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Special issue Lithium: new observations on an old medication

Mini review: Recent advances on epigenetic effects of lithium

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Abstract

Lithium (Li) remains the first line long-term treatment of bipolar disorders notwithstanding a high inter-individual variability of response. Significant research effort has been undertaken to understand the molecular mechanisms underlying Li cellular and clinical effects in order to identify predictive biomarkers of response. Li response has been shown to be partly heritable, however mechanisms that do not rely on DNA variants could also be involved. In recent years, modulation of epigenetic marks in relation with the level of Li response has appeared increasingly plausible. Recent results in this field of research have provided new insights into the molecular processes involved in Li effects. In this review, we examined the literature investigating the involvement of three epigenetic mechanisms (DNA methylation, noncoding RNAs and histone modifications) in Li clinical efficacy in bipolar disorder.

Keywords

Epigenetics, lithium, bipolar disorder, response variability, methylation, non-coding RNA

1-Introduction

Bipolar disorder (BD) is a frequent (1-5% of the adult population), severe and burdensome psychiatric disorder defined by the recurrence of major depressive and (hypo)manic episodes interspaced by remission periods. The treatment of acute phases and the long-term prevention of recurrence mainly rely on mood stabilizers (anticonvulsants, atypical antipsychotics and lithium salts). Long-term therapy with lithium is the first line choice of treatment in BD and has proven efficacy not only in the prevention of mood recurrences but also in the reduction of suicide risk [1,2]. Naturalistic studies repeatedly showed that only 30% of the patients with BD are excellent responders during maintenance treatment while 70% of patients show various degrees of response to Li [3–6]. However, meta-analyses have shown that long-term Li efficacy (at least partial) was obtained in 47-60 % of individuals with BD [7,8]. Nevertheless, Li response can take up to two years to be characterized and the lack of predictive markers lead to lengthy trial and potential error processes, and/or delayed optimal care of patients [9].

The heritability of Li response has been evidenced by family and genetic studies [5,10,11]. Candidate-gene studies have been performed with more than 60 genes to identify those associated with Li response [12]. Unfortunately, most of the positive result obtained could not be reproduced in independent samples. Furthermore, large-scale genetic studies show that complex traits are characterized by the sum of modest effects of common genetic variants, supporting a polygenic nature of Li response variability [13]. The largest genome wide association study published so far, from The International Consortium on Lithium Genetic (ConLiGen), identified two long non coding RNAs (lncRNA) as associated with Li response in BD [14]. Furthermore, a recent transcriptomic analysis of whole blood samples from BD patients compared to non-affected controls identified 976 differentially regulated genes linked to Li treatment in the BD patients but only 6 differentially regulated genes linked to the BD diagnosis after accounting for medication use and cell type heterogeneity [15]. These results suggested that, beyond genetics, regulators of gene expression may also play an important role in Li response.

The best known mechanisms of gene expression regulation are epigenetic mechanisms, among which the most studied are DNA methylation, histone modifications and regulation by non-coding RNAs such as lncRNAs and miRNAs. All of them mediate the interplay

between genetic and environmental factors. Li-induced gene expression regulation of numerous genes has been evidenced both in cellular models and in preclinical models [16]. Interestingly though, several recent studies reported that Li can modulate epigenetic mechanisms at several levels of regulation. Moreover, preclinical and clinical evidences suggest that pre-existing or Li-induced epigenetic mechanisms may play a role in therapeutic response. In this mini-review, we will focus on the reported effects of Li treatment on DNA methylation, histone modification, miRNAs and their potential influence on the therapeutic response. The following search string was used in two different databases (PubMed, Google Scholar): (“epigenetic” OR “epigenomic” OR “DNA methylation” OR “DNA hydroxymethylation” OR “methylome” OR “histone acetylation” OR “histone deacetylation” OR “histone methylation” OR “microARN” OR “miRNA” OR “non-coding RNA”) AND (“lithium” OR “mood stabilizer” OR “valproic acid”) AND “bipolar disorder” leading to 228 articles that were screened for inclusion in this mini-review. Only peer-reviewed studies published in English were included. This search was complemented with examining references in identified articles. Table 1 summarizes the main findings of the articles included in this minireview.

