



- (51) **International Patent Classification:**
C12Q 1/68 (2006.01)
- (21) **International Application Number:**
PCT/IL2009/001185
- (22) **International Filing Date:**
13 December 2009 (13.12.2009)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/201,515 11 December 2008 (11.12.2008) US
- (71) **Applicant** (*for all designated States except US*):
RAMOT AT TEL-AVIV UNIVERSITY LTD. [IL/IL];
Tel Aviv University Campus, The Senate Building,
Floor-1, P.O. Box 39296, 61392 Tel Aviv (IL).
- (72) **Inventors; and**
- (75) **Inventors/Applicants** (*for US only*): **NAVON, Ruth** [IL/
IL]; 24 Hameiri Street, 52651 Ramat Gan (IL).
BARUCH, Yekutiel [IL/IL]; 208 Bnei Efraim St., 69985
Tel Aviv (IL). **SILBERBERG, Gilad** [IL/IL]; 25 Hibner
Street, 49408 Petach Tikvah (IL).
- (74) **Agent:** **REINHOLD COHN AND PARTNERS;**
P.O.Box 13239, 61131 Tel Aviv (IL).
- (81) **Designated States** (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT,
TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

- (54) Title:** GENETIC MARKERS OF SCHIZOPHRENIA

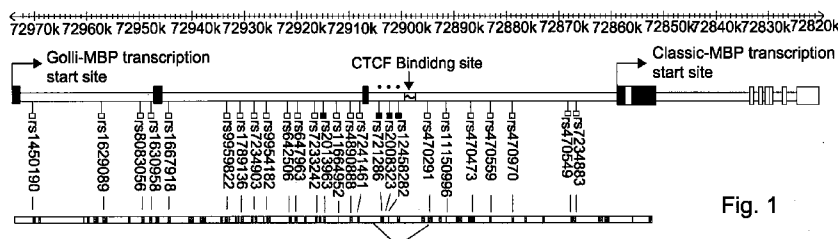


Fig. 1

- (S7) Abstract:** The present invention provides a method of diagnosing a predisposition of a subject to schizophrenia, by detecting the presence of single nucleotide polymorphisms (SNPs) in the *golli-MBP* region of the *MBP* (myelin basic protein) gene. This can be achieved by determining allelic variants of the *golli-MBP* region of the *MBP* gene (*golli-MBP*), wherein the presence of a risk-haplotype is associated with predisposition to schizophrenia and the presence of a protective-haplotype is associated with non-schizophrenic subjects. The invention further provides nucleic acid probes which allow detection of the SNPs, as well as kits comprising them. The invention further relates to the association of these SNPs with brain morphology changes.

GENETIC MARKERS OF SCHIZOPHRENIA

FIELD OF THE INVENTION

5 This invention relates to methods for genetic diagnosis and assessment of predisposition of a subject to mental or psychiatric disorder, particularly to schizophrenia.

BACKGROUND OF THE INVENTION

10 Schizophrenia is a severe and common psychiatric disorder with a lifetime risk of approximately 1% in the general population worldwide. It is a multi-factorial disorder, and its etiology includes strong genetic factors characterized by a complex inheritance pattern. Although the disease is predominantly hereditary, little is known about genetic factors and their effects on the pathophysiology of the disease.

15 Multiple studies have reported oligodendrocyte and myelin abnormalities, as well as dysregulation of their related genes, in brains of schizophrenia patients (Tkachev et al 2003).

 Myelin basic protein (*MBP*) encodes two families of proteins: classic-MBPs and golli-MBPs. While the classic-MBPs are predominantly located in the myelin sheaths
20 of the nervous system, the golli proteins are more widely expressed and are found in both the immune and the nervous systems.

Oligodendrocytes are responsible for the formation of myelin sheaths in the vertebrate central nervous system (CNS). Myelin basic protein (MBP), which is the second most abundant protein in the CNS myelin, is the only structural protein found so far to be
25 essential for its formation (Boggs 2006). The human MBP protein family arises from a genetic unit called *genes of the oligodendrocyte lineage* (golli), via use of various alternative splicing combinations among its 10 exons. The golli proteins are generated from the 5' transcription start site while the classic-MBPs are produced from a downstream transcription start site of the gene. Although they share some common
30 MBP epitopes, the golli products differ from the classic-MBPs due to the presence of 133 additional amino-acids encoded by exons upstream to the *classic-MBP* transcription

start site. While the classic-MBPs are predominantly located in the myelin sheaths of the nervous system, the golli proteins are more widely expressed and are found in both the immune and the nervous systems (Feng 2007). Golli knock-out (KO) mice, in which only the golli products of the MBP gene were ablated, have been reported to exhibit behavioral abnormalities reflective of neuronal abnormalities (Olmstead et al. 2000). Several studies have shown changes in *MBP* gene expression levels in schizophrenia and bipolar disorder in different brain regions (Chambers and Perrone-Bizzozero 2004, Haroutunian et al. 2007, Tkachev et al. 2003).

