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▶ **To cite this version:**

Sigrid Le Clerc, Laura Lombardi, Bernhard T Baune, Azmeraw T Amare, Klaus Oliver Schubert, et al.. HLA-DRB1 and HLA-DQB1 genetic diversity modulates response to lithium in bipolar affective disorders. Scientific Reports, 2021, 11 (1), 10.1038/s41598-021-97140-7 . hal-03500675

HAL Id: hal-03500675
https://cnam.hal.science/hal-03500675

Submitted on 22 Dec 2021

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HLA-DRB1 and HLA-DQB1 genetic diversity modulates response to lithium in bipolar affective disorders

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Bipolar affective disorder (BD) is a severe psychiatric illness, for which lithium (Li) is the gold standard for acute and maintenance therapies. The therapeutic response to Li in BD is heterogeneous and reliable biomarkers allowing patients stratification are still needed. A GWAS performed by the International Consortium on Lithium Genetics (ConLiGen) has recently identified genetic markers associated with treatment responses to Li in the human leukocyte antigens (HLA) region. To better understand the molecular mechanisms underlying this association, we have genetically imputed the classical alleles of the *HLA* region in the European patients of the ConLiGen cohort. We found our best signal for amino-acid variants belonging to the *HLA-DRB1*11:01* classical allele, associated with a better response to Li ($p < 1 \times 10^{-3}$; FDR < 0.09 in the recessive model). Alanine or Leucine at position 74 of the HLA-DRB1 heavy chain was associated with a good response while Arginine or Glutamic acid with a poor response. As these variants have been implicated in common inflammatory/autoimmune processes, our findings strongly suggest that HLA-mediated low inflammatory background may contribute to the efficient response to Li in BD patients, while an inflammatory status overriding Li anti-inflammatory properties would favor a weak response.

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Bipolar affective disorder (BD) is a severe and often chronic psychiatric illness, characterized by recurrent dysregulation of mood with alternating episodes of mania and depression. BD affects around 48.8 million people[1] accounting for 9.9 million years of life lived with disability worldwide[1]. All-cause mortality and risk of suicide[2] are substantially increased in people with the disorder. Both genetic and environmental factors have been identified to contribute to the pathogenesis of BD[3] but the underlying molecular processes remain poorly understood. Nevertheless, well codified mood stabilizer-based treatments have totally changed BD patient management.

In this context, Lithium (Li) has gained a status as the 'gold standard' amongst mood-stabilizers used for both acute- and maintenance therapy in BD[4–6]. Li display strong anti-manic properties[7,8]. Accordingly, it is the only mood stabilizer with protective effects against further episodes of both manic and depressive polarity[6], making it the most efficient therapeutic option for preventing relapses[9]. Even if Li is recommended as a first-line option for anti-manic and maintenance treatment[10–13], its routinely use is hampered by several caveats. Firstly, the therapeutic response to Li in BD is highly heterogeneous. In acute mania, about 65% of patients respond at least partially to Li monotherapy while 35% are refractory[14,15]. In maintenance treatment, an efficient long-term response is reported only for approximately 30% of patients[16], whereas 30% has intermediate outcomes and 30% poor response. Secondly, Li is toxic at high doses and interindividual variation in metabolism requires a stringent monitoring of Li plasma levels[17]. And thirdly, Li has long-term adverse effects including alteration of thyroid and parathyroid functions and an elevated risk of renal failure[5].

Amongst biological Li modulator markers, genetic variants (i.e., single nucleotide polymorphisms, SNPs[16,18]) and genetic traits (i.e., polygenic risk scores, PRS) have been identified that differentiate Li response groups. We recently reported that BD patients with high genetic load for schizophrenia and major depressive disorders (MDD) are less likely to have favorable Li treatment outcomes than those with lower genetic load of these traits[19,20]. Moreover, we showed in cross-trait meta-GWAS and pathway analyses that genetic signals tagging the major histocompatibility complex (MHC) and pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin 4 (IL-4) and interferon gamma (IFNγ) could have a biological role in Li treatment response in BD[19]. More specifically, the most significant finding of the cross-trait GWAS and the SNP from the post-GWAS functional analyses suggested that the human leukocyte antigen (HLA) cluster which is hosted by the major histocompatibility complex (MHC) region could be implicated in the genetic susceptibility to schizophrenia (SCZ) and Li treatment response[19]. The MHC/HLA region is the most robust immunogenetic finding in SCZ[21], and could be marking a schizophrenia-type pathogenesis associated with Li non-response. The extensive linkage disequilibrium (LD) in the *HLA* region, the presence of non-*HLA* genes surrounding the cluster and pitfalls in study designs may explain previous inconsistent data concerning *HLA* association with Li responsiveness in

