levels were determined using Total Lab analysis. Note: there was not a band for the 50 ug/ml PJE treatment so the relative expression could not be computed.



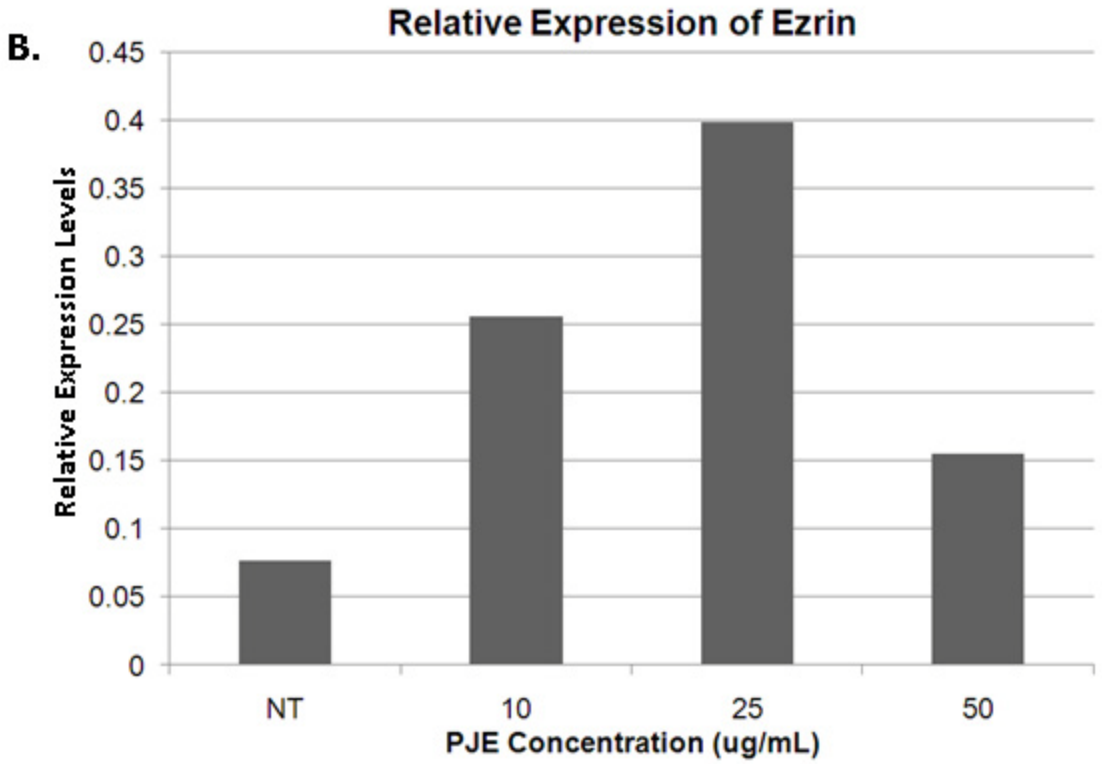


Figure 2: Ezrin protein expression was increased by PJE treatment in pancreatic cancer cells. 3a. Pancreatic cancer cell lysates (10ug) were prepared from cells alone (NT); 10 ug/mL PJE; 25 ug/mL PJE; and 50 ug/mL PJE for 48 hrs. They were then subjected to 7.5% SDS-PAGE, electrotransferred to a membrane, and blotted with the anti-ezrin antibody (1:500 dilution) or anti ERK-2 antibody (1:1,330 dilution) which was used as a loading control. The western blot was detected using the western breeze kit. 3b. Expression levels were determined using Total Lab analysis.

There was a dose dependent decrease in Gl-1 when cells were treated with PJE (Figure 2). Ezrin protein levels had a dose dependent increase up to 25 µg/mL PJE treatment (Figure 3). There was an increase in the PJE treatment of 50 µg/mL, however, it was weaker compared to the 25 µg/mL PJE treatment (Figure 3).

Discussion

We have shown in past research that the most prominent polyphenols in POM Wonderful PJE are Quinnic acid, Ellagic acid, Punicalin, and Punicalagin (data not shown). Ellagic acid has pro-apoptotic effects in pancreatic cancer (23). Formerly, we found that cancer cells treated with PJE led to decreased cell growth. This data partially explains why the cancer cells became more adherent to the culture dish. Since Ellagic acid is the only commercially available polyphenol found in pomegranates, we replicated the above experiments with this single polyphenol and found that the cancer cells also adhered more strongly to culture dishes and led to decreased cell growth, but to a much lesser degree than with PJE. This data suggests a need for the interaction between all polyphenols found in PJE.

It is known that Gl-1 is over-expressed in most cancers (15-17). In our study, we showed that pancreatic cancer cells treated with PJE decreased Gl-1 expression, suggesting that PJE can inhibit the HH pathway. Based on the data, it is suggested that the polyphenols are interfering with the HH pathway by down regulating the Gl-1 transcription factors. Since transcription of E-cadherin is inversely dependent on the Gl-1 transcription factor, the decrease in the Gl-1 expression increases the E-cadherin expression (16). While there is no published data to support that ezrin is directly regulated by Gl-1, the similarities between E-cadherin and ezrin could suggest a similar interaction between Gl-1 and ezrin in the HH signaling pathway. This relation is due to the fact that both are cell adhesion proteins and both have been shown to be involved in pancreatic cancer (19-22). We have demonstrated that both adhesion proteins have an increased expression when pancreatic cells were treated with PJE.

Our data contradicts the current studies on ezrin expression, including pancreatic cancer. In these studies, a high expression of ezrin correlates with a poor outcome (20-22). Specifically, ezrin leads to an increase in the spread of the cancer through its interaction with signaling events that are involved in the regulation of cell survival, proliferation, and migration (20-22). Like our data, a few studies have shown that weak ezrin expression correlates with poor patient outcome (24,25). Instead of pancreatic cancer, however, these studies examined serous ovarian carcinoma and lung adenocarcinoma (24,25). Our findings are the first to show that increased expression of ezrin leads to an inhibition of pancreatic cancer growth. Based on this study, we suggest that ezrin is regulated by the HH signaling pathway, similar to E-cadherin. When Gl-1 levels are low, ezrin levels are high. In conclusion, this study suggests that PJE has possible benefits for pancreatic cancer. However, our experiments need to have increased replication to confirm these novel results.

Acknowledgments

This project would not have been possible without the generous donation of the COLO-357 pancreatic cancer cell line from Dartmouth University, Chapman University's undergraduate student research grant and Faculty Research Grant.

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Treating Cocaine Dependency with Psychopharmacotherapy and Behavioral Therapy

Robyn Liebman

Abstract

Cocaine is an addictive drug that affects more than 14 million people globally, according to the United Nations. This paper is a conceptual meta-analysis of numerous studies that tested the effects of psychopharmacological therapy along with behavioral therapy in the treatment of cocaine addiction. It is hypothesized that cocaine dependent individuals treated with a combination of psychopharmacological and behavioral therapies will be less likely to use cocaine. Measurements of cocaine use throughout the experiments were generally assessed by urine screenings. Results indicate that there is more evidence that a combination of psychopharmacological and behavioral therapies will reduce cocaine use. There are no indications that any specific type of psychopharmacology is more effective than others. This literature review suggests that, while there is no specific category of medication that is most effective in the treatment of cocaine addiction, more studies should be conducted, as it is a promising option that could be utilized along with behavioral therapy.

Keywords: Cocaine Dependence, Behavioral Therapy, Psychopharmacotherapy, Treatment

Introduction

Cocaine is a highly addictive stimulant drug that affects the central nervous system. It is the most potent stimulant that is derived from natural origin. Cocaine was introduced in the 1880's as a local anesthetic used for blood vessel constriction to limit bleeding in eye, nose, and throat surgeries (Drug Enforcement Administration). Sigmund Freud, among other doctors, recommended cocaine as a treatment for many ailments such as; upset stomachs, tuberculosis, depression, and hay fever (Courtwright, 1991). Cocaine was also used to help treat opiate addictions, before it was discovered to be an addictive substance itself (Musto, 1989). Due to the substantial use of cocaine to treat ailments in the late 19th century, a wave of dependency and addiction affected men, women and children.

A. Background & History

The use of cocaine in America has undergone three stages. First cocaine was introduced in the 1880's as a treatment option for ailments, and as it was shown to be effective, it gained acceptance and popularity as it entered its middle stage, only to be shortly recognized and feared as an illicit drug and treated as an illegal substance, which was its final stage (Musto, 1989). Cocaine has become the second most widely used recreational drug in America (Drug Enforcement Administration).