10 **References**

Ashburner, J., Friston, K.J., (2000) Voxel-based morphometry--the methods. *Neuroimage* 11, 805-821.

Boggs, J.M. (2006) Myelin basic protein: a multifunctional protein. *Cell Mol Life Sci*, 63, 1945-1961.

15 **Chambers, J.S. and Perrone-Bizzozero, N.I.** (2004) Altered myelination of the hippocampal formation in subjects with schizophrenia and bipolar disorder. *Neurochem Res*, 29, 2293-2302.

Crum, W.R., Griffin, L.D., Hill, D.L., Hawkes, D.J. (2003) Zen and the art of medical image registration: correspondence, homology, and quality.

20 *Neuroimage* 20, 1425-1437. **Feng, J.M.** (2007) Minireview: expression and function of golli protein in immune system. *Neurochem Res*, 32, 273-278.

Haroutunian, V., Katsel, P., Dracheva, S., Stewart, D.G. and Davis, K.L. (2007) Variations in oligodendrocyte-related gene expression across multiple cortical regions: implications for the pathophysiology of schizophrenia. *Int J Neuropsychopharmacol*, 10, 565-573.

25 **Korostishevsky, M., Kaganovich, M., Cholostoy, A., Ashkenazi, M., Ratner, Y., Dahary, D., Bernstein, J., Bening-Abu-Shach, U., Ben-Asher, E., Lancet, D.,**

Ritsner, M. and Navon, R. (2004) Is the G72/G30 locus associated with schizophrenia? single nucleotide polymorphisms, haplotypes, and gene expression analysis. *Biol Psychiatry*, 56, 169-176.

5 Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage* 19, 1233-1239.

Olmstead, C., Pribyl, T., Kampf, K., Jacobs, E., Campagnoni, C., Handley, V.S., E., Messing, A., Lazareff, J. and Campagnoni, A. (2000) Targeted ablation of the Golli products of the myelin basic protein gene: learning deficits. *Soc Neurosci Abstr* 26.30.17.

10 Shifman, S., Bronstein, M., Sternfeld, M., Pisante-Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R.D., Swartz-Vanetik, M., Knobler, H.Y., Shinar, E., Beckmann, J.S., Yakir, B., Risch, N., Zak, N.B. and Darvasi, A. (2002) A highly significant association between a COMT haplotype and schizophrenia. *Am J Hum Genet*, 71, 1296-1302.

15 Tkachev, D., Mimmack, M.L., Ryan, M.M., Wayland, M., Freeman, T., Jones, P.B., Starkey, M., Webster, M.J., Yolken, R.H. and Bahn, S. (2003) Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet*, 362, 798-805.

20 Torrey, E.F., Webster, M., Knable, M., Johnston, N. and Yolken, R.H. (2000) The stanley foundation brain collection and neuropathology consortium. *Schizophr Res*, 44, 151-155.

25 SUMMARY OF THE INVENTION

The present invention is based on the finding that single nucleotide polymorphisms (SNPs) in the *golli-MBP* region of the *MBP* gene are associated with schizophrenia. Specifically, the SNPs include rs12458282, rs2008323, rs721286 and any combination thereof. The SNPs of the present invention further include rs2013963, 30 rs470549, rs7234883, rs9960721, rs685975, and rs7233574.

Accordingly, these SNPs can serve as a basis for diagnosing a predisposition of a subject to schizophrenia. This can be achieved by determining allelic variants of the *golli-MBP* region of the *MBP* gene (*golli-MBP*), wherein the presence of a risk-

haplotype is associated with predisposition to schizophrenia and the presence of a protective-haplotype is associated with non-schizophrenic subjects.

Accordingly, in the first of its aspects, the present invention provides a method of diagnosing a predisposition of a subject to schizophrenia, wherein said method comprising testing a sample obtained from the subject for the presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (v) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (vi) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (vii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (viii) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- (ix) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and
- (x) any combination thereof;

wherein the presence of each of said single allele or haplotype is indicative of a predisposition of the subject to schizophrenia.

