Journal of Affective Disorders 152-154 (2014) 395–402

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

Polymorphism of Toll-like receptor 4 gene in bipolar disorder

José Oliveira [a,b,f], Marc Busson [a], Bruno Etain [b,d,e,f], Stéphane Jamain [b,d,e,f], Nora Hamdani [b,d,e,f], Wahid Boukouaci [a], Kahina Amokrane [a,c], Mériem Bennabi [a,f], Emmanuel Le Guen [a,b,f], Aroldo Ayub Dargél [a,b,f,g], Josselin Houenou [b,d,e,f], Rayna Ivanova [c], Frank Bellivier [b,d,e,f], Chantal Henry [b,d,e,f], Jean-Pierre Kahn [f,h], Dominique Charron [a,c,f,i], Rajagopal Krishnamoorthy [a,j], Laetitia Vervoitte [k], Marion Leboyer [b,d,e,f,1], Ryad Tamouza [a,c,f,i,*,1]

[a] INSERM, UMRS 940, Hôpital Saint-Louis, Paris F75010, France
[b] INSERM, U 955, IMRB, Psychiatrie Génétique, Créteil F94000, France
[c] Laboratoire Jean Dausset, Hôpital Saint-Louis, 1, avenue Claude Vellefaux, Paris F75010, France
[d] Université Paris-Est Créteil, UMR_S955, UPEC, Créteil F94000, France
[e] AP-HP, Pôle de Psychiatrie, groupe hospitalier Henri Mondor, Créteil F94000, France
[f] Fondation FondaMental, fondation de coopération scientifique, Créteil, France
[g] Laboratório de Psiquiatria Molecular, Centro de Pesquisas Experimentais, Hospital de Clínicas de Porto Alegre, Programa de Pós-Graduação em Medicina: Psiquiatria, Universidade Federal do Rio Grande do Sul, UFRGS, CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasil
[h] Service de Psychiatrie et Psychologie Clinique, CHU de Nancy, Hôpitaux de Brabois, F54500 Vandoeuvre Les Nancy, France
[i] Université Paris Diderot, Sorbonne Paris cité, Paris, France
[j] INSERM, U 763, Robert Debré Hospital, Paris F-75019, France
[k] Inserm U955, Centre d'Investigation Clinique 006 and plateforme de ressources biologiques, Hôpital Henri-Mondor, Creteil F-94000, France

ARTICLE INFO

Article history:
Received 10 July 2013
Received in revised form
29 September 2013
Accepted 30 September 2013
Available online 17 October 2013

Keywords:
Bipolar disorders
Innate immunity
TLR-4
Polymorphism

ABSTRACT

Background: Bipolar disorder (BD) is considered as a multifactorial disorder involving complex interactions between genetic and environmental factors, where immune dysfunction is thought to play a key etiopathogenic role. In particular, excess of winter births associated with early-life infections raise the possibility of the implication of innate immunity. Given the pivotal role of Toll-like receptor 4 (TLR-4), a major innate immune sensor molecule, we hypothesized that genetic variations of TLR-4 may be associated to BD.

Methods: Genomic DNAs from 572 BD patients and 202 healthy controls (HC) were analyzed for the distribution of six single nucleotides polymorphisms (SNPs) scattered along the TLR-4 locus (*rs*1927914, *rs*10759932, *rs*4986790, *rs*4986791, *rs*11536889 and *rs*11536891). Associations between BD and these polymorphisms were examined using the Chi-square test.

Results: We found that *rs*1927914 AA and *rs*11536891 TT genotype are more frequent in BD patients than in controls (corrected p; *pc*=.02 and .02 respectively) particularly in early-onset BD patients (*pc*=.004 and .006) born during the summer season (*pc*=.02 and .002 respectively). We also found that *rs*4986790 AG and *rs*4986791 CT genotypes were significantly associated with presence of autoimmune thyroiditis (*pc*=.002).

Limitations: Our results are to be confirmed by replication in independent BD cohorts.