	GWAS1	GWAS2
All_individuals	847	1292
Age at interview	48.15 (13.78)	46.90 (14.01)
Sex		
Men	360 (43%)	565 (44%)
Women	487 (57%)	727 (56%)
Alda scale A score	6.14 (3.05)	6.48 (2.77)
Alda scale total B score	2.16 (1.63)	2.86 (1.69)
Alda scale total score	4.32 (3.36)	3.97 (2.99)
Non responders	186	201
Age at interview	45.56 (11.82)	44.88 (12.66)
Sex		
Men	82 (44%)	84 (42%)
Women	105 (56%)	117 (58%)
Alda scale A score	1.63 (1.19)	1.73 (1.22)
Alda scale total B score	2.66 (1.64)	3.56 (1.80)
Alda scale total score	0.38 (0.76)	0.18 (0.49)
Responders	418	685
Age at interview	50.91(14.29)	47.50 (14.70)
Sex		
Men	170 (41%)	317 (46%)
Women	248 (59%)	368 (54%)
Alda scale A score	8.78 (1.05)	8.61 (1.08)
Alda scale total B score	1.66 (1.51)	2.50 (1.60)
Alda scale total score	7.12 (2.07)	6.11 (2.12)
Continous (B < 4 exluded)	772	1080
Age at interview	48.49 (13.73)	47.08 (14.05)
Sex		
Men	324 (42%)	471 (44%)
Women	448 (58%)	609 (56%)
Alda scale A score	6.25 (3.04)	6.66 (2.74)
Alda scale total B score	1.83 (1.29)	2.36 (1.16)
Alda scale total score	4.65 (3.31)	4.48 (2.89)

Table 1. Phenotypic characteristics of individuals used for the analyses. Data are n, n (%), or mean (SD). Alda scale refers to the Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder; Non-resp = Li non responders (ALDA subscale A less than or equal to 3); Resp = Li responders (ALDA subscale A greater than or equal to 7).

HLA protein, position, amino-acid(s)	Freq (%) GWAS1 (N = 772)	Beta GWAS1	*P*-value GWAS1	Q-value GWAS1
HLA-DRB1, 37, tyrosine and leucine	33.35	0.57	4.78×10^{-4}	0.12
HLA-DRB1, 71, arginine	50.06	0.51	6.78×10^{-4}	0.12
HLA-DQB1, 26, leucine	68.8	− 0.53	8.30×10^{-4}	0.12
HLA-DRB1, 67, phenylalanine	16.65	0.68	9.80×10^{-4}	0.12

Table 2. Best results of association analysis of HLA with response to Li as continuous trait for group GWAS1 in additive model. Freq: frequency; Q-value: the *q*-value gives the expected positive false discovery rate.

BD[22–24]. Nevertheless, the above-mentioned tags of both HLA and pro-inflammatory cytokines reasonably focus attention towards the possible implication of immune processes in responses to Li.

Accordingly and given the role of *HLA* alleles in antigen presentation to downstream effector immune cell subsets, we took advantage of the above-mentioned GWAS[19] for an in-depth analysis of genetic variants of the *HLA* region in relation to Li treatment response. To this end, we imputed the *HLA* class-I and class-II genetic variants corresponding to HLA amino-acids and classical alleles and investigated their distribution in responders and non-responders to Li treatment.

HLA protein, position, amino-acid(s) or *HLA* allele	Meta-analysis continous scale			Meta-analysis dichotomous scale			AF GWAS1 (%)			AF GWAS2 (%)		
	Beta	*P*-value	Q	OR	*P*-value	Q	All (N = 772)	Non-responder (N = 186)	Responder (N = 418)	All (N = 1080)	Non-responder (N = 201)	Responder (N = 685)
HLA-DRB1, 74, alanine and leucine	0.32	1.94×10^{-3}	0.27	1.32	2.22×10^{-3}	0.27	70.27	65.24	72.37	71.19	67.41	73.07
HLA-DRB1, 74, arginine and glutamic acid	−0.37	3.54×10^{-3}	0.27	0.72	2.87×10^{-3}	0.27	16.13	19.52	13.52	16.31	19.40	15.47
*HLA-DQB1*02*	−0.33	3.96×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 74, alanine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 71, lysine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 55, leucine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 52, leucine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 47, phenylalanine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 46, glutamic acid	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 37, isoleucine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 30, serine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, 28, serine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61
HLA-DQB1, -10, serine	−0.33	3.87×10^{-3}	0.27	0.75	5.40×10^{-3}	0.27	21.70	26.20	20.33	20.57	22.89	18.61

Table 3. Results of the meta-analysis for continuous and dichotomous response outcome in additive model. AF: allele frequency; Q-value: the *q*-value gives the expected positive false discovery rate; Non-resp = Li non responders (ALDA subscale A less than or equal to 3); Resp = Li responders (ALDA subscale A greater than or equal to 7).

City/Country	N		A/L Allele frequency		Recessive model		Dominant model	
	Non- resp	Resp	Non-resp (%)	Resp (%)	Non-resp (%)	Resp (%)	Non-resp (%)	Resp (%)
GWAS1								
Cagliari/Italy	52	91	63.46	71.43	38.46	51.65	88.46	91.21
Halifax/Canada	55	150	58.18	71.00	32.73	52.00	83.64	90.00
San Diego/USA	79	58	68.99	75.00	46.84	62.07	91.14	87.93
Wuerzburg/Germany	25	30	62.00	76.67	36.00	56.67	88.00	96.67
GWAS2								
Barcelona/Spain	19	40	50.00	65.00	21.05	40.00	78.95	90.00
Canada	17	52	67.65	65.38	35.29	42.31	100.00	88.46
Paris/France	47	114	62.77	71.05	34.04	50.88	91.49	91.23
Romania	18	82	77.78	79.27	55.56	59.76	100.00	98.78
Sweden	39	200	71.79	77.25	51.28	59.50	92.31	95.00

Table 4. Country by country distribution of the patients carrying the HLA-DRB1 alleles A or L at position 74. Non-resp = Li non responders (ALDA subscale A less than or equal to 3); Resp = Li responders (ALDA subscale A greater than or equal to 7); N = number of patients in the group; recessive model = percentage of patients carrying only the A or L allele; dominant model = percentage of patients carrying at least one allele A or L.