In an embodiment, the haplotype comprises allele C of the C/T polymorphism rs12458282, and allele T of the C/T polymorphism rs721286. In another embodiment, the haplotype comprises allele C of the C/T polymorphism rs12458282, and allele A of the A/G polymorphism rs2008323. Additionally, the haplotype may comprise allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286. The haplotype alternatively comprises allele C of the C/T polymorphism rs12458282, allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286. Additionally, the haplotype can comprise allele C of the C/A single nucleotide polymorphism (SNP) rs9960721, allele A of the C/A single nucleotide polymorphism (SNP) rs685975, and allele G of the A/G single nucleotide polymorphism (SNP) rs7233574.

In another aspect, the present invention provides a method of diagnosing a subject as having low predisposition to schizophrenia, wherein said method comprising

testing a sample obtained from the subject for the presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

- (i) allele C of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele G of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele T of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele G of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (v) allele G of the A/G single nucleotide polymorphism (SNP) rs470549;
- (vi) allele G of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (vii) allele A of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (viii) allele C of the C/A single nucleotide polymorphism (SNP) rs685975;
- (ix) allele A of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and
- (x) any combination thereof;

wherein the presence of each of said single allele or haplotype is indicative of having low predisposition of the subject to schizophrenia.

The haplotype may comprise allele T of the C/T polymorphism rs12458282, and allele C of the C/T polymorphism rs721286. Additionally, the haplotype may comprise allele T of the C/T polymorphism rs12458282, and allele G of the A/G polymorphism rs2008323. The haplotype may further comprise allele G of the A/G polymorphism rs2008323, and allele C of the C/T polymorphism rs721286. The haplotype may comprise allele T of the C/T polymorphism rs12458282, allele G of the A/G polymorphism rs2008323, and allele C of the C/T polymorphism rs721286. Additionally, the haplotype can comprise allele A of the C/A single nucleotide polymorphism (SNP) rs9960721, allele C of the C/A single nucleotide polymorphism (SNP) rs685975, and allele A of the A/G single nucleotide polymorphism (SNP) rs7233574.

In another aspect, the present invention provides a method of diagnosing a predisposition of a subject to schizophrenia, wherein said method comprising testing a sample obtained from the subject for the presence of at least one genotype of the *golli-MBP* region selected from the group consisting of:

- (i) T/T genotype of a single nucleotide polymorphism (SNP) rs721286;
- (ii) A/A genotype of a single nucleotide polymorphism (SNP) rs2008323;

- (iii) C/C genotype of a single nucleotide polymorphism (SNP) rs12458282;
- (iv) A/A genotype of a single nucleotide polymorphism (SNP) rs2013963;
- (v) A/A genotype of a single nucleotide polymorphism (SNP) rs470549;
- (vi) A/A genotype of a single nucleotide polymorphism (SNP) rs7234883;
- 5 (vii) C/C genotype of a single nucleotide polymorphism (SNP) rs9960721;
- (viii) A/A genotype of a single nucleotide polymorphism (SNP) rs685975;
- (ix) G/G genotype of a single nucleotide polymorphism (SNP) rs7233574;
- and
- (x) any combination thereof;

10

wherein the presence of each of said genotype is indicative of a predisposition of the subject to schizophrenia.

In accordance with the invention the subject may be human. Additionally, the subject may be an embryo, a fetus, a child, or an adult.

15

In one embodiment, the sample is a body fluid, preferably blood, saliva, cerebrospinal fluid, urine, or sperm.

20

In an embodiment, the testing for polymorphism is performed by SNP genotyping. In one embodiment, the SNP genotyping is performed by a method selected from the group consisting of (a) a primer extension assay; (b) PCR assay; (c) an allele-specific PCR assay; (d) a nucleic acid amplification assay; (e) a hybridization assay; (f) a mismatch-detection assay, (g) an enzymatic nucleic acid cleavage assay, and (h) a sequencing assay.

25

Additionally, the methods of the present invention can further comprise measuring a clinical symptom of the subject. Clinical symptoms may be those known by physicians from literature or diagnosis manuals such as Diagnostic and Statistical Manual of Mental Disorders DSM-IV (American Psychiatric Association, 1994). The clinical symptoms may be used for increasing the confidence level results of the methods of the present invention.

30

The methods of the invention may further comprise performing morphological analysis of the subject's brain for detecting aberrant brain morphology and microstructure changes. In one embodiment the morphological analysis is performed using magnetic resonance imaging (MRI). In one particular embodiment the

morphological analysis is performed using functional magnetic resonance imaging (fMRI).