2-DNA methylation studies

DNA methylation and hydroxymethylation on cytosine at CpG islands are the most studied epigenetic mechanisms and might be linked to modifications in transcriptional activity [17]. A Li-induced decrease of global DNA methylation was found in LCL from Li-responders BD patients (N=14) compared to control subjects (N=16) [18]. In another study of global DNA methylation performed in the leukocytes of BD patients, a significant decrease of DNA methylation level was observed in patients on lithium monotherapy (N=29), compared to untreated controls (N=26) or patients treated with a combination of lithium and valproate (N=32) [19]. However, the authors could not correlate lithium response level (as assessed with the Alda scale) with the global DNA methylation level [19]. Gene-specific methylation studies have also been undertaken to better characterize Li-induced effects. Since the *BDNF* gene has been repeatedly associated with the pathophysiology and symptoms of BD [20–23], its promoters have been the most studied. Incubation with lithium for 48 h was found to significantly decrease DNA methylation at the *BDNF* promoter IV in cultured rat hippocampal

neurons; in addition, simultaneous increase of BDNF mRNA levels was also observed [24]. The *BDNF* promoter I also seems to be targeted by Li treatment. Hence, patients treated with lithium (or valproate) displayed a trend [25] or a significant decrease [26] of DNA methylation levels at the *BDNF* promoter I as compared to other medications. This medication (Li and valproate)- induced decrease of DNA methylation was also found significant at the prodynorphin promoter [27].

The polygenicity of the variability of lithium response suggests that genome-wide studies allow a more integrative view of the biological pathways and mechanisms involved. At therapeutic concentrations, lithium carbamazepine, and valproate induced similar DNA methylation patterns at 117 genes in neuroblastoma cell lines SK-N-SH, in addition Li-specific DNA methylation modulation at 365 genes were also observed [28]. In this study the authors did not find any Li-induced DNA methylation modulation at the promoters of *BDNF*. Differential DNA methylation patterns were observed for quetiapine and valproate in a genome-wide DNA methylation study in blood samples from 172 patients with BD but no significant effect of Li treatment was found [29]. Recently, we performed a genome wide analysis of the peripheral blood DNA methylation profiles of 15 excellent responders and 11 non responders to Li prophylactic treatment. This led to the identification of a signature of 7 differentially methylated regions able to discriminate between responders and non-responders to Li [30]. The small sample size and retrospective design of this study does not allow definitive conclusions regarding the predictive value of this methylomic signature however these results provide candidates that can be tested in future prospective studies such as the R-Link H2020 funded EU-project (www.r-link.eu.com) [31].

3-miRNA studies

Translational repression through microRNAs (miRNAs) is another major epigenetic mechanism. Results from several preclinical and clinical studies suggest that Li may exert some of its molecular effects through this mechanism (Table 1). A genome wide miRNA analysis, on 2,563 patients characterized for their response to Li, investigated common variants at miRNA loci [32]. They identified 15 miRNAs significantly associated with two Li response phenotypes. Even if these results did not remain significant after correction for multiple testing, this genetic study suggest that miRNAs might be involved in Li response in

