Drug Response-Related DNA Methylation Changes in SCZ, BP, and MDD.

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DNAm has been proposed to be involved in both the pathology and drug treatment of these disorders (Schroeder et al., 2012; Vialou et al., 2013; Jaffe et al., 2016). Emerging data indicates that DNAm could be used as a predictor of drug response for psychiatric disorders.

Authors performed a systematic review to evaluate the reproducibility of published changes of drug response-related DNAm in SCZ, BD and MDD. 37 publications

1. Medication-induced DNAm changes (n=8)
2. the relationship between DNAm and clinical improvement (n= 24)
3. comparison of DNA status across different medication (n= 14).

Findings: Only BDNF was consistent with the DNAm changes detected in 4 independent studies for MDD. It was positively correlated with clinical improvement in MDD BDNF DNAm is promising as a predictor of antidepressant txt response for MDD.

What are the future research strategies, including experimental , analyatical procuedures and statistical criteria?

Background:

Severe psychiatric disorders, coferring lifelong disability. A majority of these psychiatric disorders receive medication as the first-line treatment, (Pratt et al., 2017)

Therapeutics for SCZ, BD, and MDD are generally based onsimilar classes of molecules, targeting similar pathways, with distinct doses and proper combinations. However, drug selection is clinically subjective and treatment typically requires weeksof symptom evaluation to determine treatment efficacy. A largeproportion of patients fail to respond to first-line drug treatment. This illustrates the importanceof developing biomarkers that can support decisions regardingoptimal drug choice, and identify likely poor responders asquickly as possible. This emphasizes the need for a betterbiomarker-based stratification of patients that could facilitatetreatment planning.

Pharmacogenetic approaches for guiding the treatment of psyiatric disoders has been a rapidly expanind area of research in the last two diecades (Nelson etal ., 2016) The main hypothesis of these studies was that genetic variats could predict the influence of drug treatment. Numerous pharmacogenetic studies have investigated the genetic contribution to the treatment reponse of these disorders. (Bau et al, 2008, Drandl et al., 2016, Shi et al, 2017, Yu et al., 2018)

Although psychiatric disorders are highly heritable, gene-environment interactions are relevant, and results of pharmacogenetic studies have been inconsistent, limiting the clinical utilization of this approach.

Emerging evidence suggests that epigenetic marks cold be used as a apredictor of drug response for psychiatri disorders (Chan and Baylin, 2012, Heerboth et al,m 2014). Dysregulation of epigenetic events can be pathological, and associated with SCZ, BD, and MDD (Mill et al 2008, Sabunciyan et al., 2012, Guintivano et al., 2013, Chen et al., 2014, Liu et al, 2018). This implies that biological pathways and cellular processes are under the impact of epigenome status. Unlike genetics, epigenetic status is dynamic and could better reflect various environmental events during disease progress and drug treatment. Because of the reversibility of epigenetic events, could the modulation of epigenetic regulators be valuable for therapeutic potential?? How do we understand periods of remission in terms of this reversibility or dynamic behavior?

DNAm is the product of the interaction between genetic variants and environmental influence, and for this reason might be a better predictor of txt outcomes (Kubota et al,m 2012; Moore et al., 2013).

Drugs may exert their effects by reversing these DNAm deregulations. Clozapine and sulpiride, have demonstrated the ability to activate brain DNA demethylation (Dong et al., 2008). Therefore, DNAm is a promising molecular approach to study mechanisms and prediction of drug response in psychiatric disorders (Goud Alladi et al., 2018, Lisoway et al., 2018)

1. reproducibility of reported changes of DNAm-related genes associated with drug response in each disorder – they examined the “stability” of the reported changes whereby stability is defined as the presence of significant results ( p < 0.05)
2. can the results of candidate gene studies be reproduced in GWAS –

Pharmacoepigenetics research in psychiatry focuses on the effects of txts on DNAm and their potential influences on txt response. The main aim of pharmacoepigenetics is the identification of DNAm of specific genes that are associated with txt outcomes of psychiatri disorders, with the ultimate goal of translating this information into strategies that can support personalized medicine.