When abused, cocaine creates a euphoric sensation and causes blood pressure, body temperature, and heart rate to rise (Drug Enforcement Administration). The brain experiences an increase of dopamine release in the nucleus accumbens, activating the meso-cortico-limbic system (Karila et al., 2008). The dopamine system is important in motivation, emotion, information processing, as well as a reinforcement mechanism. This is why the drug is extremely addictive. Cocaine manipulates other neurotransmitters, such as glutamate, GABA, endocannabinoid, corticotrophin, and norepinephrine (Karila et al., 2008). Due to the way cocaine affects numerous neurotransmitters, there are various psychopharmacological approaches currently being tested in the treatment of cocaine dependency.

History of Treatment

Most of the time, patients do not experience the effects of the medications until a few weeks after they begin pharmacological treatment. For this reason, it is important to utilize cognitive behavioral therapy along with psychopharmacotherapy in order to begin the recovery process before the medication is effective.

Over the past 30 years, behavioral therapies have been widely studied for use in the treatment of drug and alcohol dependency. There are various types of behavioral therapies that have been researched, and the most effective are Cognitive Behavioral Therapy and Contingency Management (Carroll, 1998). By utilizing behavioral therapy treatment approaches, cocaine dependent individuals have demonstrated improvement (McKay et al., 2010).

a. Behavioral Therapy

Cognitive Behavioral Therapy is considered effective for the treatment of cocaine dependence for various reasons. It is a short-term therapy option that is effective in the treatment of cocaine addiction and it is especially efficacious for those that are severely dependent on the drug (Carroll et al., 2004). This behavioral therapy focuses on the immediate problems that patients are dealing with, and is a structure and goal-oriented approach in treating and coping with these direct issues. It is easily adaptable and flexible to the individual's needs in therapy and controlling dependency behaviors (Carroll & Onken, 2005). In general, those who are dependent on cocaine utilize the drug as a coping mechanism for underlying problems. The skill training of cognitive behavioral therapy teaches individuals to deal with their habits and to be aware of what they are doing in response to their

interpersonal and intrapersonal issues. Cognitive behavioral therapy is highly effective in combination with pharmacotherapy for other issues, for example, depression.

b. Contingency Management

Another type of behavioral therapy, contingency management, has also been effective in the treatment of cocaine dependency. Contingency management is a behavioral therapy that systematically reinforces desired behaviors, and withholds or punishes undesired behaviors (Higgins & Petry, 1999). The most effective type of contingency management to treat drug dependency is a reward-based technique that systematically provides the participant with an incentive, or voucher, that can be cashed in for a monetary value or a prize. The patient receives these vouchers after demonstrating the desired behavior, based on a previously determined criteria and schedule. Both prize and monetary vouchers produce significant patient outcomes, as demonstrated in a study conducted by Olmstead and Petry (2009), and therefore both are utilized in numerous cocaine dependency studies as a behavioral therapy technique. A study conducted by Higgins et al. (2005), found that contingency management was effective in numerous ways; it was well accepted by patients, had high retention within subjects, was effective in achieving initial cocaine abstinence, and was able to retain abstinence in cocaine dependent individuals. Contingency management, although effective on its own, is often combined with cognitive behavioral therapy for the treatment of those who are dependent on cocaine, in order to ensure a successful recovery.

All behavioral therapies have been shown to be effective in treating drug dependence, however, there are currently no psychopharmacological treatments for cocaine dependency. Currently no FDA approved pharmacological treatments for cocaine dependency exists. Those that are dependent on cocaine are often treated for co-existing psychiatric disorders, and this is the only time the psychopharmacotherapies for cocaine substance abuse are usually utilized (Carroll & Onken, 2005). Many cocaine dependent individuals self-medicate concurrent psychiatric issues, and thus if the underlying issue is treated, the cocaine dependency will ease. Therefore the combination of psychopharmacological and behavioral therapy is a promising outlet for the treatment of cocaine dependency.

Prevalence

Substance Abuse and Mental Health Services (2008) estimates that approximately 36.8 million Americans have used cocaine, representing 14.7% of the population. Although statistically cocaine use in the United States has decreased by an estimated 25% since 2006, the National Drug Intelligence Center (2010) attributes this decrease to the increasingly high demand of cocaine in other parts of the world. Cocaine is an outstanding problem among Americans. Although recent studies suggest cocaine use is decreasing slightly, the amount of people who use it is still astronomically high, indicating that a treatment option is necessary to lower the rate and prevalence of cocaine dependency and abuse (National Drug Intelligence Center, 2010).

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The history of cocaine indicates what can happen in the future if cocaine dependency and addiction issues are not dealt with. Epidemics such as the widespread addiction among men, women and children in the 19th and early 20th centuries lead to legal and social consequences including crime and gateways to other addictive substances, such as heroin (Courtwright, 1991).

A. Problem

1. Cost

The treatment of cocaine can be very costly. It is estimated that the United States spends over $414 billion annually on substance abuse disorders, and a large portion of that is dedicated to the treatment of cocaine dependency (Quality Enhancement Research Institute, 2008). Due to the highly addictive nature of this drug, there is a great deal of costs involved with the treatment and rehabilitation to assist and maintain those that are dependent. Although the treatment costs for each individual vary based on time in treatment, needs, and type of therapy, there are various categories that these costs are divided into. Some common categories include treatment personnel, administration and office personnel, space, equipment, transportation, communication services, and insurance, to name a few (National Drug Intelligence Center, 2010). The cost involved in treating those with a cocaine dependency can be steep. Treatment is not the only cost involved with the issue of cocaine use. The transportation and illegal trade of drugs is a huge cost that America pays for every year.

2. Social Complications

The use of illegal drugs, especially cocaine, has many negative social effects. Cocaine can cause permanent physical and emotional damage, which in turn negatively affects family members, coworkers, and friends (National Drug Intelligence Center, 2010). Drug abuse leads to health complications, and sometimes death, which can cause subsequent child abandonment, if that individual had children, and an increase in the number of children placed into foster care.

3. Cocaine Related Crime and Violence

The most common arrest category and population in prisons and jails is criminals that have broken the law pertaining to drug use and abuse. The Bureau of Justice estimates that 53% of the federal prisoners are incarcerated on a drug related offense (National Drug Intelligence Center, 2010). These criminals are then placed into the legal system, monopolizing time, money and resources that are needed for the prosecution of other violent offenders. Drug related crimes are the largest category of arrests made and they account for 12.2% out of 14 million arrests, which has increased over the last 20 years (National Drug Intelligence Center, 2010). Studies have shown that when stimulants are used, human behavior increases in competitiveness, excitability and

volubility (Hoaken & Stewart, 2003). Overall cocaine has negative social effects, health complications, and increased rates crime and aggression.

4. Infectious Diseases

Cocaine use leads to the transmission of infectious diseases. Users who inject cocaine are at risk of contracting HIV and viral hepatitis. Along with these risks cocaine reduces immunity and increases susceptibility to infectious diseases (Friedman, Pross & Klein, 2006). Besides the known health risk of using cocaine, a new epidemic is threatening the population of cocaine users. A pharmaceutical agent called levamisole is appearing in higher quantities in cocaine samples obtained. This drug is typically used in the deworming of livestock, and is harmful to humans causing agranulocytosis, a fatal blood disorder (Drug Enforcement Administration, 2005). Individuals are not always aware of what the cocaine is laced with, and the substances that are mixed with cocaine can be fatal.

C. Purpose and Goal

The purpose and goal of this paper is to assess the effectiveness of pharmacotherapy and behavioral therapy, compared to behavioral therapy only, in the treatment of those who are dependent on cocaine. Various classes of psychopharmacotherapy have been tested in the effectiveness of treating cocaine dependency, yet there is no indication as to which drug category has been shown to be most successful throughout treatment. The various categories that have been tested include antidepressants, anticonvulsants, antabuse, analeptics, glutamate inhibitors, anti-inflammatory, and various others, and no single category has been more effective than others (Karila et al, 2008).

D. Significance

A protocol for treating cocaine dependent individuals is necessary to reduce cocaine use and abuse. This conceptual meta-analysis examines the various categories of psychopharmacotherapy, when combined with behavioral therapy in comparison to behavioral therapy alone in the efficacy of treating cocaine dependency. Overall these treatments will be examined in order to provide a general overview of the methods and efficacy in order to determine what is the best approach.