BD. In a model of mice exposed to cerebral ischemia, Li was found to increase miR-124 expression in brain [33]. In rat brain, chronic Li treatment has been shown to significantly decrease let-7b, let-7c, miR-105, miR-128a, miR-24a, miR-30c, miR-34a and miR-221 while miR-144 was increased in the hippocampus [34]. Among the potential effectors, regulation of *Dpp10* (dipeptidyl peptidase like 10), *Grm7* (glutamate metabotropic receptor 7) and *Thrb* (thyroid hormone receptor beta) were confirmed. It is important to note that miR-34a and miR-221 expression were also found regulated by Li in lymphoblastoid cell lines (LCLs) from individuals with BD [35]. Furthermore, the same study, combining genetics and epigenetics data, showed that the effects of Li on miR-221 and miR-34a were correlated with an inverse regulation of the expression of several of their predicted target genes. Hence, differential interactive networks of mRNAs and miRNAs modulated by Li were found in LCLs from Li responders vs non responders [36]. The same team reported that concomitant treatment with Li and valproate significantly modulated miR-222 levels in a neuronal culture system of rat cerebellar granule cells [37]. Finally, Li was found to decrease the expression of miR-4286 in human neural progenitor cells [38]. Indeed, expression levels of miRNAs have been linked to Li response, Pisanu and collaborators reported differential expression level of 31 miRNAs in LCLs from excellent responders compared to non-responders. These modulation were inversely correlated with 418 differentially expressed genes in the two groups [39]. Among those they could validate the modulation of miR-320a and miR-155-3p and three of their target genes: *CAPNS1* (Calpain Small Subunit 1), *RGS16* (Regulator of G Protein Signaling 16) and *SP4* (Sp4 Transcription Factor). These results suggest that few miRNAs could be proposed as biomarkers of response variability to Li in BD. This is further supported by a study of miR-134 plasma level in BD patients. This miRNA was found decreased during a manic episode, but upon introduction of medication (combination of antipsychotics and mood stabilizers including Li) and improved mood, miR-134 plasma level increased [40].

4-Histone modification studies

Histone modification is the least studied and understood epigenetic mechanism in the field of Li response (Table 1). Li has been shown to globally increase histone H3 acetylation in primary mixed glial cultures from rat [41]. Treatment with Li (14 days of intraperitoneal injection) induced significant global increase of acetylated histone H3 and decrease of the

protein expression of HDAC4, 5,7 and 8 in the nucleus accumbens of mice [42]. In two Huntington disease transgenic mouse models, Li was found to increase the acetylation of histone H3 in the striatum and the cortex [43]. These global effects have been associated with gene expression regulation in few studies. Hence, acute Li was found to increase the global level of acetylation and phospho-acetylation of histone H3 in the central nucleus of the amygdala 30 min to 1 hour after intraperitoneal injection and this was associated with increased *cFos* expression levels [44]. Li-induced histone H3 methylation and acetylation in the leptin receptor gene have been evidenced in rat hippocampus leading to an increase of expression level of this gene [45]. The mechanisms involved have not been fully investigated. Histone deacetylases (HDACs) remove the acetyl group from histone proteins on DNA, making the DNA less accessible to transcription factors therefore playing critical roles in transcriptional regulation of genes. HDAC1 expression can be downregulated by lithium, in HeLa, 293T and SW480 human cell lines, resulting in a decreased HDAC1 activity in cells [46]. Protein levels of HDAC4, 5, 7 and 8 have also been found decreased after Li treatment in the Nucleus accumbens of mice [42]. After 2 weeks of Li treatment, the sleep deprivation-induced HAT/HDAC imbalance in rat hippocampus was partially corrected [47]. All these results suggest that Li could act on the histones acetylation levels through the regulation of the expression level of histone acetylases and deacetylases proteins. However, little is known on the potential mechanisms that might be involved.

5- Future directions

The involvement of both genetic risk and environmental factors in Li prophylactic response variability of individuals with BD has long been proposed. A significant amount of work, using different analytical approaches in different models and tissue types, has been published on the epigenetic effects of Li. Li targets multiple intracellular targets including the Protein kinase A, MAPK (Mitogen-activated protein kinases) and Glycogen synthase kinase-3, a key translator of the activation of Wnt and Akt, pathways [48]. All these pathways could be involved in Li-induced epigenetic modifications observed and it remains unclear how Li may induce these epigenetic mechanisms. Regarding DNA methylation modifications, a recently identified novel role for Gsk-3 activity is the regulation of this marker via N-myc and the de