The present invention further provides nucleic acid probes comprising a nucleotide sequence which hybridizes with at least a portion of golli-MBP region and which allows detection of at least one SNP. In preferred embodiments said at least one SNP is selected from the group consisting of rs12458282, rs2008323, rs721286, rs2013963, rs470549, rs7234883, rs9960721, rs685975, rs7233574 or any combination thereof. In one embodiment said probe is used in diagnosis of schizophrenia or predisposition to schizophrenia

In another embodiment, the nucleic acid probe can also be used in correlating at least one SNP of golli-MBP region with the outcome of a schizophrenia treatment.

The present invention further provides the use of nucleic acid probes comprising a nucleotide sequence which hybridizes with at least a portion of golli-MBP region and which allow detection of at least one SNP therein in diagnosis of schizophrenia or predisposition to schizophrenia. In preferred embodiments said at least one SNP comprises rs12458282, rs2008323, rs721286, rs2013963, rs470549, rs7234883, rs9960721, rs685975, rs7233574 or any combination thereof.

In another embodiment, the present invention relates to a kit for carrying out any the methods provided herein. In one embodiment, the kit is compartmentalized to receive a reagent for measuring single nucleotide polymorphism (SNP) in the *golli-MBP* region in a nucleic acid sample of a subject, said kit comprising at least one oligonucleotide that interacts with a single nucleotide polymorphism (SNP) in the *golli-MBP* polynucleotide region wherein said SNP polymorphism is selected from the group consisting of rs12458282, rs2008323, rs721286, rs2013963, rs470549, rs7234883, rs9960721, rs685975, rs7233574 and any combination thereof.

The present invention further provides an array comprising a substrate having a plurality of segments, wherein at least one segment comprises a probe for detecting at least one single nucleotide polymorphisms (SNP) from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele C of the C/T single nucleotide polymorphism (SNP) rs721286;
- (v) allele G of the A/G single nucleotide polymorphism (SNP) rs2008323;

- (vi) allele T of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (vii) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (viii) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (ix) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- 5 (x) allele G of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (xi) allele G of the A/G single nucleotide polymorphism (SNP) rs470549;
- (xii) allele G of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (xiii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xiv) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- 10 (xv) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- (xvi) allele A of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xvii) allele C of the C/A single nucleotide polymorphism (SNP) rs685975;
- (xviii) allele A of the A/G single nucleotide polymorphism (SNP) rs7233574;

15 for use in diagnosis of schizophrenia or assessment of predisposition to schizophrenia.

The present invention further provides use of the array in diagnosis of schizophrenia or predisposition to schizophrenia.

In another aspect, the present invention provides a method for obtaining information regarding a predisposition of a subject to develop schizophrenia, said
 20 method comprising testing a sample obtained from the subject for the presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- 25 (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282.
- (iv) allele C of the C/T single nucleotide polymorphism (SNP) rs721286;
- (v) allele G of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (vi) allele T of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (vii) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- 30 (viii) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (ix) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (x) allele G of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (xi) allele G of the A/G single nucleotide polymorphism (SNP) rs470549;

- (xii) allele G of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (xiii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xiv) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- (xv) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- 5 (xvi) allele A of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xvii) allele C of the C/A single nucleotide polymorphism (SNP) rs685975;
- (xviii) allele A of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and

- (xix) any combination thereof;

10 wherein the presence of each of said single allele or haplotype is indicative of the predisposition of the subject to schizophrenia.

The information can be used for various purposes, for example to classify a subject in a clinical trial, or to screen populations.

15 Additionally, the present invention provides a method of diagnosing a subject having schizophrenic symptoms as a schizophrenic, comprising:

(a) diagnosing a predisposition of a subject to schizophrenia, comprising testing a sample obtained from the subject for the presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

1. allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- 20 2. allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
3. allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
4. allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
5. allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
6. allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- 25 7. allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
8. allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
9. allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and
10. any combination thereof;

30 (b) identifying at least one schizophrenia symptom and optionally at least one schizophrenia risk factor in said subject;

wherein presence of each of said single allele or haplotype in step (a) combined with the presence of at least one symptom or risk factor in step (b) is indicative of the subject as being schizophrenic.

It is therefore contemplated that the above described methods of the invention
5 may be utilized in combination with additional schizophrenia-identifying markers (e.g. genetic markers) other than those disclosed in the present application.

In another aspect the present invention provides a method of determining a predisposition of a subject to having an aberrant brain morphology and microstructure changes, said method comprising testing a sample obtained from the subject for the
10 presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282; and
15 (iv) any combination thereof;

wherein presence of each of said allele or haplotype is indicative of a predisposition to having an aberrant brain morphology.