E. Hypothesis

It is therefore hypothesized that if a cocaine dependent individual is treated with a combination of psychopharmacological and behavioral therapy, then that individual will have more success in reducing cocaine use than if the individual is treated with only behavioral therapy.

1. Operational Definitions

a. Cocaine Dependence

The American Psychiatric Association (*DSM-IV-TR*) describes 7 symptoms for cocaine dependence (2000). If the participant has any three of them they are considered dependent on cocaine. These symptoms include; demonstrating excessive or inappropriate use of cocaine, preoccupation with cocaine, having an increased or decreased tolerance to cocaine, having trouble stopping or reducing drug use once started, withdrawal symptoms when cocaine intake is reduced or stopped, continuing use of cocaine even though it causes issues within everyday life, and sacrifice of important activities or friendships because of cocaine use. All of the studies require that the participants meet the criteria for this diagnosis of cocaine dependence.

The independent variables are the treatment conditions, psychopharmacology and behavioral therapy versus behavioral therapy alone. The treatment success is the dependent variable and will be measured by utilizing the number of participants who have reduced cocaine use. Cocaine use in every study is measured through urine samples provided by participants. These samples are tested for benzoylecgonine, a cocaine metabolite that would indicate that the individual had cocaine in his or her system (Alvarez, Farre, Fonseca & Torrens, 2009). Numerous other studies also assessed cocaine use through self-report of the participants as well as retention in the study.

b. Psychopharmacotherapy

Psychopharmacotherapy is defined as a medication-based therapy that utilizes medication in order to address the underlying pathophysiology of cocaine dependency (Johnson, Roache, Ait-Daoud, Javors, Harrison, et al., 2006).

c. Behavioral Therapy

Behavioral therapy consists of cognitive behavioral therapy (CBT), contingency management (CM), or a combination of the two (Garcia-Rodriguez et al., 2009). Each study specifies the measurements that are utilized within the specific experiment, and that will be clarified throughout the results section. The meta-analysis assesses CBT and CM as forms of behavioral therapy and does not discriminate between the two to determine which one is more effective when combined with psychopharmacotherapy.

II. Results

Many studies have shown that the use of psychopharmacotherapy in combination with behavioral therapy decreases cocaine use in individuals who are dependent. Some of these empirical studies have tested multiple different types of medications, while others look at one. The studies indicate how significant the difference was between the control group, behavioral therapy alone, and the experiment groups, which consisted of behavioral therapy with psychopharmacotherapy.

A. Attention Deficit Disorder Treatment

Methamphetamine is a stimulant psychopharmacological agent that is most often prescribed to those who suffer from Attention Deficit Disorder and Attention Deficit Hyperactivity Disorder. This psychoactive drug increases levels of dopamine, norepinephrine, and serotonin and is often abused as high levels create a euphoric feeling. The FDA has approved the use of Desoxyn, the trademark name for methamphetamine, in small doses that are taken in an immediate or sustained release form (Mooney, Herin, Schmitz, Moukaddam, Green, & Grabowski, 2009). The use of methamphetamines is hypothesized to be effective in the treatment of cocaine dependency due to the similar mechanism of action.

Participants were randomly assigned to one of three conditions, methatmphetamine sustained release, immediate release, or a placebo condition. Each group received CBT throughout the entire trial, and a CM program was added to the behavioral therapy at week five. Cocaine use was measured through urine samples that were collected weekly. The authors hypothesized that the participants that received sustained release and immediate release methamphetamine would produce less cocaine positive samples than the placebo condition. Results indicated a significant different between the treatment conditions $F (2, 344) = 14.7$, $p < 0.0001$, the Sustained Release condition decreased positive cocaine urine samples to 29%, immediate release resulted in 66% positive, and the placebo condition 60%. The non-significant results for the immediate release treatment condition can be attributed to the fact that the majority of the doses were not taken and were returned to the clinic. Overall, more research needs to be completed to look at the effects of methamphetamine as a treatment option, along with behavioral therapy, for cocaine dependence. This article supports the overall hypothesis as the sustained release treatment condition along with CBT and CM reduced cocaine use compared to the placebo condition (Mooney et al., 2009).

B. Amyotrophic Lateral Sclerosis Treatment

The only FDA approved medication to treat Amyotrophic Lateral Sclerosis (ALS) is Riluzole. This psychopharmacological agent inhibits presynaptic glutamate release. Cornish et al. (1999) hypothesizes that the glutamatergic systems are involved in the regulation and reduction of drug seeking behaviors in those that are cocaine dependent; therefore riluzole could be an option in treating those drug-seeking behaviors (as cited in Ciraulo et al., 2005). Riluzole was included in a study comparing five different psychopharmacological treatments to a placebo condition, where all conditions received CBT simultaneously. Results were measured through urine analysis provided by the participants. Overall, riluzole, combined with CBT, did not have a significant effect on reducing cocaine positive urine samples, or overall cocaine use by those that are cocaine dependent. These results indicate that riluzole is not an effective treatment, as it did not have a significant outcome compared to the other medications or placebo treatment groups (Ciraulo et al., 2005).

C. Antabuse

Antabuse is a medication traditionally used in the treatment of alcoholism and has also been shown to reduce the breakdown of dopamine in the brain. This mechanism of action suggests that it is efficacious in the treatment of cocaine dependence. At first Disulfiram, an antabuse, was used to treat co-morbid alcohol and cocaine dependence (Carroll, Nich, Ball, McCance, Rounsavile, 1998). However, it is hypothesized that disulfiram may be effective in the treatment of cocaine dependence, regardless of co-morbid alcohol dependence. Medication and cognitive behavioral therapy were tested in the efficacy of treating participants who are solely cocaine dependent. Two types of behavioral therapy, CBT and interpersonal psychotherapy (IPT), were tested along with the medication conditions. Researchers used a 2x2 factorial design, which allowed four treatment groups; disulfiram plus CBT, disulfiram plus IPT, placebo plus CBT and placebo and IPT. Results were measured utilizing urine toxicology screens and self-report of use. The hypothesis stated that, if a cocaine dependent individual was treated with a combination of disulfiram and cognitive behavioral therapy, then there would be greater efficacy than those not treated with disulfiram and cognitive behavioral therapy.

Results of this study showed that the disulfiram and CBT conditions were most efficacious compared to the placebo and IPT conditions. Participants that were treated with medication along with behavioral therapy produced significantly less cocaine samples than those who were in the placebo treatment group ($z = -3.74$, $p < 0.01$). Disulfiram has shown to be efficacious in the treatment of cocaine dependency along with behavioral therapy, although more research is needed in order to determine what type of behavioral therapy best accompanies this psychopharmacotherapy (Caroll et al., 2004).

D. Anti-Convulsant

There are many medications that are prescribed for the treatment of seizures. However, for many of these drugs their mechanism of action is unknown. It is assumed that anticonvulsants would control impulses, often times working through the GABA receptors and dopaminergic system (Alvarez et al., 2009). Gabapentin, Tiagabine, Topiramate, Valproate, Lamotrigine have all been assessed for their efficacy in treating cocaine dependency.

Topiramate, which increases GABA activity in the brain, has been effective in the treatment of other addictions and dependencies, such as alcoholism. In a small study, 40 participants were divided into the control or treatment conditions. The treatment condition received a dose of topiramate each day, which slowly increased over the course of 13 weeks. Every participant received CBT twice a week. Results were measured through urine analysis, and there was a significant difference in the groups, the topiramate treatment condition was more likely to be abstinent from cocaine use over the duration of the study ($Z = 2.67$, $P = 0.01$). This indicates that the odds of cocaine use in the placebo group increased throughout the duration of the study, whereas the odds that the treatment group would use cocaine decreased. Due to the small sample size of this study, it is not clear as to

whether topiramate is a strong treatment choice for cocaine dependency, although it is promising. More research on this medication should be completed (Kampman et al., 2004).

Two anticonvulsant medications were tested against each other to determine efficacy in the treatment of cocaine dependence. Tiagabine and gabapentin were compared to the placebo control condition in a study utilizing 76 participants. The hypothesis of this study was that if a cocaine dependent individual is treated with either tiagabine or gabapentin, then that individual will have greater efficacy in the reduction of cocaine use than those who receive the placebo. This study is relevant to the overall hypothesis as it tested the efficacy of treating a cocaine dependent individual with medication and cognitive behavioral therapy compared to a placebo and cognitive behavioral therapy. The results were measured through urine samples and self-reported cocaine and indicated that tiagabine had significantly more cocaine-free samples than the gabapentin or placebo treatment conditions. Tiagabine had mean of 43% cocaine-free samples, gabapentin resulted in 31%, and the placebo condition had 35% (Gonzalez et al., 2007).