novo DNA methyltransferase 3a, isoform 2 (Dnmt3a2), in mouse embryonic stem cells [49]. Since Li has been described as an inhibitor of GSK3beta, this latter may mediate the links between exposure to lithium and regulation of DNMTs. Regarding histone modifications, it has been suggested that Li may prevent the imbalance between histone acetyl transferase/histone deacetylase (HAT/HDAC) activity [50]. Furthermore, the potential associations of the observed epigenetic modifications with Li response variability only received little attention. All the published results studied only one epigenetic mechanism at a time, however complex interplays between miRNA, histone modifications, and DNA methylation have been demonstrated. Thus more studies combining several epigenetic mechanisms or integrating transcriptomics and epigenetics are still needed. Furthermore, many of these results involved a small number of samples and are sometimes inconsistent. Increasing the sample size and phenotype uniformity in large international research groups is important to improve the power of the studies. Another research avenue is that the few SNPs (single nucleotide polymorphism) found associated with Li response variability were intronic or located in a lncRNA [14,51]. This suggests that genetic markers that could impact epigenetic markers such as mQTL (methylation quantitative locus) or SNPs in miRNAs might be of particular interest. Furthermore, all the results published so far, were obtained after retrospective assessment of Li response and in subjects or biological materials that were not naïve to Li exposure. Therefore it is not possible to differentiate whether the observed modifications represent a predisposition to good/poor Li response variability or if they result from differential Li biological effects in good and poor responders. Further efforts should be made to analyze the identified epigenetic markers in prospective BD samples before and after the initiation of Li treatment. Despite all these limitations, the search for epigenetics markers of Li response variability could make a major contribution to personalization of patients care in BD. Peripheral epigenetic measures could predict Li response phenotype but more likely shorten the time to identify the response status or allow a follow-up of the response once the treatment is installed.

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Conflict of interest

F. Bellivier has received honoraria and financial compensation as an independent symposium speaker from Sanofi-Aventis, Lundbeck, AstraZeneca, Eli Lilly, Bristol-Myers Squibb and Servier. B. Etain has received honoraria from Sanofi-Aventis. All other authors have no conflicts of interest relevant to the subject matter of this article.

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Table 1: Summary of studies on epigenetic effects of lithium. BDNF : Brain-Derived Neurotrophic Factor, pDYN : prodynorphyn, CBZ : Carbamazepine, VPA : valproate, DMR : Differentially Methylated Regions, PCR (Polymerase Chain Reaction), RT-qPCR (Reverse Transcriptase quantitative polymerase chain reaction), miRSeq (miRNA sequencing), Ach3 (acetylated histone 3), Ach4 (Acetylated histone 4), pACH3 (phosphor- acetylated histone 3)

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Tissue/Cell lines	Sample characteristics	Drug exposure	Method	Genes	Li effects	References
DNA methylation						
Lymphoblastoid cell lines	CTL (n=16) Li-R (n=14) Unaffected relatives (n=14) Affected relative (16)	LiCl 0.75mM for 7 days	ELISA	Global methylation	Not studied	[18]
Human Leukocytes	CTL (n=26) Li monotherapy (n=29) Combination therapy (n=32)	NA	ELISA	Global methylation	Hypomethylation	[19]
rat hippocampal neurons	NA	LiCl 1 or 2mM for 7 days	Methylation specific PCR	BDNF promoter I, II, IV and VI	Li decreased methylation at exon IV	[24]
Human PBMCs	BD 1 (n=61) BD 2 (n=50) MDD (43)	NA	real-time PCR	BDNF promoter I	Hypomethylation	[25]
Human PBMCs	BD 1 (n=49) BD 2 (n=45)	NA	real-time PCR	BDNF promoter I	Hypomethylation	[26]
Human PBMCs	BD 1 (n=54) BD 2 (n=45)	NA	real-time PCR	pDYN promoter	Hypomethylation	[27]
SK-N-SH cells	NA	8 days VPA 0.3 and 0.6 mM LiCl 0.6 and 1.2 mM CBZ 0.05 and 0.1 mM	HumanMethylation27K BeadChip	Genome-wide	345 genes hypermethylated 138 genes hypomethylated	[28]
Human peripheral blood	BD (n=172)	NA	HumanMethylation27K BeadChip HumanMethylation450K BeadChip	Genome-wide	Quetiapine and VPA showed significant DNA methylation alterations	[29]
Human peripheral blood	BD Li-R (n=15) BD Li-NR (n=11)	NA	SeqCap Epi	Genome-wide	Signature of 7 DMR associated with Li response	[30]
miRNA						
Human peripheral blood	BD (n = 2,563)	NA	versatile gene-based test for GWAS	Genome-wide	15 miRNAs associated with Li response	[32]
Mice brain	NA	LiCl 1 mg/kg bolus then 2 mg/kg/day for 7 day.	RT-qPCR	miR-124	Li increased miR-124 expression	[33]
Rat hippocampus	NA	Chows : 1.2 g/kg Li2CO3 or 10g/kg VPA (1 week) then : 2.4 g/kg Li2CO3 or 20g/kg VPA	mirVana miRNA Probe Set 1564V2	Genome-wide	Li and VPA modulate miRNAs expression miR-34a contributes to the effects of lithium and VPA on metabotropic glutamate receptor 7	[34]