Conclusions: We report for the first time a genetic association between BD and TLR-4 a major player of innate immunity. Possible mechanisms underlying bipolar disorders linking altered TLR-4 expression and increased susceptibility to infections and/or autoimmunity are discussed.

1. Introduction

Bipolar disorder (BD) is a highly heritable and chronic mood disorder, known to be associated with substantial functional impairment, high health care costs and premature mortality (Leboyer and Kupfer, 2010). Classically described as a cyclical illness, with full blown manic or depressive episodes interspaced with normal euthymic periods, evidence now suggests that patients experience a more subtle chronic course than initially

* Corresponding author at: Laboratoire Jean Dausset, Hôpital Saint Louis, 1, avenue Claude Vellefaux, 75010 Paris, France. Tel.: +33 1 42 49 48 90; fax: +33 1 42 49 46 41.
E-mail address: ryadtamouza@yahoo.fr (R. Tamouza).
[1] RT and ML are co-senior authors.

0165-0327/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jad.2013.09.043

thought, characterized by residual symptoms, emotional dysregulation, sleep and circadian rhythm disturbances, cognitive impairment, and increased risk of psychiatric and medical comorbidities including autoimmune thyroid diseases (Leboyer and Kupfer, 2010). Accumulating knowledge tend to view BD as a progressive and multi-systemic disorder in which immune response dysfunctions seem to parallel the onset, progression and occurrence of medical comorbid disorders, in particular cardio-vascular disorders and diabetes (Berk et al., 2011; Leboyer et al., 2012). Such dysimmunity is mainly reflected by chronic low-grade inflammation behaving both as trait and state markers at the systemic level reflecting altered central nervous system (CNS) integrity (Dickerson et al., 2007; Goldstein et al., 2009; Hope et al., 2011; Modabbernia et al., 2013; Rao et al., 2010; Söderlund et al., 2011; Berk et al., 2010) and causal of cognitive decline with progressive atrophy of specific brain areas along disease progression.

Given the observed association between BD and environmental factors such as excess of winter births (5.8% winter excess of births) (Torrey et al., 1996), severe psychological stressors including childhood traumatic events observed in more than half of the patients (Etain et al., 2010) and associations with infectious events induced by neurotropic pathogens including *Toxoplasma gondii*, influenza virus or herpes simplex virus (HSV) (Dickerson et al., 2004, 2006; Gerber et al., 2012; Hamdani et al., 2012; Machón et al., 1997; Moore et al., 2001; Pearce et al., 2012; Tedla et al., 2011), we hypothesized that innate immune responses and their immunogenetic control could participate to the underlying mechanisms associated with BD.

Among the central players of immune first line defense, the Toll-like receptors (TLRs) are of particular interest because of their capacity to recognize and sense a wide range of pathogen-associated molecular patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs). Belonging to the pattern recognition receptor (PRR) family, the TLRs consist of eleven functional members (TLR-1–TLR-11) (Mukhopadhyay et al., 2004), classified into two subgroups based on their cellular localization. While the TLR-3, TLR-7, TLR-8, TLR-9, and TLR-10 are expressed exclusively in the intracellular compartments, the TLR-1, TLR-2, TLR-4, TLR-5, TLR-6 and TLR-11 are cell surface molecules (Akira et al., 2006; Venkatasubramanian and Debnath, 2013) and all of them are pivotal molecules for the induction of innate immune responses (Mogensen, 2009).