Tiagabine was also tested in an 8-week trial compared to sertraline and donepezil, that divided a sixty-seven participant sample size into 17 participants per treatment. Each participant received CBT simultaneously. Although results were not significant, tiagabine resulted in less positive urine samples than the other two medications, and the placebo condition. Tiagabine resulted in 73% less positive urine results, compared to the placebo condition which had a 32% increase in positive urine samples. The results for the efficacy of tiagabine as a psychopharmacological treatment, combined with behavioral therapy, in the treatment of cocaine dependency are mixed and need to be researched further in order to determine whether or not tiagabine should be used to treat patients (Winhusen et al., 2005).

Results reported by Gonzalez et al. (2007) indicate that gabapentin was not effective, however, other studies have tested the efficacy of gabapentin in the treatment of cocaine dependency. Gabapentin is known to increase GABA levels in the human brain (Petroff et al., 1998). In one study involving gabapentin, Ninety-nine participants were randomly assigned into either the gabapentin 3200 mg per day treatment or the placebo condition for twelve weeks. Every participant in both treatment conditions also received weekly behavioral therapy, which included cognitive behavioral relapse-prevention therapy. Results of this study indicated that there was no significant difference in cocaine use between treatment conditions ($z = -1.25, .22$). Participants were classified into either a low-use or high-use group depending on their reported cocaine-use during a two-week lead in period. Out of these two classifications, those in the high-use group reduced cocaine use across both treatments. The low-use classification group on average maintained levels of cocaine-use across treatments. These results indicate that those who have a high-level of cocaine use will reduce cocaine-use when receiving cognitive behavioral therapy independent of psychopharmacological therapy. These findings refute the overall hypothesis because there is no significant difference between those treated with psychopharmacology and behavioral therapy compared to those treated with placebo and behavioral therapy (Bisaga et al., 2006).

Another study testing gabapentin, and other medications, found that gabapentin did not result in a significant change in cocaine-negative urine samples compared to the placebo condition, when combined with behavioral therapy. Three medications were evaluated in this study and individuals were placed into one of four conditions, one of the three pharmacotherapies or the placebo. The authors hypothesized that if a cocaine dependent individual is treated with a combination of psychopharmacotherapy and behavioral therapy then it will be more effective than the placebo condition. Results indicated that gabapentin did not produce significant changes in positive urine results, compared to the placebo condition. Gabapentin has not been efficacious in the treatment of cocaine dependency when combined with behavioral therapy, despite what researchers hypothesized based on the effect that gabapentin has on the GABA neurotransmitter levels (Berger et al., 2005).

Valproate, another anti-convulsant, was tested in the treatment of cocaine dependency. This medication has an unknown mechanism of action, but it does enhance GABA levels. In a study examining how effective Valproate is in the treatment of cocaine dependency compared to a placebo condition, each participant was placed into a treatment or placebo group and received CBT throughout the 8-week study. Overall, results indicated that there was no difference in reducing cocaine-negative urine samples between the treatment and placebo conditions. Each group demonstrated overall improvement, however none of the conditions were significantly different than the others. Although there were no significant differences between groups, there is a need for more research in the treatment of cocaine dependency using valproate, as it did lead to a decrease in cocaine positive urine samples overall, along with all the other treatment conditions (Reid et al., 2005a).

In another study, valproate was tested with four other medications and a placebo condition, for its efficacy in the treatment cocaine dependency along with CBT. Sixty-eight participants were randomly assigned to valproate, one of the three psychopharmacological agents, or the placebo condition while concurrently receiving CBT. Results were measured through urine analysis and there was no significant difference in the reduction of cocaine use across treatment conditions. Although the valproate treatment condition has reduced overall cocaine use, when combined with behavioral therapy, the results have not been significant, indicating that valproate may not be the best psychopharmacological agent in treating cocaine dependency (Reid et al., 2005b).

Anti-convulsants have been highly tested in the treatment of cocaine dependency. Although many of the studies indicated that there was no difference between treatment conditions, there is a need for more research utilizing behavioral therapy in combination with anti-convulsants. The results did not show that they increased cocaine use, one study demonstrated that the anti-convulsant reduced overall cocaine use, as did the other psychopharmacological treatment conditions (Reid et al., 2005b).

E. Anti-Depressants

The use of anti-depressants for the treatment of cocaine dependency has been thoroughly examined. There are three types of anti-depressants that have been studied, which work on different neurotransmitters.

1. Selective Serotonin Reuptake Inhibitor

The first one to address is the selective serotonin reuptake inhibitors (SSRI). These anti-depressants have shown mixed results, as they are effective in rats, but have not been effective in human cocaine dependence treatment. Overall 157 individuals with cocaine dependency were randomly assigned to receive 20 mg of citalopram or a placebo medication for 12 weeks, along with behavioral therapy. Two types of behavioral therapies were assessed, cognitive behavioral therapy and contingency management, which were combined with the medication treatment conditions. The amount of cocaine was measured through urine samples collected from the participants in both treatment conditions. The medication treatment effect was significant compared to the placebo condition; the mean number of consecutive cocaine free days was 5.06 for the treatment condition and 2.13 for the placebo. Those that received the citalopram 20 mg per day and the behavioral therapy produced less positive urine samples than those who received the placebo medication along with the behavioral therapy. Overall the results strongly supports the hypothesis that psychopharmacotherapy combined with behavioral therapy is an effective treatment for cocaine dependency (Moeller et al., 2007).

Another SSRI, sertraline, was tested, along with CBT for efficacy in treating cocaine dependency. This serotonin 5-HT reuptake inhibitor is traditionally used to treat depression, obsessive-compulsive disorder, and panic disorders. Two other psycopharmacotherapies and a placebo condition were also used as grouping variables so samples sizes were n=17. Participants were assessed through urine samples throughout the 8-week trial. The sertraline did not show significant differences in positive urine samples compared to the placebo and other medication treatment conditions. However, altogether each treatment condition was very similar, showing that there was an overall decrease in positive urine samples, none of which were significant (Winhusen et al., 2005).

One other SSRI that was examined is paroxetine. This medication was measured in comparison to four other medications and a placebo condition. Ciraulo et al. (2005) hypothesized that if the cocaine dependent participant was treated with a psychopharmacological agent along with behavioral therapy, then that participant would be more successful in producing less cocaine positive urine results. Each participant received CBT along with the medication or placebo, based on random assignment. Efficacy was measured by urine samples and self-report. Paroxetine did not show a significant difference in efficacy of treatment compared to the other treatment and placebo conditions. Overall, one SSRI was effective when combined with behavioral therapy in the treatment of cocaine dependence. Therefore the results for the SSRI category were mixed. Although other medications did not support the hypothesis, by demonstrating a non-significant response, SSRI medications should still be researched further as a treatment option for cocaine dependency (Ciraulo et al., 2005).

2. Serotonin and Norepinephrine Reuptake Inhibitor

Another category of anti-depressants is the serotonin and norepinephrine reuptake inhibitor, assumed to inhibit serotonin and norepinephrine reabsorption. Venlafaxine was examined in comparison to four other medications and a placebo condition, and all participants received behavioral therapy concurrently. Participants provided urine samples and self-report questionnaires in order to determine cocaine-use. Venlafaxine was not effective when combined with behavioral therapy in reducing cocaine use in participants that are dependent (Ciraulo et al., 2005).

3. MAOI

Lastly, monoamine oxidase inhibitors (MAOI) were assessed in the treatment of cocaine dependency. MAOI's are hypothesized to increase levels of dopamine, serotonin and norepinephrine by breaking down the neurotransmitters. Three hundred participants were randomly assigned to be in the placebo or treatment condition. Selegilline was administered through patches every day, and the placebo condition received a placebo patch instead of the treatment. Each was also supplied with behavioral therapy once a week, despite treatment condition. Cocaine use was measured through urine samples supplied by the participants. Results indicated that both treatment conditions decreased cocaine use among participants. However there was no significant difference between groups. Although there was no significant difference, it is important to note that both conditions had a decrease in cocaine use (Elkashef et al., 2006).