Results

Genotyping data from 2139 BD patients who had long-term Li treatment were obtained from the International Consortium ConLiGen for *HLA* imputation (847 in GWAS1 and 1292 in GWAS2). The descriptive statistics of the phenotypes of the participants in each analysis are presented in Table 1. The main goal of the present study was

to uncover HLA amino-acids or classical *HLA* alleles potentially associated with responses to Li in BD. We first performed association analyses with *HLA* variants separately for the GWAS1 and GWAS2, and then performed a meta-analysis of the two studies.

For the association analyses of both GWAS1 and GWAS2, no HLA variant reached the Bonferroni significance (threshold of 5.2×10^{-5}) in the additive model, neither for the continuous nor for the extreme phenotypes used to measure Li response with the Alda scale (comparison of extreme phenotypes, see "Materials and methods"). Nevertheless, several suggestive associations exhibiting a FDR < 0.25 were observed and are presented in Supplementary Table 2.

In the analysis of GWAS1 for the continuous trait (A score, individuals with B score > 4 were removed), the top ranked variants (p-values $< 1 \times 10^{-3}$ FDR = 0.12) were located within the *HLA-DRB1* and *HLA-DQB1* genes. For HLA-DRB1, Tyrosine/Leucine at position 37, Arginine at position 71, and Phenylalanine at position 67 were associated with a better response to Li (Table 2). Interestingly, these amino-acids notably belong to the HLA-DRB1 heavy chain encoding the *HLA-DRB1*11:01* allele. Whereas weak response to Li was found to be associated with Leucine at position 26 of the *HLA-DQB1* locus (Table 2). None of these signals were replicated in the GWAS2. The amino-acid frequencies in the GWAS1 and GWAS2 were in agreement with those published in the reference cohorts from Allele frequency net database[25] (Supplementary Table 3).

In a second step, the meta-analysis of GWAS1 and GWAS2 (in the additive model) identified two signals almost reaching the 0.25-FDR threshold for suggestive associations (Table 3). Importantly, the associations were of similar magnitude and direction of effect for both the continuous and dichotomous traits. Once again, the two types of signals were related to the *HLA-DRB1* and *HLA-DQB1* loci and the frequency of their variants were also comparable to those of the reference cohorts from Allele frequency net database[25] (Supplementary Table 3). The first signal in the *HLA-DQB1* locus which consisted of several amino-acids belonging to the *HLA-DQB1*02* heavy chain was associated with no response to Li (Table 3). The second signal tags the amino-acid 74 of the HLA-DRB1 heavy chain. While Alanine or Leucine at position 74 are related to a better response to Li, Arginine or Glutamic acid at position 74 were associated to a weaker one (Table 3). For the analysis of the dichotomous phenotypes (defined by the Alda score ≥ 7 for the responders and ≤ 3 for non-responders), the frequency of the amino-acids Alanine or Leucine at position 74 was higher among the responders as compared to the non responders [72% vs 65% (GWAS1) and 73% vs 67% (GWAS2) in responders vs non-responder respectively]. Since the distribution of HLA alleles is known to exhibit South-North and/or East–West gradients even in European countries, we have specifically analysed the distribution of the alleles (A/L vs others) at position 74 in each country separately. For each country, we thus compared the allelic frequency under a dominant (patients carrying at least a A or a L allele) or a recessive (patients carrying only A or L alleles) model of the dichotomous phenotype of responders and non-responders (when N ≥ 15 in each group). Surprisingly, we observed that, in nine countries out of nine with sufficient number of patients in each dichotomous phenotype (Alda score ≥ 7 and Alda score ≤ 3), the frequency of patients carrying only A or L allele in the recessive model is systematically lower in non-responders than in responders (Table 4), suggesting a recessive effect. The probability of getting such a systematic difference in 9 countries out of 9 by chance is statistically very weak ($p = 0.004$). When comparing extreme groups (Alda score ≥ 9 and Alda score ≤ 3, see "Materials and methods") in GWAS1 and GWAS2 for the allele A/L at position 74 in the recessive model, we obtained a FDR of 0.09 (Supplementary Tables 1 and 5), stronger than the one obtained for the additive model and confirming the recessive effect. Finally, it is worth mentioning that the *HLA-DRB1*11:01* allele bears an Alanine 74 which confirms the above-mentioned favourable effect of such variant on responses to Li. Additionally, it is important to note that the HLA-DRB1 position 74 was previously reported to be in LD with the positions 37, 67, and 71 that came out strongly in GWAS1 (Table 2)[26].