Methyl groups are added to DNA nucleotides, primarly cytosine, and adenine. DNAm has a prominent influence on the structure and functions of DNA, particularly in the regulation of gene expression. However, DNAm regulates gene expression differently according to its genomic context.

1. DNAm levels of CpG sites near transcription start sites repress gene expression levels, while DNAm levels of gene body CpGs could activate the expression of their target genes (Jones, 2012; Moore et al., 2013)
2. Conflicting results were reported regarding intergenic CpGs: come claimed that they might regulate gene expression less frequently (Chen et al., 2014; Wagner et al., 2014; Liu et al., 2018), while others suggested

DNAm observed at non-CpG sites (CpA, CpT, and CpC in mammals (Pinney, 2014) although CpGs are the primary sites for DNAm. Non-CpG methlationsites are common in brain (Lister et al., 2013; Guo et al., 2014). The functions of non-CpG m are still largely unknown.

Given that DNAm signals can be tissue-specific, it is important to establish the pros and cons of different types of tissue for investigation (Mill and Petronis, 2007).

Correlations of DNAm in blood and multiple regions of the brain – inconsistent results (Horvath et al. 2012, Walton et al., 2016) – but look at the hypercoordinated paper

Which tissue is more representative? Braun et al (2019) performed genome-wide DNAm comparison across the human brain, blood, saliva and buccal cells. They proposed that todetermine the optimal surrogate tissue for representing brain DNAm, the DNAm patterns specific to the genomic region ofinterest between these two tissues must be considered (Braunet al., 2019). More importantly, the proxy tissue should havesimilar responsive changes to environmental influences such asdrugs. Building a reliable peripheral-brain relationship will becritical in proving the biological relevance and illustrating theunderlying biological mechanisms of availablein vivohumantissue for clinical application.

Research strategies:

1. Target analyses for candidate genes
2. The discovery of genome-wide DNAm with microarrays,
3. The assessment for global methylation
4. Methylated DNA immunoprecipitation sequencing (MeDIP)
5. Reduced Representation Bisulfite Sequencing (RRBS)
6. Whole-genome bisulfite sequencing – gold standard for measuring CpG and non-CpG methylation, but very expensive.

Read Moran, 2016 for info regarding EPIC for methylation profiling.

Research strategies:

1. Candidate gene method: focus on a limited number of genes, bases on their established association with a disorders´pathogenesis or hypothesized relation to response to drug treatment. most studies had limited statistical power, a concern especiallyfor dense array data. Because of this limitation, the candidategene method can provide little comprehensive understandingregarding the mechanism of action of drugs, but can onlyconfirm previous knowledge or test relevance of a particular genemethylation (Harrison, 2015).
2. Array These genome-wideanalyses offer a non-biased experimental approach to identifynovel candidates (Kurdyukov and Bullock, 2016), but wefound only 3 studies that that implemented genome-wideanalysis, using microarrays to evaluate the drug response.These studies identified several loci associated with clinicalimprovement, almost all of which were not previouslypredicted to be relevant. Unfortunately, genome-wide DNAmmeasurement faces two challenges, first its cost (added tocosts of a clinical trial) and second, the challenge of havinglarge samples in a study of patients receiving controlledtreatment regimens. These problems limit the size of datasets, and therefore statistical power, for array analysis of drug-related treatment effects. However, genome-wide approachesusing a microarray do provide more comprehensive dataand could identify novel and unexpected DNAm effects(Barros-Silva et al., 2018).
3. GlobalDNAm reflects the DNAm status of total genomic content within a sample. Several methods exist to assess global DNAm status, including enzyme-linked immunosorbent assays (ELISA), theuse of a previously validated protocol of Linear InterspersedNuclear Element 1 (LINE- 1) and the use of restrictionenzymes (Burghardt et al., 2020). However, this approach onlyassesses DNAm changes at the genome level, and thus doesnot further provide data on region-specific DNAm changes tocharacterize critical regulatory regions (e.g., CpG islands and promoter regions)

Response is definedas a clinically meaningful reduction in symptoms (e.g., areduction of at least 50% in baseline symptom levels) (Aaronsonet al., 2017).