F. Anti-Emetic

Ondansetron is a dopamine receptor antagonist, and has the potential of eliminating the reinforcing effects of cocaine. By eliminating the reinforcing effects, individuals will be less probable to relapse. Therefore this psychopharmacological agent is worth studying in order to determine the efficacy in treating cocaine dependence. It is hypothesized that if ondansetron, with CBT, is used to treat cocaine dependence, it will be more effective than treating solely with CBT. Sixty-three participants were divided into one of four psychopharmacotherapy treatment groups; 0.25 mg, 1.0 mg, 4.0 mg and placebo. Each participant received weekly CBT and urine samples and self-report measures were collected on a weekly basis to assess the efficacy. The 4.0 mg treatment condition showed a significant rate of change compared to the placebo condition. The other two experimental conditions did not show a significant difference from the placebo condition, indicating that more research needs to be completed in order to determine the most effective dosage of ondansetron, along with behavioral therapy, in the treatment of cocaine dependence (Johnson et al., 2006).

G. Anti-Hypertension

Reserpine, a medication used since the 1950's to treat hypertension, was assessed for success in treating cocaine dependency, with a combination of behavioral therapy. This psychopharmacological agent disrupts storage of catecholamines, dopamine and norepinephrine, releasing these neurotransmitters to be metabolized quickly, therefore depleting dopamine until new vesicles become available, which could take several weeks. Therefore, when individuals who are taking reserpine use cocaine, they will no longer experience the euphoric effects of the drug. One study divided participants into two treatment conditions, those that received reserpine and those that received the placebo medication. Along with the psychopharmacological treatment, each participant received weekly, individualized CBT. A total of 79 participants completed the 12-week trial and cocaine use was measured through urine analysis and self-report of craving. No significant outcomes were measured across treatment conditions. Those that received the placebo or the reserpine psychopharmacotherapy, along with CBT, did not result in any significant change of cocaine-positive urine samples over the course of the 12-week study (Winhusen et al., 2007).

Another study conducted on the effectiveness of reserpine compared this medication to three other psychopharmacotherapies over the course of 10 weeks. Participants were assigned to one of four treatment conditions, where they received a placebo, reserpine or one of the other two treatments. All of the subjects participated in individual weekley CBT along with the psychopharmacological treatment. The authors stated that individuals who were treated with a combination of psychopharmacotherapy and behavioral therapy would be more successful in reducing cocaine positive urine samples compared to the placebo and behavioral therapy condition. Results were measured through urine analysis collected weekly and self-report. The reserpine treatment condition, along with CBT, yielded a significant decrease in cocaine positive urine samples. The reserpine and placebo conditions were the only treatments to show a significant change in the amount of cocaine positive urine samples (Berger et al., 2005).

These mixed findings for reserpine indicate that more research should be completed on this psychopharmacological agent. Between the two studies, the treatment time and the subject pools were different and this could be attributed to the difference in results. It is important to further investigate this medication, as it has shown potential for being efficacious in the treatment of cocaine dependency when combined with behavioral therapy.

H. Anti-Inflammatory

Celecoxib is an anti-inflammatory medication that is mainly used to treat arthritis. This medication was tested for the treatment of cocaine abuse because this psychopharmacological agent creates an enhanced sensitivity to cocaine. It works by inhibiting clooxygenase-2 activity, which in turn will affect the level of neuroplasticity in the brain of an individual who chronically uses cocaine. It has been hypothesized that regular cocaine use can cause neuroplasticity, or neuroadaptations, in areas of reward-related learning and memory processes, which in turn can

lead to impulsive decision making and abnormal habits that are not sensitive to negative consequences of these actions (Thomas, Kalivas & Shaham, 2008). Twenty-three participants were divided into two treatment conditions, celecoxib and placebo. Each participant received a cognitive behavioral treatment program throughout the 8-week trial. Results were measured through urine samples and indicated that there was no significant difference between treatment conditions for those treated with celecoxib compared to the placebo condition. Both treatment conditions demonstrated a decrease in cocaine use overall, but there was no difference between the groups in that change. The use of celecoxib and behavioral therapy has not been shown to be efficacious although more studies should be conducted on other types of anti-inflammatory medications (Reid et al., 2005a).

I. Anti-Narcoleptic

A wake-promoting agent used in the treatment of narcolepsy has been effective in the treatment of cocaine dependence in combination with behavioral therapy. Modafinil can help in various ways; first of all it is a stimulant and therefore would alleviate some withdrawal symptoms. Secondly, modafinil has been effective in the treatment of Attention Deficit Hyperactivity Disorder through the glutamate/GABA and hypocretin systems (Anderson et al., 2009). Another empirical study that tested the effects of modafinil on 62 participants found that there was a significant difference between treatment conditions (Dackis, Kampman, Lynch, Pettinati, O'Brien, 2005). There were two treatment conditions, including modafinil 400 mg and the placebo condition. Each participant received twice-weekly CBT. Results indicated that there was a significant difference between treatment conditions. The modafinil plus cognitive behavioral therapy condition resulted in significantly less positive urine samples over the course of 8 weeks. The mean percentage of negative urine samples was 42.3% for the medication treatment condition and 24.0% for the placebo treatment. Overall, supporting the hypothesis that psychopharmacotherapy plus behavioral therapy is more effective in producing less positive urine samples than therapy alone.

Another experimental study hypothesized that modafinil plus CBT treatment would be more successful in reducing cocaine use in those that are cocaine dependent compared to participants that were given the placebo condition. Three treatment conditions were tested; modafinil 200mg, 400 mg, and placebo. Along with these medication treatments, each participant underwent one hour of manually guided CBT each week. The rate of change between treatment conditions was not significant, however the maximum number of consecutive non-use days was significant. The average number of non-use days; 400 mg modafinil averaged 12.0 days, 200 mg averaged 12.6 days, and the placebo condition averaged 8.8 days. Those that were in the modafinil treatment conditions had a significantly higher amount of consecutive non-use days compared to the placebo condition. This supports the hypothesis that a combination of psychopharmacotherapy and CBT can reduce the number of non-use days (Anderson et al., 2009).

J. Anti-Parkinson

Levodopa is a medication used to treat Parkinson's Disease. It works as a dopamine precursor. Due to the effect that levodopa has on dopamine in the brain, it is hypothesized that it will be efficacious in the treatment of cocaine dependency, as it could help reduce cravings and chronic use. The study by Schmitz et al. (2008) also tested the efficacy of levodopa with three different types of behavioral therapy; clinical management, CBT, and voucher-based reinforcement (CM). This was a 3x2 study and therefore participants were divided into 6 different treatment conditions. Cocaine use was measured through urine analysis collected three times per week. Overall, there was a significant reduction in cocaine use in the Levodopa (M = 61.6%) compared to the placebo condition (M = 79.1%), despite what type of behavioral therapy was used. The voucher-based reinforcement treatment condition combined with levodopa had the lowest cocaine positive samples (M = 59%), compared to CBT and levodopa (M = 84%), however each behavioral therapy condition was significantly lower combined with the levodopa than with the placebo. This study supports the hypothesis that behavioral therapy combined with psychopharmacotherapy is the most effective treatment option in reducing cocaine use among those that are dependent (Schmitz et al., 2008).

Levodopa-Carbidopa is a combination of levodopa, which is a precursor of dopamine, and a decarboxylase inhibitor, which increases dopamine activity. The theory behind using L-dopa is that it will stabilize dopamine stores, which are often depleted with the chronic use of cocaine. The first treatment condition received 200/50 mg of L-dopa, which doubled to 400/100 mg by the end of the trial, and the second received 400/200 that doubled to 800/200 mg. The third treatment condition received a placebo throughout the entire 8 weeks. Every participant in each treatment condition received 1 hour of cognitive behavioral therapy each week as well as the medication or placebo. The hypothesis states that the L-dopa treatment condition will be more efficacious on treating those with cocaine dependence than the placebo condition. The outcome of this study indicated that there was no significant difference in the percentages of cocaine use of the L-dopa treatment (40.0%) compared to the placebo (51.0%) conditions. Urine levels were not significantly different across treatments, despite time in treatment or medication dose. Although the differences in cocaine use were not significant across treatment conditions, there was a slight decrease in the L-dopa treatment group, which suggests that more studies should be conducted in order to determine whether L-dopa could be an effective treatment in combination with behavioral therapy (Mooney et al., 2007).