In order to test dependence between the observed *HLA-DRB1* and *HLA-DQB1* signals, we evaluated the LD between their variants. We found a high level of correlation (r or D' > 0.9) between *HLA-DQB1*02* and two variants of *HLA-DRB1* (Supplementary Table 4). The r^2 was found to be the higher ($r^2 > 0.5$), between Alanine or Leucine at position 74 of HLA-DRB1 and *HLA-DQB1*02* (Supplementary Table 4), which suggests a putative link between the *HLA-DRB1* and *HLA-DQB1*. We used Haploview[27] to determine the haplotype between Alanine or Leucine at position 74 of HLA-DRB1 and *HLA-DQB1*02* and we observed that all individuals carrying Alanine or Leucine at position 74 of HLA-DRB1 did not carry *HLA-DQB1*02* and reciprocally. Given that Alanine and Leucine 74 have been found to be associated with good responders, while *HLA-DQB1*02* with non-responders, it was not possible to determine whether the signal was due to the *HLA-DRB1* or to the *HLA-DQB1*, or both of them.

Discussion

Understanding the mechanisms underlying inter-individual responses for a given therapeutic compound is a leading step towards precision medicine as, whatever the treatment efficacy, a subset of patients does not respond. Regarding BD, Li constitute the cornerstone of long-term therapy and management of the disease, but only around one-third of Li treated patients respond positively[28]. A great deal of research has thus been done to identify predictors of the degree of prevention against recurrences of mania and depression during long-term treatment. While several studies showed that Li response was polygenic, a large GWAS-based survey performed by the ConLiGen consortium in 2017, demonstrated that responses to Li were influenced both by the allostatic load of inter-individual SCZ polygenic scores and by genetic markers tagging the HLA antigen presentation pathway and genes encoding pro-inflammatory cytokines[19].

Li has been demonstrated to exhibit potent anti-inflammatory properties mediated at least by the well-known inhibitory effects exerted on the Glycogen synthetase kinase-3-beta (GSK-3β) enzyme and downstream on the NF-κB pathway with consequent reduction of the pro-inflammatory cytokines TNF-α and IL-6 levels among others[29,30]. In addition, it has a potent anti-inflammatory action mediated both by the inhibition of IL-1β

production and by the reduction of cyclooxygenase-2 expression while enhancing expression of IL-2 and IL-10 anti-inflammatory mediators. Finally, Li is also thought to modulate glial cellular inflammatory processes[31,32]. Given that the HLA system is central for the control of inflammatory processes, we imputed putative functional *HLA* variants within individuals from the above-mentioned GWAS and analyzed their potential association with response to Li.

Although no *HLA* variant per se reached the stringent Bonferroni significance threshold, we observed that several amino-acid positions had a low FDR score. Interestingly, all belonged to the HLA class-II cluster, suggesting the involvement of immune humoral processes in response to Li especially as some of them are located within the 3rd hypervariable (3HVR) sequences (amino-acids 67–74). Of interest, the 3HVR is the primary T-cell recognition site which conditions the immune adaptive processes mediated by the HLA class-II alleles[33]. Accordingly, the 3HVR is the core-associated region with immune processes including chronic inflammation and autoimmunity. It is thus important to mention that the *HLA* signal observed in the ConLiGen study[19] tags the *HLA-DM* gene which encodes molecules pivotal for the HLA class-II antigen processing pathway by facilitating antigenic peptide loading to *HLA* class-II alleles[34].

More precisely, in the analysis of patients belonging to GWAS1, we observed that within the HLA-DRB1 heavy chain, Tyrosine or Leucine at position 37, Arginine at position 71 and Phenylalanine at position 67, amino-acids all belonging to the *HLA-DRB1*11:01* classical allele, are associated to a better response to Li. Such a finding could be discussed either at amino-acid or allele level. Indeed, Arginine 71 has previously been demonstrated to modulate disease severity in inflammatory/autoimmune settings. For example, in the context of inflammatory polyarthritis, a study evaluating the relationship between amino-acids at position 11, 71 and 74 and clinically defined inflammation showed that Arginine 71 belongs to haplotypes conferring protection against severe inflammation. In this study, while Valine at position 11 is the primary risk factor for severe clinical inflammation, a Serine at this position confers protection against clinically-defined inflammation and disease progression[35]. Of interest, *HLA-DRB1*11:01* bears the reported protective haplotype against inflammation namely, Serine 11, Arginine 71 and Leucine 74. In addition, another study evaluating whether clinical severity in rheumatoid arthritis (RA) correlated with HLA genetic heterogeneity, demonstrated that among patients having the prototypic at risk shared epitope, consisting of specific amino-acids combinations at positions 70 to 74 of the 3HVR, Arginine 71 characterized patients who were non-producers of the rheumatoid factor autoantibody (RF), a subset of patients usually characterized with less aggressive and destructive disease as compared to those who produce RF[36].