In one embodiment the haplotype comprises allele C of the C/T polymorphism rs12458282, allele A of the A/G polymorphism rs2008323, and allele T of the C/T
20 polymorphism rs721286.

In some embodiments, aberrant brain morphology is manifested by at least one parameter selected from the group consisting of:

- (i) an increase in apparent diffusion coefficient (ADC);
- (ii) a change in fractional anisotropy (FA); and
25 (iii) a change in gray matter volume.

In one embodiment, the apparent diffusion coefficient (ADC) increase is observed in a brain region selected from the group consisting of: Right – middle temporal gyrus; Left - post central gyrus; Cerebellum; Post central lobule; Precuneus; Posterior cingulate cortex; and the anterior cingulated cortex (ACC).

30 In one embodiment, the fractional anisotropy (FA) change is observed as a decrease in FA in a brain region selected from the group consisting of: Left – superior longitudinal fasciculus; Right – superior frontal gyrus; Right - middle temporal gyrus; Right – medial frontal gyrus; anterior cingulated cortex and the Cerebellum.

In one embodiment, the fractional anisotropy (FA) change is observed as an increase in FA in a brain region selected from the group consisting of: Left – superior longitudinal fasciculus; Left Insula and the Claustrum.

In one embodiment, the change in gray matter volume is observed as a gray matter volume decrease in a brain region selected from the group consisting of: Left - medial frontal gyrus; Left - middle temporal gyrus; Left Insula; Left – superior temporal gyrus; Cerebellum; Lingual Gyrus; Right – superior frontal gyrus; Left – inferior frontal gyrus; Right - medial frontal gyrus; post central gyrus; Post central lobule and the anterior cingulate cortex.

In one embodiment, the change in gray matter volume is observed as a gray matter volume increase in a brain region selected from the group consisting of: Left – parahippocampal gyrus; Left - Claustrum and left amygdala.

In a further aspect, the present invention relates to a nucleic acid probe comprising a nucleotide sequence which hybridizes with at least a portion of golli-MBP region which allows detection of at least one polymorphism therein for use in correlating at least one polymorphism of golli-MBP region with the outcome of a schizophrenia treatment. In some embodiments, the polymorphism of golli-MBP region is an allele or haplotype of the *golli-MBP* region selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (v) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (vi) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (vii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (viii) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- (ix) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574; and
- (x) any combination thereof;

wherein the presence of each of said single allele or haplotype is indicative of a predisposition of the subject to schizophrenia.

In another aspect, the present invention is directed to a method for obtaining information regarding a predisposition of a subject to develop schizophrenia, the method comprising testing a sample obtained from the subject for the presence of at

least one allele or haplotype of the *golli-MBP* region selected from the group consisting of: allele T of the C/T single nucleotide polymorphism (SNP) rs721286; allele A of the A/G single nucleotide polymorphism (SNP) rs2008323; allele C of the C/T single nucleotide polymorphism (SNP) rs12458282; allele C of the C/T single nucleotide polymorphism (SNP) rs721286; allele G of the A/G single nucleotide polymorphism (SNP) rs2008323; allele T of the C/T single nucleotide polymorphism (SNP) rs12458282; allele A of the A/G single nucleotide polymorphism (SNP) rs2013963; allele A of the A/G single nucleotide polymorphism (SNP) rs470549; allele A of the A/G single nucleotide polymorphism (SNP) rs7234883; allele G of the A/G single nucleotide polymorphism (SNP) rs2013963; allele G of the A/G single nucleotide polymorphism (SNP) rs470549; allele G of the A/G single nucleotide polymorphism (SNP) rs7234883; allele C of the C/A single nucleotide polymorphism (SNP) rs9960721; allele A of the C/A single nucleotide polymorphism (SNP) rs685975; allele G of the A/G single nucleotide polymorphism (SNP) rs7233574; allele A of the C/A single nucleotide polymorphism (SNP) rs9960721; allele C of the C/A single nucleotide polymorphism (SNP) rs685975; allele A of the A/G single nucleotide polymorphism (SNP) rs7233574; and any combination thereof;

wherein the presence of each of the allele or haplotype is indicative of the predisposition of the subject to schizophrenia, thereby obtaining information regarding a predisposition of a subject to develop schizophrenia.

In some embodiments, the information classifies a subject in a clinical trial. In other embodiments, the information classifies a population of subjects. In a specific embodiment, information correlates a specific haplotype/genotype with an outcome of a therapy for schizophrenia.

BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Figure 1 is a schematic illustration of the genomic structure of the *MBP* gene.