Lymphoblastoid cell lines	BD 1 (n=20)	LiCl 1 mM 4, 8, and 16 days	RT-qPCR	13 miRNAs	Day 4: Li increased miR-221, miR-152, miR-15a, miR-155 and decreased miR-494 Day 16: Li increased miR-221, miR-152, miR-155 and miR-34a	[35]
Lymphoblastoid cell lines	BD 1 Li-R (n=8) BD 1 Li-NR (n=8)	LiCl 1mM 7 days	miRNA chips 3.0	Genome-wide	Li decreased Let-7 miRNA family expression in the Li-R	[36]
Rat cerebellar granule cells	NA	LiCl 3 mM, VPA 0.8 mM, or LiCl 3 mM + VPA 0.8 mM for 6 days	Affymetrix GeneChip	Genome-wide	121 miRNAs regulated by Li+VPA combination	[37]
Lymphoblastoid cell lines	BD committed suicide (n=7) BD low suicide risk (n=11)	LiCl 1mM 7 days	nCounter® miRNA expression Assay (Nanostring)	Genome-wide	After Li treatment miR-4286 was increased while miR-186-5p and -miR-423-5p were decreased in LCL from patients who committed suicide	[38]
Lymphoblastoid cell lines	BD Li-R (n=12) BD Li-NR (n=12)	LiCl 1mM 7 days	miRSeq	Genome-wide	31 miRNAs differentially expressed between R and NR.	[39]
Human plasma	BD (n=21) drug free CTL (n=21)	NA	RT-qPCR	miR-134	Increase of miR-134 after 4 weeks of medication (combination of antipsychotics and mood stabilizers)	[40]
<i>lncRNA</i> Human peripheral blood	BD (n = 2,563)	NA	GWAS	Genome-wide	4 snps in AL157359.3 and AL157359.4 associated with Li response	[14]
<i>Histone modifications</i> Rat primary microglia culture	NA	LiCl 5 mM 24h	Western Blot	Ach4 Ach3	Li increased Ach3 levels	[41]
Mice	NA	LiCl 200mg/kg i.p. for 14 days	Western Blot	Ach3	Li increased Ach3 in Cingulate cortex and Nucleus accumbens but not in Amygdala or Striatum Li decreased HDAC4, 5, 7 and 8 in Nucleus accumbens	[42]
Mice	NA	Chows : 3 g/kg Li2CO3 or 25g/kg VPA (4 or 8 weeks)	Western Blot	Ach3	Li increased Ach3 in cortex and striatum	[43]
Rats	NA	Acute LiCl (0.15 M, 12 ml/kg, i.p.)	Western Blot	Ach3 pAch3	Li increased Ach3 and pAch3 in the amygdala	[44]

Rats	NA	Chows : 0.1% Li2CO3 or 0.2% VPA (30 days)	Chromatin immunoprecipitation	AcH3 3MeAcH3	Li decreased 3MeAcH3 in hippocampus	[45]
HEK 293T, HeLa and SW480 cells	NA	LiCl 20 mM12h and 24h	Western Blot	HDAC1	Li decreased HDAC1	[46]