Our meta-analysis showed that patients having Alanine or Leucine at position 74 were more represented in the group of responders. Of note, *HLA-DRB1*11:01* allele which bears Leucine at position 74 was also likely associated with good response. Patients with Arginine or Glutamic acid at position 74 belong to the non-responder subgroup. These associations were also observed in the analysis of extreme groups where homozygous subjects for the A/L alleles were always found in smaller numbers in the extreme non-responders as compared to responders (Table 4, Supplementary Table 1), altogether suggesting a recessive effect. The beneficial effect exerted by the *HLA-DRB1*11:01* allele can be discussed regarding its involvement in immune disease settings. A Brazilian study of the genetic influence on multiple sclerosis, a chronic autoimmune condition, showed that beside the canonical at risk *HLA-DRB1*15* allele, the *HLA-DRB1*11* variant exerts a strong protective effect against the disorder[37]. Such protective effect against multiple sclerosis was also reported in ethnically distant populations-groups[38]. Moreover, a recent study allowed us to establish a link between our finding and the signal identified in the primary ConLiGen GWAS study concerning the association with the HLA-DM region[19]. Indeed, the authors, analyzing the mechanism underlying the protective status conferred by *HLA-DRB1*01:01* and *HLA-DRB1*11:01* alleles against multiple sclerosis, demonstrated the involvement of the HLA-DM-mediated lower efficiency to bind antigenic peptide to the antigen peptide groove of the protective allele with consequent failure of peptide presentation at the cell surface and possible alleviation of inflammatory/autoimmune processes[39]. In another immune condition, namely chronic Hepatitis C Virus infection, the *HLA-DRB1*11* was demonstrated to mediate protection against liver damage[40]. Altogether, these data suggest that good Li responders are associated with HLA variants conveying low inflammatory characteristics.

Concerning the non-responder patient group, an association was found with Glutamic acid or Arginine at position 74 in *HLA-DRB1*. The latter has been shown to drive the risk of developing Grave's disease, a common inflammatory/auto-immune disorder either in patients bearing the usually associated *HLA-DRB1*03* specificity or in affected non-DR3 Graves patients[41]. In addition, we also observed an association between weak response to Li and multiple amino-acids scattered along the heavy chain of the *HLA-DQB1* gene, almost all corresponding to the *HLA-DQB1*02* allele. This association may suggest the involvement of the well-known *HLA-8.1* ancestral haplotype (*A*01 ~ B*08 ~ DRB1*03 ~ DQB1*02*), which is characterized by a steady state pro-inflammatory status in healthy subjects and is the most associated *HLA* haplotype with inflammatory/autoimmune disorders. Another possibility explaining the association with *HLA-DQB1*02* could be merely a LD with another *HLA* variant, not uncovered in the present study such as *HLA-DRB1*03* known to be in LD with *HLA-DQB1*02* and to bear the non-response associated Arginine 74. This hypothesis needs to be confirmed in larger cohorts of patients as it may have practical therapeutic implications such as add-on treatments of anti-inflammatory drugs in non-responders to Li.

Our study was performed on patients coming from multiple countries, which inevitably introduce heterogeneity in terms of data collection, methods of diagnosis/evaluation of the phenotype and information on patients such as comorbidities, overall constitutes possible confounding effects. Within this line, the fact that the distribution of Alda scores in the GWAS1 group was slightly more variable than that observed in the GWAS2 group and that patients belonging to the GWAS1 were slightly older than that of the GWAS2 group (Table 1), may also contribute to differences in the analysis of the two groups. In spite of these limits, the association between the HLA-DRB1 position 74 A/L allele and responders was consistently observed under the recessive model with extreme phenotypes in all the countries tested in the GWAS1 and in the GWAS2. We have already mentioned

that the recessive effect for HLA-DRB1 cannot be dissociated from a dominant effect for the *HLA-DQB1*02* allele in terms of genetic association. Such recessive-based observation may reflect the characteristics of the HLA system (and other innate immune sensors) in terms of structure, function and evolution where the heterozygosity advantage was selected to better fight pathogen diversity, a recessive status may prevent overwhelmed inflammatory processes[42–44].

Altogether, beside the well-documented anti-inflammatory effect exerted by Li on the innate GSK-3β pathways and consequently on circulating pro- and anti-inflammatory cytokines, our observations indicate that response to Li in BD patients may be underpinned by adaptive HLA-mediated inflammatory processes, fitting with the known anti-inflammatory effect driven by Li that may contribute to its therapeutic efficiency[31]. Our genetic findings showing an association between HLA-mediated lower inflammation in patients with efficient response to Li, may suggest that non-responders among BD patients could have inflammatory backgrounds that override Li mediated anti-inflammatory properties.