Figure 2 shows a portion of the *golli-MBP* nucleotide sequence (SEQ ID NO:1) that comprises rs721286 in accordance with an embodiment of the present invention;

Figure 3 shows a portion of the *golli-MBP* nucleotide sequence (SEQ ID NO:2) that comprises rs2008323 in accordance with an embodiment of the present invention;

Figure 4 shows a portion of the *golli-MBP* nucleotide sequence (SEQ ID NO:3) that comprises rs12458282 in accordance with an embodiment of the present invention;

Figure 5 shows a portion of the golli-MBP nucleotide sequence (SEQ ID NO:4) that comprises rs2013963 in accordance with an embodiment of the present invention;

Figure 6 shows a portion of the golli-MBP nucleotide sequence (SEQ ID NO: 5) that comprises rs470549 in accordance with an embodiment of the present invention;

5 **Figure 7** shows a portion of the golli-MBP nucleotide sequence (SEQ ID NO:6) that comprises rs7234883 in accordance with an embodiment of the present invention;

Figure 8 shows a portion of the golli-MBP nucleotide sequence (SEQ ID NO:7) that comprises rs9960721 in accordance with an embodiment of the present invention;

10 **Figure 9** shows a portion of the golli-MBP nucleotide sequence (SEQ ID NO:8) that comprises rs685975 in accordance with an embodiment of the present invention;

Figure 10 shows a portion of the golli-MBP nucleotide sequence (SEQ ID NO:9) that comprises rs7233574 in accordance with an embodiment of the present invention;

15 **Figure 11** provides statistical parametric maps showing gray matter volume changes in risk-haplotype carriers compared with non-carriers. (A) Focusing on the ACC gray matter and (B) focusing on the left Amygdala gray matter.

Figure 12 provides statistical parametric maps showing FA changes in risk-haplotype carriers compared with non-carriers. (A) Focusing on the ACC and (B) focusing on the left SFG.

20 **Figure 13** provides a statistical parametric map showing ADC elevation in risk-haplotype carriers compared with non-carriers.

DETAILED DESCRIPTION OF EMBODIMENTS

Terms and definitions

25 As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides and reference to "the SNP" includes reference to one or more SNPs and so forth.

30 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

For the purposes of the present invention, an “*allele*” is a particular form of a genetic locus, distinguished from other forms by its particular nucleotide sequence, or one of the alternative polymorphisms found at a polymorphic site.

5 The term “*gene*” refers to a segment of genomic DNA that contains the coding sequence for a protein, wherein the segment may include promoters, exons, introns, and other untranslated regions that control expression.

The term “*genotype*” is a 5' to 3' sequence of nucleotide pair(s) found at a set of one or more polymorphic sites in a locus on a pair of homologous chromosomes in an individual. As used herein, genotype includes a full-genotype and/or a sub-
10 genotype. “*Genotyping*” shall mean a process for determining a genotype of an individual. The term “*haplotype*” shall mean a 5' to 3' sequence of nucleotides found at a set of one or more polymorphic sites in a locus on a single chromosome from a single individual. “*Haplotyping*” shall mean a process for determining one or more haplotypes in an individual and includes molecular techniques, and/or statistical inference.

15 The term “*golli-MBP region*” or “*golli-MBP*” refers to a genetic unit called *genes of the oligodendrocyte lineage*.

The term “*risk-haplotype*” shall refer to an allele or haplotype, the presence of which is associated with predisposition to schizophrenia according to the present invention.

20 The term “*protective-haplotype*” shall refer to an allele or haplotype, and the presence of which is associated with non-schizophrenic subjects.

A “*predisposition*” or “*susceptibility*” of a subject to schizophrenia indicates that a subject has a tendency with a specified degree of statistical confidence to develop the disorder. The degree is determined by effect-sizing parameters (such as odds-ratio)
25 relevant to the studied ethnic group.

A “*genetic locus*” refers to a location on a chromosome or DNA molecule corresponding to a gene or a physical or phenotypic feature, where physical features include polymorphic sites.

The term “*polymorphism*” shall refer to a sequence variation observed in an
30 individual at a polymorphic site. Polymorphisms include nucleotide substitutions, insertions, deletions, and microsatellites and may but need not, result in detectable differences in gene expression or protein function. A “*single nucleotide polymorphism*” or “*SNP*” is a single nucleotide variation at a polymorphic site.