In a study comparing levodopa-carbidopa to two other medications and a placebo condition, participants were randomly assigned to a medication treatment condition and received weekly CBT treatment for the 8-week trial. Urine screenings and self-report was used to evaluate cocaine use and were collected three times per week. Shoptaw et al. (2005) hypothesized that if a participant received one of the medications along with behavioral therapy, then that participant would demonstrate higher reduction in cocaine use than those in the placebo condition. However, results did not indicate a significant difference in the reduction of cocaine use between the levodopa-carbidopa group and the placebo group (Shoptaw et al., 2005). Levodopa should be further examined as the results indicate mixed results in the efficacy of reducing cocaine use.

A third type of medication used to treat Parkinson's disease is called pramipexole, which is a dopamine agonist. As seen before, this study tested this psychopharmacological agent along with four others. Pramipexole was one medication that was compared to a placebo condition to assess the efficacy in reducing cocaine use. Each participant received CBT along with the psychopharmacological or placebo treatment. Results were measured through urine analysis and self-report. Pramipexole did not reduce cocaine use significantly compared to the other treatment conditions (Ciraulo et al., 2005).

Anti-Parkinson medications have a mechanism of action that work as either an agonist or precursor to dopamine (Schmitz et al., 2008). Because of this action, it has been hypothesized that these medications will have a significant effect on the treatment and reduction of cocaine use in individuals who are dependent on cocaine. However, after examining Levodopa, Levodopa-carbidopa and pramipexole there have mixed results that indicate that these psychopharmacological agents could be affective, in combination with behavioral therapy, in the treatment of cocaine dependence.

K. Anti-Psychotic

Atypical antipsychotic medications are a second generation of antipsychotics that work with the dopamine system, although the specific mechanism of action for these medications is unknown, and differs between each one (Reid et al., 2005b). One anti-psychotic in particular, olanzapine, has an unknown mechanism of action, but has been hypothesized to be a dopamine antagonist. This type of psychopharmacological agent can be effective in the treatment of cocaine dependence because it would potentially assist in cocaine cravings of those individuals that are dependent. One study utilized examined the efficacy of olanzapine compared to three other psychopharmacological treatments and a placebo condition. A total of 68 participants were randomly assigned to one of the five medication treatment conditions, and each participant received CBT counseling throughout the treatment. Efficacy of the treatment conditions was measured through urine analysis collected from the participants. Results demonstrated that there were no significant changes across treatment conditions in the reduction of cocaine positive urine samples. These results indicate that the use of olanzapine is not efficacious, when combined with CBT, in the reduction of cocaine use in those that are dependent on cocaine.

L. Arterial Obstruction

Pentoxifylline is a phosphodiesete inhibitor that increases blood flow and is mainly used to treat patients with muscle pain or fatigue. It is hypothesized to be effective in the treatment of cocaine dependency, as it will suppress the self-administration of cocaine in those who are dependent. This psychopharmacological agent was compared to four other medications and a placebo condition, combined with CBT in the efficacy of reducing cocaine positive urine samples. Results indicated that over the 8-week trial, pentoxifylline, combined with CBT, did not have a significant effect on reducing cocaine use in participants who are considered cocaine dependent. These

results refute the overall hypothesis and there was no significant difference between psychopharmacological and placebo treatment conditions (Ciraulo et al., 2005).

M. Dementia

Hydergine is a medication used to enhance cognition and improve memory in those patients that suffer from dementia. Although the mechanism of action for hydergine is unknown, it is assumed that it increases cerebral glucose metabolism in the cortex, enhance cerebral blood flow, and effect selective dopamine receptor sites. In an experimental study over 8 weeks, cocaine dependent participants were randomly assigned to one of four treatment conditions, including a placebo treatment; each treatment conditions had 15 participants. Each participant also underwent CBT throughout the trial. Measurements of the efficacy of the psychopharmacological agents were assessed through urine analysis and self-report. Results indicated that the participants in the hydergine treatment condition produced more negative cocaine urine samples compared to the placebo, however the difference was not significant. Hydergine, however, was the only medication out of the three tested in this experiment to demonstrate a decrease in urine positive results, although not significant. These results lead to the conclusion that hydergine should be further examined, in combination with behavioral therapy for the treatment of cocaine dependent individuals (Shoptaw et al., 2005).

N. Hyperprolactonemia

Hyperprolactonemia is a disease that causes excessive levels of proactin in the blood. Webster et al. (1994) states that cabergoline is mainly used to treat those with this disorder and has been shown to bind to dopamine receptors and inhibit the release of proactin secretion (as cited in Shoptaw et al, 2005, p. 79). The use of cabergoline has also been effective in the improvement of motor functions for those suffering from Parkinson's disease. In a study comparing cabergoline to two other medications and a placebo condition, 60 participants were randomly assigned into four treatment conditions. Each treatment condition received CBT treatment as well, and urine samples were utilized to assess the efficacy of the psychopharmacological treatments in comparison to the placebo condition in reducing cocaine use in the participant pool. Results indicated that the cabergoline treatment condition produced significantly less positive urine samples (55.8%) compared to the placebo condition (38.6%; $F = 4.02$, $df = 3$; $P = 0.02$). These results are significant and indicate that cabergoline could be an effective treatment, combined with CBT, in the treatment of cocaine dependency by reducing use among participants. Although this study indicated that results were significant, more research in order to replicate this study should be completed (Shoptaw et al., 2005).

O. Muscle Relaxant

Baclofen is a psychopharmacological agent that is a GABA receptor agonist that is a skeletal muscle relaxant. It is most commonly prescribed for multiple sclerosis, spinal cord disease and injury. Baclofen is hypothesized to effect

cocaine-evoked dopamine release, therefore would be able to reduce cocaine use and cravings in those that are dependent on cocaine. A study comprised of 160 subjects was divided into two treatment conditions, one placebo condition and one where the participants received baclofen. Every participant despite treatment condition also received weekly CBT therapy for one hour. This study was 8 weeks long and results were measured through urine samples. Results of the urine analysis indicated that baclofen did not have a significant impact on the amount of cocaine-negative urine samples compared to the placebo condition. Overall this particular psychopharmacological agent, combined with behavioral therapy did not have a significant impact on cocaine use in individuals that are dependent, however muscle relaxants should not be ruled out completely for the treatment of cocaine dependence (Kahn et al., 2009).

P. Vitamins and Supplements

The use of vitamins and supplements in the treatment of cocaine dependency has not been widely tested. Many researchers focus on those psychopharmacological agents that are produced by drug companies to treat major illnesses. However, it is worth examining these vitamin and supplements to see if there they have any impact on cocaine use in those that are dependent.

Chronic cocaine use may impact cell membranes and lead to damage, a consequence of mitochondrial dysfunction, which is caused by impairment of cerebral metabolism. Coenzyme Q10 (CoQ10) is a substance that is found in the mitochondria and is an important component of the electron transport chain, participating in aerobic cellular respiration. Corwin et al., 1998 argues that without CoQ10, there can be mitochondrial dysfunction, which could possibly lead to cell membrane damage, which would in turn cause neuronal degeneration (as cited in Reid et al., 2005b, p. 45). This degeneration could then cause neuropsychological deficits, leading to the reduction in impulse control in those that use cocaine frequently. Therefore, the CoQ10 supplement is tested for efficacy in the ability to reduce cocaine use to determine its efficacy in treating cocaine dependence. CoQ10 was tested along with two psychopharmacological agents and a placebo condition. Participants were randomly assigned to one of the four conditions and received simultaneous CBT treatment. Results indicated that there were no significant differences in the CoQ10 treatment condition compared to the placebo condition regarding negative cocaine samples. The self-reported cocaine use however decreased in all treatment groups but there were no significant differences between conditions. The use of CoQ10 in combination with behavioral therapy was not shown to be effective or supportive of the overall hypothesis that a combination of pharmacotherapy and behavioral therapy would be more effective in the treatment of cocaine addiction than the behavioral therapy treatment alone (Reid et al., 2005b).

Cocaine has been shown to alter the serotonin levels in the brain as well as the dopamine levels. Although many studies have looked at the results of altering the dopamine system, research on the serotonin system is equally important. Tryptophan, an amino acid that is ingested has been shown to increase production of serotonin.