Results for MDD – which means that work still needs to be done for SCZ and BP

Twenty-one candidate genes were evaluated and DNAm of 12genes showed a significant correlation with clinical improvement(p<0.05). Only BDNF (Carlberg et al., 2014; Tadi ́c et al.,2014; Lieb et al., 2018; Wang et al., 2018b; Hsieh et al., 2019;Wagner et al., 2019), 5-HTT (Kang et al., 2013; Domschke et al.,2014; Okada et al., 2014; Iga et al., 2016), and HTR1B (Gassóet al., 2017; Wang et al., 2018a) were assessed in multiple MDDstudies. No replication efforts were found in studies of othermajor psychiatric disorders. Baseline DNAm levels of BDNFin MDD patients in both blood and leukocytes was positivelycorrelated with remission of depressive symptomatology afterantidepressant treatment in 4 of 6 independent studies (Tadi ́cet al., 2014; Lieb et al., 2018; Wang et al., 2018b; Hsieh et al., 2019).

Try to understand the difference of nominal p values and correction for multiple testing

Failure to replicate – difference in platforms, limitations in statistical power, 450K did not have the same sites for escitalopram than EPIC does

We did find one study involving schizophrenia, whichevaluated the correlation between DNAm changes in blood after1-year of clozapine treatment and changes of PANSS scores for 21SCZ patients (Kinoshita et al., 2017). They found that DNAm ina site located at the CREBBP gene was negatively correlated withclinical improvement after multiple testing correction. However,this finding remains to be replicated. Further analysis showed thatnone of these significant candidate genes tested for antipsychotics(e.g., HTR1A, CYP3A4, COMT), especially clozapine, reached anominal significance in genome-wide analysis.

However, the drug medications for eachdisorder are not mutually exclusive, as antipsychotic drugs mayalso be used in BD and MDD patients (Cruz et al., 2010; Patkarand Pae, 2013; Poo and Agius, 2014). The therapeutic efficacyof individual drugs is quite variable, and treatment trials toestablish efficacy are laborious, so establishing biomarkers toimprove guidance for treatment planning of individual patientsis an important aim of psychiatric research.

Future –

Insufficient statistical power is a limitation in psychiatric studies of DNAm

cost of using methylation panels, the high cost of clinicaltrials where drug therapy is controlled along with diversedrugs used across studies, and the large number of sites formethylation investigation, together conspire to limit samplesizes and statistical power to detect effects of interest. These factors limit our ability to evaluate reproducibility of current pharmacoepigenetic findings. Although several interesting associations between DNAm and drug treatment outcomesoccurred in relatively small sample sizes, findings from thesestudies should be considered preliminary until replication in alarger sample size is pursued. So, given that collecting clinical data with a large-sample is challenging and costly, small-sample studies remain the focus of most investigators in this area viahypothesis-generating studies.

To fill this gap, multi-site collaborations with ethnicallydiverse groups are needed in studies of the pharmacoepigeneticsof psychiatric disorders. Prominent consortia have beenestablished which looked at pharmacogenomics in psychiatricdisorders, like the STAR∗D project (Rush et al., 2004), theGenome-based Therapeutic Drugs for Depression (GENDEP)project (Uher et al., 2010), the Chinese AntipsychoticsPharmacogenomics Consortium (CAPC) (Yu et al., 2018),and others. These consortia benefit ongoing and futurepharmacoepigenetic studies by providing preliminary findingsthat can be examined in new samples. For example, Powell et al.(2013) found that DNAm in IL11 could predict the clinicalresponse to antidepressants using samples from the GENDEPproject. Large international consortia, as have been developedfor genetic association studies, are needed to study adequatenumbers of patients with samples collected using a uniformprocedure and with standardized drug treatment.