An "*oligonucleotide probe*" or a "*primer*" refers to a nucleic acid molecule of between 8 and 2000 nucleotides in length, or is specified to be about 6 and 1000 nucleotides in length. More particularly, the length of these oligonucleotides can range from about 8, 10, 15, 20, or 30 to 100 nucleotides, but will typically be about 10 to 50 (e.g., 15 to 30 nucleotides). The appropriate length for oligonucleotides in assays of the invention under a particular set of conditions may be empirically determined by one of skill in the art.

The term "*schizophrenia*" refers to a psychiatric disorder diagnosis that describes a mental disorder characterized by abnormalities in the perception or expression of reality. Typically, schizophrenia is diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association 1994). As used herein the term "Schizophrenia" shall also encompass schizophrenia related disorders such as but not limited to schizoaffective disorder, schizophreniform disorder, schizotypal personality disorder, schizotypy, a typical psychotic disorder, delusional disorder, brief psychotic disorder, avoidant personality disorders, bipolar disorder, and attention deficit hyperactivity disorder. It is to be understood that the methods, probes, arrays and kits of the present invention can also be used with respect to these conditions and disorders as well as for schizophrenia.

A schizophrenic person, i.e. a person suffering from schizophrenia, may exhibit numerous symptoms such as, but not limited to, abnormalities in the perception or in the expression of reality. Schizophrenia is typically manifested by various symptoms such as, for example, auditory hallucinations, paranoid or even bizarre delusions. Schizophrenia may also be manifested in disorganized speech and unusual thinking characterized by significant social or occupational dysfunction. Disorganized speech may be in the form loosely connected sentences and meaning, incoherence (word salad). A schizophrenic person may also suffer from loss of train of thought (thought disorder) and subject flow. In one uncommon species of schizophrenia, the person may be mute, motionless, or exhibit purposeless agitation, and other signs of catatonia. As a result the person may be socially impaired.

The standardized criteria for diagnosing schizophrenia are derived from the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, version DSM-IV-TR, and also the World Health Organization's International Statistical Classification of Diseases and Related Health Problems, the ICD-10.

The current classification of psychoses holds that symptoms need to have been present for at least one month in a period of at least six months of disturbed functioning.

The factors and symptoms of schizophrenia shall be referred to herein as "*schizophrenia symptoms*".

5 As used herein a "*sample*" refers to any biological sample obtained from a subject which is suitable for isolation of nucleic acids. Such biological sample may be obtained from e.g. blood, saliva, cerebrospinal fluid, urine, feces or sperm.

SNPs are common single nucleotide variations that occur in a population. SNPs are obtainable from various repositories such as Ensembl, a joint project between
10 EMBL - European Bioinformatics Institute (EBI) and the Wellcome Trust Sanger Institute (WTSI), or the dbSNP, the SNP repository maintained by NCBI, The Human Genic Bi-Allelic Sequences Database (HGVBBase) and The SNP Consortium Ltd.(TSC).

International collaborations have envisaged classifying SNPs for genomes of numerous species including Homo sapiens, Mus musculus, and plant genomes. As a
15 mere example, HapMap project seek to identify the genetic patterns of human DNA sequence variation. Information such as SNP genotypes, recombination rates may be downloaded from the HapMap website (www.hapmap.org).

SNPs are conventionally identified by their relative position within a nucleotide sequence. Typically, following identification of an SNP a database reference is provided,
20 "rs" number/SNP ID number. Consequently, sequence and other information related with a given "rs" number/SNP ID number may be obtained by browsing, for example, the dbSNP of the Entrez SNP which is provided by the NCBI, at www.ncbi.nlm.nih.gov.

As used herein "*magnetic resonance imaging*" or "*MRI*" - refers to an imaging method to visualize detailed internal structure and function of the brain.

25 In the form of Functional MRI (fMRI) it enable measurement of signal changes in the brain that are due to changing neural activity. In the form of diffusion tensor imaging (DTI) it allows an accurate measure of myelin and myelination disruptions. DTI enables quantification of fractional anisotropy (FA) and apparent diffusion coefficient (ADC) of water molecules and can provide high-contrast images of the
30 brain's white matter.

As used herein "*brain morphology*" shall refer to the brain morphology of a subject and shall include at least one of the following structural features: morphological volume measurements of brain structures, gray matter, white matter, myelination of

brain tissue, fiber organization, axon morphology, fractional anisotropy (FA) and apparent diffusion coefficient (ADC).

As used herein "*aberrant brain morphology*" shall refer to at least one morphological brain feature selected from the group consisting of: myelin and myelination disruption, aberrant fiber organization or aberrant axon morphology, abnormally small axons, aberrant water content in the brain's regions, decrease of cellular compartment or increase of extra-cellular volume.