Materials and methods

Study population. This study includes GWAS genotype and phenotype data of Li-treated BD patients from 22 participating sites belonging to the International Consortium on Lithium Genetics (ConLiGen)[16]. The whole cohort consisted of 2588 patients resulting from two GWAS of 1187 and 1401 individuals named GWAS1 and GWAS2 in the original publication[16]. Samples were collected and genotyped in two distinct steps, determining the GWAS1 and GWAS2 groups [detailed rationale is available in the appendix of Hou et al.[16]]. The various cohorts were initially developed separately and various chips were used for genotyping[16]. We kept the same distribution of the samples and the names GWAS1 and GWAS2. After removal of Japanese, Taiwanese and Sardinian patients given the poor applicability of *HLA* imputation in these population-groups on the one hand, and the difference in HLA frequencies among the Sardinian population on the other, 862 and 1309 patients belonging respectively to the GWAS1 and GWAS2 were subjected to analysis. We performed a quality control of the genotypes for each patient. As cryptic relatedness between individuals can introduce a bias in association analysis and in the following meta-analysis, we computed identity-by-descent (IBD) between all pairs of individuals for the two groups and removed one individual of the related pair if a second degree related can be detected (N = 14 for GWAS1 and N = 11 for the GWAS2). We also removed one patient in the GWAS1 and six patients in the GWAS2 because of an excess of heterozygosity, suggesting a poor DNA quality. After these two steps, 847 patients from GWAS1 and 1292 from GWAS2 remained for analysis.

The Conligen consortium used data obtained through an international collaboration. The University of Heidelberg Ethics Committee provided the central ethics approval for the consortium. Written informed consent was obtained from each patient (they were all aged > 18) according to the study protocols of the participating cohorts and their respective institutions. All procedures were performed in accordance with the guidelines of the institutional research committees and of the Helsinki Declaration.

Study cohort phenotypes. From the discovery GWAS participants[16], long-term treatment responses to Li were evaluated using the Alda scale. Briefly, the Alda scale used criterion A to rate the degree of response (activity of illness while on adequate Li treatment) on a 10-point scale and criterion B which establishes the relationship between improvement and treatment (i.e. number of episodes off treatment, frequency of episode off treatment, duration of treatment, compliance, use of additional treatment). The total score is obtained by subtracting score B from score A and negative scores are set to 0, so that the total score ranges from 0 to 10.

In the present study, we performed 3 types of phenotype analyses in order to dissect the genetic associations with Li response:

(1) A continuous phenotype (range 0–10) using the A score, excluding all individuals with a total B score greater than 4 for a dimensional assessment of Li response. In this study, there were 772 patients studied in GWAS1 and 1080 patients studied in GWAS2 by linear regression with the continuous phenotype in the additive model.

(2) A dichotomous phenotype comparing good vs non responders to Li, defined by an A Alda score ≥ 7 for responders and ≤ 3 for non-responders. In this comparative study (in the additive model), there were 186 non-responders compared with 418 responders from GWAS1, and 201 non-responders compared with 685 responders from GWAS2. To take into account the variations of distribution of HLA alleles according to countries, we also checked country by country the frequency of patients carrying the HLA-DRB1 A or L alleles at position 74 in the non-responder and responder groups, when these groups had enough patients (N ≥ 15) to compute a reliable frequency. We added the Sardinian population (comprising 52 non-responders compared with 91 good responders, Table 4), even if it was excluded from the statistical analysis.

(3) We also performed a country-by-country analysis in extreme phenotypes defined by an A Alda score ≥ 9 for responders and ≤ 3 for non-responders: there were 186 non-responders compared with 275 responders from GWAS1, and 201 non-responders compared with 362 responders from GWAS2. In Sardinian population, there were 52 non-responders compared with 39 good responders (Supplementary Table 1). We also computed a FDR value comparing the non-responders with responders in the recessive model with this extreme phenotype (Supplementary Table 5).

Imputation of HLA variants. The SNP2HLA software[45] has been developed to impute directly *HLA* class-I and class II-classical alleles at the 4-digit level from the chromosome 6 genotypic data of each patient. In addition, the software computes directly the amino-acid variants from the imputed 4-digit HLA alleles, allowing to test associations also at the level of the HLA protein amino-acid variants (including the ones constituting the peptide antigen presenting groove) thus facilitating the biological interpretation. A total of 1708 imputed vari-

ants (433 classical *HLA* alleles and 1275 amino-acid variations) were generated. The variants with minor allele frequency < 0.05 and/or *p*-value for Hardy–Weinberg departure test $< 1 \times 10^{-6}$ were removed. The resulting 85 classical *HLA* alleles and 880 amino-acid variations were subjected to the statistical analysis pipeline. To validate the accuracy of the imputation, we compared the frequency of the variants with those listed in the Allele frequency net database[25]. Given that the studied populations were from European descent, we choose available representative populations from France (France, Rennes, N = 200; France, west, N = 100) and from Germany (Germany, Essen, N = 174) in the Allele Frequency Net Database with alleles given in at least four digits. The best variants obtained from our association analysis had a rather strong MAF, and since the MAF found in the control populations were similar it was sufficient to confirm the quality of the HLA imputation for thee variants, even if these control populations had a relatively modest size. For the amino-acid frequencies we used the tool of translation from Allele frequency net database[25].

Statistical analysis. For the 965 HLA imputed variants having passed the quality controls, associations between HLA amino-acids or alleles and response to Li were tested. The statistical analysis was performed by a multivariate linear or logistic regression using PLINK software[46] under additive model for response to Li (continuous or binary phenotypes). To correct for possible population stratification, we performed principal component analysis (PCA) on the genotypes and used the top two principal components as covariates in statistical analyses. To avoid spurious association, we added age and gender of patients as covariates. Finally, a classical meta-analysis was performed between associations resulting from GWAS1 and GWAS2 for two phenotypes (continuous (1) and dichotomous (2)) using PLINK. We used the Bonferroni threshold (for 965 tests − 85 classical *HLA* alleles + 880 amino-acid-, *p*-value $< 5.2 \times 10^{-5}$) to determine the significance of the associations and we also took the false discovery rate (FDR) < 0.25 for the suggestive associations.