In this context, the prominent TLR-4 molecules constitute an excellent candidate to be studied in BD given (i) their specificity to recognize lipopolysaccharide (LPS) from gram-negative bacteria and various ligands from viruses, fungus, and mycoplasma as well as products of oxidative stress/inflammation (Piccinini and Midwood, 2010; Sirisinha, 2011), (ii) their capacity to induce specific intra-cellular signaling cascade that activate nuclear factor-kappa B (NF-κB) and other transcription factors which precede the synthesis of pro-inflammatory cytokines and inflammatory mediators (Janeway and Medzhitov, 2002), (iii) their expression in circulating immune cells as well as in brain by microglia, astrocytes, oligodendrocytes and neurons or thyroid cells (Hanke and Kielian, 2011; Nishimura and Naito, 2005; Okun et al., 2011; Suh et al., 2009), and (iv) their implication in CNS homeostasis and CNS immune surveillance (Rivest, 2009) during diseases of infectious or non-infectious origin including neurodegenerative pathological processes (Glass et al., 2010). TLR-4 molecules are encoded by a highly polymorphic gene exhibiting polymorphisms scattered along the promoter, coding and 3′-untranslated regions (3′UTR) (Netea et al., 2012). Furthermore, polymorphisms of the TLR-4 gene have been studied in a variety of clinical entities, eg. *viz* increased susceptibility to bacterial infection (Agnese et al., 2002), Alzheimer's disease (Balistreri et al., 2009; Minoretti et al., 2006; Wang et al., 2011), Crohn's disease (Shen et al., 2010), atherosclerosis (den Dekker et al., 2010) or cancer (Kutikhin, 2011) but not yet shown in psychiatric disorders. Only one recent work addressed the impact of various TLRs, including TLR-4, on pro-inflammatory cytokine production in supernatants of cultured blood cells from schizophrenia and BD patients after selective TLR agonist-induction (McKernan et al., 2011).

Given the high likelihood of innate-immune involvement in BD, as discussed above, we investigated, in a case-control study, the potential impact of TLR-4 polymorphism in susceptibility to BD.

2. Methods

2.1. Sample composition

Five hundred and seventy-two BD patients meeting DSM-IV criteria (American Psychiatric Association, 1994) for BD type I or II or not otherwise specified, consecutively admitted into three French university-affiliated psychiatry departments (Paris-Créteil, Bordeaux and Nancy), were interviewed by trained psychiatrists, using the French version of the Diagnostic Interview for Genetic Studies (DIGS version 3.0) (Nurnberger et al., 1994). All patients were euthymic at inclusion [*i.e.* having a Montgomery-Asberg Depression Rating Scale (Montgomery and Asberg, 1979) score and a Mania Rating Scale (Bech et al., 1978) score no more than five]. A set of demographic and/or clinical variables (Table 1) were assessed and recorded. Two hundred and two healthy controls (HC) were recruited and interviewed with the DIGS to assess personal and familial history of psychiatric disorders using the National Institute for Mental Health Family Interview for Genetic Studies (Maxwell, 1992). Only those, with neither personal nor family history (first degree) of psychiatric disorders, affective disorders or suicidal behavior were included. All patients and controls were of French descent, with at least three grandparents from the mainland of France and were consecutively recruited between February 2006 and January 2010. Among the clinical variables of interest, the age at onset (AAO) of BD was defined as the age at which the first mood episode (depressive, manic or hypomanic) occurred, as determined by reviewing medical records and information obtained with the DIGS. The threshold for early-onset BD (AAO before the age of 22 years) was defined on the basis of previous admixture analyses in four independent samples (Bellivier et al., 2003, 2001; Lin et al., 2006; Manchia et al., 2008) which identified three AAO subgroups: early-, intermediate- and late-onset. In order to have comparable subgroup sample size and taking into consideration the genetic homogeneity (Grigoroiu-Serbanescu et al., 2001) intermediate- and late-onset samples

Table 1
Demographic and clinical characteristics of study subjects.

	BD patients	Healthy controls
Mean age (years, y)	42 y (range: 16–67)	43 y (range: 19–64)
Sex		
Male	42% (216)	62% (101)
Female	58% (297)	38% (61)
Season of birth[a]	***n*=513**	***n*=157**
Winter	23% (118)	23% (36)
Spring	27% (138)	25% (39)
Summer	22% (113)	26% (41)
Autumn	28% (144)	26% (41)
Thyroiditis[a]	***n*=504**	***n*=202**
Yes	15% (77)	
No	85% (427)	100%

[a] Phenotype data were not available for the whole cohort.