Therefore, tryptophan has the potential to assist in the treatment of cocaine dependency, when combined with behavioral therapy in order to reduce cocaine use, which is argued by Herges & Taylor, 1998 (as cited in Jones et al., 2004, p. 422). This particular study by Jones et al. (2004) uses CM in combination with tryptophan to treat cocaine dependence. This 2x2 study tested tryptophan versus placebo conditions and contingent vouchers versus non-contingent vouchers. Participants were randomly assigned to one of the four treatment conditions for a 16-week trial. Results were measured through urine samples that were collected three times per week. The results indicated that there was no significant difference in the medication treatment condition compared to the placebo. However, there was a significant difference between therapy conditions. The contingent voucher therapy yielded less positive urine results compared to the non-contingent voucher therapy. Although these results do not support the overall hypothesis, they do reinforce the idea that CM is an effective type of behavioral therapy, and when combined with an efficacious psychopharmacological agent can be a successful treatment for cocaine dependency.

Dehydroepiandrosterone (DHEA) is an adrenal steroid hormone that is available over the counter in the United States. Shoptaw et al. (2004) looked at the efficacy of DHEA in the treatment of cocaine dependency. Fifty-seven participants were randomly assigned to either receive DHEA 100 mg per day or a placebo treatment. Along with the medication, participants received cognitive behavioral group therapy three times per week. The hypothesis of this article states that if cocaine dependence is treated with DHEA combined with behavioral therapy, then there would be less positive urine samples than those who received the placebo and cognitive behavioral therapy treatment. The results of this article indicate that there was a significant difference between conditions. The placebo group had significantly more cocaine negative urine samples (70.6%) than the DHEA treatment condition (26.8, $p < .01$). This strongly refutes the overall hypothesis as the psychopharmacological and behavioral therapy treatment condition produced less negative urine samples than the placebo and behavioral therapy condition. DHEA has shown to be not effective in the treatment of cocaine dependence and directly refutes the overall hypothesis.

Discussion

A. Summary of Findings

Overall the findings varied amongst all of the empirical studies reviewed. Some types of psychopharmacological agents were shown to be effective, when combined with behavioral therapy in the treatment of cocaine dependency while others did not demonstrate a significant difference between the placebo and treatment conditions (Appendix A). Although there is a greater number of medications that had no significant impact on the reduction of cocaine use than those that reduced cocaine use, the implication that some psychopharmacotherapies assisted in the reduction of cocaine use is vital in the formulation of a protocol for treating cocaine dependence. The results of this conceptual meta-analysis lead to implications that if a cocaine

dependent individual is treated with a combination of psychopharmacotherapy and behavioral therapy, then that person will be more successful in reducing dependency than if only treated with behavioral therapy.

1. Support

The psychopharmacological treatments that were most effective in reducing cocaine use were the monoamine and GABA enhancers. These medications have a mechanism of action that increases the monoamine and GABA levels in the brain. For example, methamphetamine reduced cocaine use based on urine analysis (Mooney et al., 2009). This medication was one of the more effective medications that was tested with behavioral therapy, compared to the placebo condition. Mooney et al. discovered that sustained release tablets of methamphetamine significantly reduced cocaine use among participants (2009). Another psychopharmacological agent, modafinil, which is a wake promoting agent that releases dopamine and norepininephrine into the brain, was also considered highly effective in the treatment of cocaine dependency, which was demonstrated within two experiments (Anderson et al., 2009; Dackis et al., 2005).

Anti-convulsant medications, that are traditionally used to treat seizures, were also efficacious in reducing cocaine use throughout multiple studies. The most successful of these medications was tiagabine and topiramate, which both are hypothesized to increase GABA through transport inhibition (Gonzalez et al., 2007; Kampman et al., 2004; Winhusen et al., 2005). Although the mechanism of action for anti-convulsant medications is not specifically known, it is predicted that they increase GABA levels. These GABA levels are vital in order to control impulses for those that are dependent on cocaine, therefore reducing times of cocaine usage which will lead to a successful treatment (Alvarez et al., 2009).

Levodopa, a medication used to treat Parkinson's disease, is proven to be a precursor for dopamine levels, and has also shown efficacy in the treatment of cocaine dependence (Schmitz et al., 2008). Overall those medications that impacted GABA and monoamine neurotransmitters in the brain were most effective in reducing cocaine use in those that are dependent.

2. Refute

Along with support for the hypothesis there are also numerous psychopharmacological options that, when combined with behavioral therapy, do not have a significant impact on the treatment of cocaine dependence. Two separate types of refutations were revealed. The first of these refutations is the treatment conditions that had no significant effect on reducing cocaine use, compared to the placebo condition when combined with behavioral therapy. Often times the placebo and treatment conditions led to a decrease in cocaine use, but the difference between the two conditions was not significant (CITATION). The second type of refutation is less common, and implies that the use of psychopharmacotherapy with behavioral therapy increased the amount of cocaine use

within the individual compared to the placebo condition. This is a direct contradiction to the hypothesis and therefore is the strongest of the refutations.

The medications that refute the hypothesis, by not demonstrating a significant change in cocaine use include; muscle relaxants, dementia medications, anti-inflammatories, MAOI's and SNRI's. There were no medications within these categories that were shown to be significantly effective. An example of this is seen in a study conducted by Reid, Cadastrone et al., (2005) when celecoxib, an anti-inflammatory, was assessed for efficacy in treating cocaine dependence. Both of the conditions, the placebo and experimental, reduced levels of cocaine use, however the difference between the two conditions was not significant.

One psychopharmacological treatment yielded results that directly disproved the hypothesis and yielded more cocaine use among individuals. The androgenic steroid, dehydroepiandrosterone, increased cocaine use among participants that were in the experiment condition compared to the placebo (Shoptaw et al., 2004). This psychopharmacological agent directly challenges the hypothesis. This psychopharmacological agent disproves the hypothesis as it demonstrated an increase in cocaine use among participants. Therefore this medication treatment is a strong refutation of the overall hypothesis that a combination of psychopharmacological and behavioral therapy will be more successful in the reduction of cocaine use, compared to behavioral therapy alone.

In summary of these findings, medications that enhance monoamine and GABA levels in the brain have been efficacious in the treatment of cocaine dependency, as measured through the reduction of cocaine use in participants that are dependent.

B. Strengths and Limitations

Each study included the strengths and weaknesses specific to the measurements, participation sizes, dropout rates and other various items. However many of the studies had similar strengths and weaknesses, which are important to note in the evaluation of these treatments so as to determine which type of treatment should be used to eliminate cocaine dependency, when combined with behavioral therapy.

1. Strengths

Throughout the various empirical studies, there have been numerous strengths indicating that results could have an impact on how cocaine dependency is treated in the future, one of the main strengths was the sizes of the studies. Sample sizes varied across all studies. It is important to have a large enough participant number in order to be able to assess the results in terms of the population that the sample is taken from.

The study that tested the efficacy of selegiline transdermal system had the largest sample size throughout every empirical article researched (Elkashef et al., 2006). Three hundred participants, in total, were divided into two

groups and each treatment condition had 150 subjects. Results indicated that selegiline was not effective, in combination with behavioral therapy, for the treatment of cocaine dependency. This large sample size is strongly supportive of the valididty of these results. With a large sample size the results are more easily applicable to the general population.

Another empirical study utilized six different testing sites to test Modafinil, an anti-narcoleptic (Anderson et al., 2009). This study recruited 35 participants per site, totaling 210 individuals recruited. This sample size was large, compared to many other similar studies, and was very diverse due to the six different locations that this study was performed at. This sample size was considered to be adequate for the study, and was larger than most others in this area of research.

An experiment, that tested the efficacy of reserpine, divided 119 participants that met the criteria for cocaine dependency into two different groups, experiment or placebo treatment conditions (Winhusen et al., 2007). The use of a large number of participants and dividing them into only 2 treatment conditions was a strength in this study as more people were examined for the efficacy of reserpine in the treatment of cocaine dependency.

Besides sample sizes, it is important to look at the length of each treatment study. Recovery from addiction is a long process, and many of the psychopharmacological agents that are utilized can take multiple weeks before participants begin to benefit from the effects. All of the articles reviewed were longitudinal studies, and the participants were treated with the behavioral therapy and psychopharmacotherapy everyday for multiple weeks.

One study in particular, completed by Bisaga et al. (2006), was over a duration of 16 weeks. The 16 weeks of treatment allowed for the adjustment of medication levels within the experimental group. By monitoring the participants for 16 weeks, researchers are able to gain a stronger measurement of cocaine use and efficacy of the treatment conditions. The length of this study allowed for the randomization and adequate testing of the effects of the medication.