Received: 24 March 2021; Accepted: 13 August 2021
Published online: 08 September 2021

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Acknowledgements

The authors thank the patients and hospital staff who kindly participated and we appreciate the contributions of the clinicians, scientists, research assistants, and study staff who helped in the patient recruitment, data collection, and sample preparation of the ConliGen studies. We are indebted to the members of the ConLiGen Scientific Advisory Board (http://www.conligen.org/) for critical input over the course of the project.
Some data and biomaterials were collected as part of eleven projects (Study 40) that participated in the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. From 2003 to 2007, the principal investigators and co-investigators were: Indiana University, Indianapolis, IN, R01 MH59545 (John Nurnberger, Marvin J Miller, Elizabeth S Bowman, N Leela Rau, P Ryan Moe, Nalini Samavedy, Rif El-Mallakh [University of Louisville], Husseini Manji [Johnson and Johnson], Debra A Glitz [Wayne State University], Eric T Meyer [Oxford University, UK], Carrie Smiley, Tatiana Foroud, Leah Flury, Danielle M Dick [Virginia Commonwealth University], Howard Edenberg); Washington University, St Louis, MO, R01 MH059534 (John Rice, Theodore Reich, Allison Goate, Laura Bierut [K02 DA21237]); Johns Hopkins University, Baltimore, R01 MH59533 (Melvin McInnis, J Raymond DePaulo Jr, Dean F MacKinnon, Francis M Mondimore, James B Potash, Peter P Zandi, Dimitrios Avramopoulos, Jennifer Payne); University of Pennsylvania, PA, R01 MH59553 (Wade Berrettini); University of California at San Francisco, CA, R01 MH60068 (William Byerley, Sophia Vinogradov); University of Iowa, IA, R01 MH059548 (William Coryell, Raymond Crowe); University of Chicago, IL, R01 MH59535 (Elliot Gershon, Judith Badner, Francis McMahon, Chunyu Liu, Alan Sanders, Maria Caserta, Steven Dinwiddie, Tu Nguyen, Donna Harakal); University of California at San Diego, CA, R01 MH59567 (John Kelsoe, Rebecca McKinney); Rush University, IL, R01 MH059556 (William Scheftner, Howard M Kravitz, Diana Marta, Annette Vaughn-Brown, Laurie Bederow); and NIMH. Intramural Research Program, Bethesda, 1Z01MH002810-01 (Francis J McMahon, Layla Kassem, PsyD, Sevilla Detera-Wadleigh, Lisa Austin, Dennis L Murphy [Howard University], William B Lawson, Evarista Nwulia, Maria Hipolito).

Author contributions

R.T., J.F.Z. and M.L. conceived and designed the study, and wrote the manuscript. S.L.C. and L.L. performed data analyses and, wrote the draft of the manuscript. A.T.A., B.T.B., J.L.S., W.B., J.R.R., P.L.C., C.B., S.P., M.C., F.T.A., Y.H.H., J.M.B., N.D., S.G., E.F.R., M.J.M., V.M., C.M.N., C.O.D., E.R. and C.P. gave critical comments on the draft and contributed to the manuscript writing. L.H., U.H., F.D., J.R.K., M.A.l., M.R., F.J.M., T.G.S., N.A., H.-C.C., S.R.C., A.J.F., M.A.F., S.H., P.H., S.J., M.M.N., T.Sh. and P.P.Z. designed the original G.W.A.S. analysis. M.A.d., M.B., S.C., P.M.C., M.D.Z., J.H., U.H., F.J.M., L.H., M.R., J.K.R., M.S., P.R.S., TGS., PD.S., and A.S. were