Recommendation for Clinical Design:Drug Selection, Treatment Duration,Evaluation, and Tissue Selection forPharmacoepigenetic StudiesNeuropsychiatric drugs are grouped into various classes withslightly different mechanisms of action. It is clear that froman efficacy perspective, first line treatments for all thesedisorders leaves considerable room for improvement, potentiallyin new drug development and in strategies for personalizedmedicine (Leucht et al., 2012; Cipriani et al., 2018; Huhnet al., 2019; Pillinger et al., 2020). Identification of new biomarkers for these disorders is difficult, primarily because of the lack of knowledge about disease pathophysiology and mechanisms of drug action.

Only 8% of studies used monotherapy forpatients. While 27% of studies claimed to use monotherapy for patients,but we noticed that besides thesame

Definition of treatment outcomes for psychiatric disordersis also a challenge, as different approaches may be used acrossstudies. A recent study suggested that integrating genomicsand phenotypic measures data could increase the accuracy ofprediction of drug response (Kauppi et al., 2018). Thus, futureresearch should consider an integrated examination of genetic,DNAm and dense phenotype assessment of drug effects not only with behavioral ratings but with direct in vivo assessment of brain physiology and treatment (Huang et al., 2019). Notably, all ofthese prediction models need to be

Predictors of drug response for psychiatric disorders ideallyneed to have biological relevance based on a solid mechanisticunderstanding of the pathophysiology of brain dysfunction.

Candidate gene studies contributed to answering specific questions in biology, but they do not provide system-wide information.

In this review, we noticed that the assessed candidate genes were generally not the top signals in genome-wide studies, and could not even reach a genome-wide significance. The genes that change the most between pre- and post-treatment, or are most strongly associated with clinical improvement, remain to be discovered. Also, pathway-based research rather than gene-based approaches may be more efficient. Different genes that converge on the same pathway may each contribute modestly, but together may robustly co-influence the functional abnormality. Therefore, system-wide research could provide better biomarkers than individual candidate genes.

Regulation of drug responses occurs at various levels including genetics, epigenetics, transcriptional, and protein modification, and also involves many functional pathways (Amare et al.,2017). To date, the underlying system of drug response-related

Data Analysis for Pharmacoepigenetic Studies

Strict statistical criteria and sufficient attention to covariances are critical for reducing false-positive rates. DNAm status isnot a constant like DNA sequence are therefore is subjectto influences from more confounders. Further, approachesfor DNAm assessment can be easily confounded by technical artifacts. DNAm levels are also affected by factors includingage (Horvath, 2013; Jaffe et al., 2016; Merid et al., 2020), sex(Maschietto et al., 2017; Xia et al., 2019), circadian rhythms(Lim et al., 2014), smoking (Shenker et al., 2013), drinking(Philibert et al., 2012), and others. All of these considerationsfor study design and data analysis need to meet standardrequirements for drug response-related DNAm studies as in mostgenomic studies.

Methylation profiling could be easily confounded by batcheffects. Systematic error can be introduced when samples areprocessed in multiple batches (e.g., the same sample measuredat different times), which cannot be eliminated unless allsamples are run in a single batch (Chen et al., 2011). It isespecially important that cases and controls not be put inseparate batches. Except for batch effects, positional effectsalso exist in the microarray and bias analysis (Jiao et al.,2018). Positional effects are emerging when the same samplein different physical positions on the array and could biasmethylation levels and lead to false findings. From our review, weobserved that only one study did the batch effects correction (Juet al., 2019), and none of these studies corrected for positionaleffects according to the method description in those papers.We cannot rule out the possibility that the data were properlyprocessed but failed to be reported in the papers, but not reporting such details at least indicated the lack of attention to the serious issues.Considering the influence of demographic information (e.g.,age, sex, BMI) is also important. In this review, nearly 49%of studies did not control for covariates in their analyses.When there was a correction for covariates, typically only sex and age was controlled. Since DNAm is highly cell-type-specific, cellular heterogeneity of blood may skew DNAm patterns, influencing findings in drug response for psychiatric disorders (You et al., 2020). All studies did not control cell-type compositions. Collecting data about potentially useful covariates,and controlling for them in analyses, should be standard practicein future research for drug response studies. However, we note that large samples will be needed to develop appropriate modeling for covariate effects, as a few outliers or complexcovariate interactions can exert effects that are challenging to deal