A handful of studies also had long treatment or maintenance therapies. Averaging around 9.26 weeks of treatment for all 22 empirical articles reviewed. A second trial, conducted by Jones et al. (2004), was also conducted over 16 weeks. Over the duration of the trial, participants received CM vouchers, psychopharmacological treatment, and urine testing twice a week. This longer study is more effective in truly assessing the efficacy of behavioral therapy along with psychopharmacotherapy. Researchers are able to measure the participants over a long period of time, so as to record any significant changes as the medication and therapy have time to positively, negatively, or not affect the participant at all. The average number of weeks that the studies lasted, 9.26, is a strong point to the overall results. Trials are long enough to allow participants to settle into the treatment group that they were randomly assigned to, in order to accurately assess their progress over the trial time.

Another major strength to the conceptual meta-analysis is the outcome measures that were used in each study. Every study utilized urine testing to determine use of cocaine by the participants. Many trials also examined the self-reports of the participants, collected frequently, to assess the efficacy of the different treatment conditions. The fact that urine was tested in each study indicates a consistent similarity in order to compare the different studies; therefore this is a direct way of measuring each experiment's outcome in comparison to the others. It is important to have the same, or similar outcome measures in order to be able to determine how the studies relate to each other and what the best type of treatment for cocaine dependency is.

Finally, another strength emerged that was only seen in two articles. This strength was the assessment of two or more different types of behavioral therapies in comparison to each other, along with the analysis of the psychopharmacotherapy compared to placebo conditions. Moeller et al. (2007) utilized a 2x2 design to test the interaction difference between treating a patient with CBT versus CM and medication versus placebo conditions. Participants were divided up into one of four treatment conditions, to allow the researchers to determine what was the most effective type of behavioral therapy. Researchers could also determine if the treatment was more effective when it was combined with psychopharmacotherapy.

Schmitz et al. (2008) utilized a 2x3 design to compare the effects of the psychopharmacotherapy administration across three types of behavioral treatments. The three types of behavioral treatments consisted of clinical management only, clinical management plus CBT, or clinical management combined with CBT and CM. This allowed researchers to determine the effects of adding different types of behavioral therapies to the psychopharmacotherapy treatment in reducing cocaine use among those that are dependent.

Although only two of the studies designed experiments that compared the differences between behavioral therapies, they are important to note in the strengths of the overall meta-analysis because they can help determine in the future, which type of behavioral therapy combined with psychopharmacotherapy is most effective in treating cocaine dependence.

2. Limitations

Despite numerous strengths, there were also several limitations to the overall meta-analysis. These limitations may impact the overall results, yet with more research there could be advances in determining the best overall treatment for cocaine dependency.

A prominent limitation across all studies was the dropout rates as well as the missed urinalysis appointments by the participants. Dackis et al. (2005) completed a study that tested the efficacy of Modafinil in treating cocaine dependence and had a significant drop out rate. The total percentage of participants that completed the study was

64.5%. Drop out rates are extremely high in cocaine studies as they are longitudinal studies that require numerous visits per week. Many other studies suffered from this limitation.

Many of the studies contained a small number of participants, therefore limiting the real world implications of the findings. One study, in particular, had 23 participants total complete the study (Reid, Angrist et al., 2005). This small sample size was then divided in half when randomly assigned to treatment conditions. The experimental condition had 10 participants, compared to the placebo condition with 13 participants total. This small sample size is a limitation of the study because each individual has a strong impact on the data, easily manipulating the overall results. More research should be conducted in order to eliminate this limitation with a greater number of participants per treatment condition.

Many of the studies that assessed more than one psychopharmacotherapy in comparison to others had small sample sizes per treatment condition. Berger et al. (2005) divided participants in order to test 3 medications and one placebo condition. Therefore when the study began with a relatively average sample size of n=60 the number has an inverse relationship with the number of treatment conditions. This study divided the 60 participants into 4 groups of 15 participants per treatment condition. Each group contained a small sample size, which places limitations on the ability to assess the efficacy of the psychopharmacological agents compared to each other. When the sample size is small, as it is in a few of the studies analyzed, the results become harder to generalize to the large cocaine dependent population in the real world.

Due to the fact that little is known about the best pharmacological treatment option to use for cocaine dependency, some of the empirical articles experimented with more than one category of medication in the trials. This can complicate results, as there is no focus on one type of medication and therefore results are sporadic. Ciraulo et al. (2005) used 5 medications and one placebo condition. These 5 different medications are in different medication categories, and are used to treat an array of ailments. Therefore, results are more difficult to generalize.

Behavioral therapy in this meta-analysis includes two types of therapies, CBT and CM. Due to the broad definition of behavioral therapy, one of the limitations discovered when reviewing the empirical articles was the unbalanced use of both therapies. Many more studies utilized CBT than CM, therefore the efficacy of combining medication with behavioral therapy is limited. There is a possibility that utilizing both behavioral therapies could be more effective than limiting it to one. Another important aspect that limits the ability to compare studies is the number of sessions of behavioral therapy and how the session was operated. Each trial utilized behavioral therapy, however there would be a discrepancy between number of session of therapy. For example one study supplied participants with behavioral therapy three times per week (Shoptaw et al., 2004), compared to another study that treated a participant once a week (Shoptaw et al., 2005). This discrepancy is a limitation in determining the efficacy

of behavioral therapy combined with psychopharmacotherapy compared to behavioral therapy alone in the treatment of cocaine dependency.

C. Conclusions

Overall, results indicated that psychopharmacotherapy and behavioral therapy are shown to be effective when used together. The psychopharmacological agents that have been most effective are the monoamine and GABA enhancers. These include anti-convulsives, methamphetamine, wake-promoting agents, and other medications that enhance GABA and other neurotransmitters such as norepinephrine, serotonin, and dopamine. Cocaine has a very complicated mechanism of action and therefore medications that have an effect on various neurotransmitters are often efficacious in the treatment of cocaine dependency (Streeter et al., 2005). The mechanism of action of cocaine is known, and a trend appears in the types of psychopharmacotherapies that are effective in treating cocaine dependency when combined with behavioral therapy. However, there is no definitive category of medication that seems to be most effective.

D. Impact

Cocaine dependency is a problem across America. The numbers of dependent individuals is astronomically high. The use of cocaine has a negative effect on society and the individual who is abusing the drug. There are numerous problems due to cocaine dependency and these include costs of treatment, social complications, the spread of infectious diseases, and cocaine related crime and violence. By reducing the cocaine dependent population, the problems that this behavior creates will lessen. The results of this meta-analysis indicate that, with more research, there can be a protocol developed to treat cocaine dependency. With this protocol, people will be able to gain better care and treatment and therefore reduce the problems that society faces due to cocaine use.

Throughout the empirical articles researched, a trend developed. This trend is significant to the scientific community to focus future research looking at psychopharmacological treatment options. Researchers have examined numerous medications to treat cocaine dependence, along with behavioral therapy, and this research is combined and organized in a way that could assist those who are conducting experiments with participants. With a focus, research time and money could be reduced when searching for an effective treatment for cocaine dependence.

E. Future Directions

Because of these findings, there is a necessity for the continuation of research on psychopharmacotherapy options that can be added to behavioral therapy treatments to help those who are cocaine dependent. Some medications were efficacious, when combined with behavioral therapy, in reducing cocaine use and therefore this avenue needs to be tested further across different populations, sample sizes, and degrees of dependency.

Further research should focus on specific categories of medication. As stated earlier, one of the limitations was testing the broad range of psychopharmacological categories instead of focusing on one in particular. A study within each medication category, that has shown efficacy, would be beneficial to the overall goal of developing a psychopharmacological agent that could treat cocaine dependency. For example, various SSRI medications should be tested in the same trial in comparison to each other. Instead of spreading out among different medication categories in one study, focusing on a specific medication will help assist researchers in discovering which mechanism of action category is effective in reducing cocaine use. This could further the narrowing down of the broad psychopharmacological spectrum to a certain type, or category, of medication that is most successful.

Two studies that were reviewed focused on testing the types of behavioral therapies, in conjunction with psychopharmacotherapies, in order to determine the best outlet for treatment. Although behavioral therapy is currently the most effective and widely used treatment to treat dependency, one type of behavioral therapy could be more effective, when combined with medication than the others.

Behavioral therapy is a successful option in the treatment of this dependency, however there is a strong potential for the use of psychopharmacotherapy in conjunction with behavioral therapy to more effectively treat cocaine dependence.

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