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Funding

This manuscript was written under the framework of Agence Nationale de la Recherche (I-GIVE ANR-13-SAMA-0004-01), INSERM (Institut National de la Santé et de la Recherche Médicale) and Fondation FondaMental. Laura Lombardi benefits from a fellowship n° FDM202006011305 from Fondation de la Recherche Médicale (FRM). Sven Cichon. and Andreas J Forstner received support from the Swiss National Science Foundation / German Research Foundation (SNSF 310030L_182731/1). This work was in part funded by the Deutsche Forschungsgemeinschaft (DFG; grant no RI 908/7-1; grant FOR2107, RI 908/11-1 to Marcella Rietschel, Michael Bauer, and Thomas G Schulze, NO 246/10-1 to MMN) and the Intramural Research Program of the National Institute of Mental Health (ZIA-MH00284311; NCT00001174). The genotyping was in part funded by the German Federal Ministry of Education and Research (BMBF) through the Integrated Network IntegraMent (Integrated Understanding of Causes and Mechanisms in Mental Disorders), under the auspices of the e:Med. This work was supported by the NIH grants P50CA89392 from the National Cancer Institute and 5K02DA021237 from the National Institute of Drug Abuse. The Canadian part of the study was supported by a grant #64410 from the Canadian Institutes of Health Research to MAl. Collection and phenotyping of the Australian UNSW sample was funded by an Australian NHMRC Program Grant (No. 1037196).The collection of the Barcelona sample was supported by the Centro de Investigación en Red de Salud Mental (CIBERSAM) IDIBAPS (grant numbers PI080247, PI1200906, PI12/00018), and Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement (2014SGR1636 and 2014SGR398). Genotyping for part of the Swedish sample was funded by the Stanley Center for Psychiatric Research at the Broad Institute. The Swedish Research Council, the Stockholm County Council, Karolinska Institutet, and the Söderström-Königska Foundation supported this research through grants awarded to Drs Backlund, Frisen, Lavebratt, and Schalling. The collection of the Geneva sample was supported by grants Synapsy–The Synaptic Basis of Mental Diseases 51NF40-158776 and 32003B-125469 from the Swiss National Foundation. The work by the French group was supported by INSERM (Institut National de la Santé et de la Recherche Médicale), AP-HP (Assistance Publique des Hôpitaux de Paris), the Fondation FondaMental (RTRS Santé Mentale), and the labex Bio-PSY (Investissements d'Avenir program managed by the ANR under reference ANR-11-IDEX- 0004-02). The collection of the Romanian sample was supported by a grant from Unitatea Executiva pentru Finantarea Invatamantului Superior, a Cercetarii, Dezvoltarii si Inovarii (Dr Grigoroiu-Serbanescu). The collection of the Czech sample was supported by the project Nr. LO1611 with a financial support from the MEYS under the NPU I program and by the Czech Science Foundation, grant Nr. 17-07070S.

Competing interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. MAd has received a grant from Servier, speaker's fees from Servier, Lundbeck, Aristo, Parexel, Gilead, ViiV, Deutsche Bank, MSD, and MyTomorrows, plus a non-fi nancial support from Lundbeck. KA has received speaker's fees from Taisho Toyama Pharmaceutical. MAl is funded by a grant of the Canadian Institutes of Health Research. MB has received speaker's fees from AstraZeneca, Pfi zer, Lilly, Lundbeck, GlaxoSmithKline, Servier, and Ferrer Internacional. BÉ received non-financial support from Labex Biopsy and Fondation Fondamental. RH received grants and speaker honoraria from Dainippon Sumitomo Pharma and Novartis plus speaker honoraria from Eli Lilly Japan, GlaxoSmithKline, Hisamitsu Pharmaceutical, Janssen Pharmaceutical, Nippon Zoki Pharmaceutical, Otsuka Pharmaceutical, Astellas Pharma, Pfi zer, and the Yoshitomiyakuhin Corporation. TK received a grant from Takeda Pharmaceutical and fees from Kyowa Hakko Kirin, Eli Lilly Japan, Otsuka Pharmaceutical, GlaxoSmithKline, Taisho Toyama Pharmaceutical, Dainippon Sumitomo Pharma, Meiji Seika Pharma, Pfi zer Japan, Mochida Pharmaceutical, Shionogi & Co, Janssen Pharmaceutical, Yoshitomiyakuhin Corporation, Agilent Technologies, Astellas Pharma, and Wako Pure Chemical Industries. IK received grants and fees from Dainippon Sumitomo Pharma, Eisai, Eli Lilly, GlaxoSmithKline, Kyowa Hakko Kirin, Meiji Seika Pharma, MSD, Novartis, Otsuka, Ono Pharmaceutical, Pfi zer, Tanabe Mitsubishi Pharma, Takeda Pharmaceutical, Shionogi, and Yoshitomi Pharmaceutical; he received grants from AbbVie GK, Asahi Kasei Pharma, Boehringer Ingelheim, Chugai Pharmaceutical, and Daiichi Sankyo and fees from Astellas Pharma and Janssen Pharmaceutical. MJM served as unpaid consultant for Pathway Genomic (San Diego, USA). SLM received a grant and fees from Naurex and Shire, further grants from Alkermes, Cephalon, Forest, Marriott Foundation, Orexigen Therapeutics, and Takeda Pharmaceutical, he further has served on the advisory boards for Bracket, Hoff mann-La Roche, MedAvante, Sunovion and received fees from Novo Nordisk. PRS received a grant from NHMRC. TGS received a grant and fees from Roche Pharmaceuticals. TSt received personal fees from Servier, Lundbeck, and Bristol-Myers Squibb. EV has received grants and served as consultant, advisor or CME speaker for the following entities (unrelated to the present work): AB-Biotics, Abbott, Allergan, Angelini, Dainippon Sumitomo Pharma, Ferrer, Gedeon Richter, GH Research, Janssen, Lundbeck, Otsuka, Sage, Sanofi-Aventis, and Takeda. FC In the last two years he has served as a speaker or in the advisory board of the following companies: Abott, Sandoz and Sanofi and he receives unrestricted research support from Telefónica Alpha.

JRDP was an unpaid consultant for Assurex Health on behalf of the National Network of Depression Centers and he owns stock in CVS. All above listed interests are outside of the submitted work. All other authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-97140-7.

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