As used herein the term "*gray matter*" (or "*grey matter*") shall refer to the component of the central nervous system, consisting of neuronal cell bodies, neuropil (dendrites and axons), glial cells (astroglia and oligodendrocytes), and capillaries. Grey matter contains neural cell bodies, in contrast to white matter, which mostly contains myelinated axon tracts.

As used herein the term "*array*" (also termed herein "*microarray*") refers to a two dimensional systematic arrangement of oligonucleotides, usually in rows and columns on a solid substrate. The array may consist of series of thousands of microscopic spots of oligonucleotides, each containing minute amounts of the oligonucleotide probes. The probes are used to hybridize with samples of nucleic acids under high-stringency conditions. Probe-target hybridization may be detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target.

Methods for the preparation of arrays are well known in the art, the probes may be attached to the solid surface by a covalent bond to a chemical matrix (for example via epoxy-silane, amino-silane, lysine, polyacrylamide or others). The solid surface can be glass, a silicon chip, or microscopic beads.

In the context of the present invention the array or microarray is used to detect SNPs.

The present invention utilizes single nucleotide polymorphisms in the *golli-MBP* region for diagnosing a subject for predisposition to schizophrenia. The invention contemplates, inter alia, SNP genotyping of *golli-MBP* region, e.g. by allele-specific PCR and can be used to develop polynucleotide-based tests for use as prognostics to predict a subject susceptibility to schizophrenia. The tests for the specific SNPs

disclosed herein in the *golli-MBP* region will enable more accurate prediction and mental and psychiatric diagnosis, thereby leading to better treatment of the subject.

The invention also provides oligonucleotides, kits, devices, and substrates useful for detecting polymorphisms associated with subjects suffering or having a genetic predisposition to suffer from schizophrenia.

Oligonucleotide primers and probes of the present invention can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical synthesis. Oligonucleotide probes and primers can comprise DNA, RNA, nucleic acid analogs such as, for example peptide nucleic acids, locked nucleic acid (LNA) analogs, and morpholino analogs. The 3' end of the probe can be functionalized with a capture or a detectable label to assist in detection of a polymorphism. Any of the oligonucleotides or nucleic acids of the invention can be labeled by incorporating a detectable label measurable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, such labels can comprise radioactive substances (^{32}P , ^{35}S , ^3H , ^{125}I), fluorescent dyes (5-bromodesoxyuridin, fluorescein, acetylaminofluorene, digoxigenin), biotin, nanoparticles, and the like. Such oligonucleotides are typically labeled at their 3' and 5' ends.

A probe refers to a molecule which can detectably distinguish between target molecules differing in structure. Detection or testing for a single nucleotide polymorphism (SNP) can be accomplished in a variety of different ways depending on the type of probe used and the type of target molecule. Thus, for example, detection may be based on discrimination of activity levels of the target molecule, but typically is based on detection of specific binding. Non exhaustive examples of such specific binding include antibody binding and oligonucleotide probe hybridization, amplification techniques or others described below. Thus, for example, probes can include enzyme substrates, antibodies and antibody fragments, oligonucleotide hybridization probes and oligonucleotide primers.

Therefore, in one embodiment, the detection of the presence or absence of the at least one variant, or single nucleotide polymorphism (SNP) involves contacting a target polymorphic site with a probe, typically an oligonucleotide probe, where the probe binds or hybridizes with a form of the target polymorphic site (e.g., the target nucleic acid containing a complementary base at the variance site as compared to hybridization

to a form of the target nucleic acid having a non-complementary base at the variance site, where the hybridization is carried out under selective hybridization conditions). An oligonucleotide probe may span two or more variance sites. Unless otherwise specified, an oligonucleotide probe can include one or more nucleic acid analogs, labels or other substituents or moieties so long as the base-pairing function is retained.

The polymorphisms associated herein with subjects having a genetic predisposition to suffer from schizophrenia disclosed in the present invention, comprise the following SNPs:

- (a) rs721286;
- (b) rs2008323; and
- (c) rs12458282.

The specific alleles indicating predisposition or risk of manifestation of a schizophrenia can be selected from the group of:

- (a) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (b) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- and
- (c) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282.

The polymorphisms associated with subjects having a genetic predisposition to suffer from a schizophrenia disclosed in the present invention, may further comprise the following SNPs:

- (a) rs2013963;
- (b) rs470549; and
- (c) rs7234883.

The specific alleles indicating predisposition or risk of manifestation of schizophrenia may further be selected from the group of:

- (a) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (b) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- and
- (c) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883.

The polymorphisms associated with subjects having a genetic predisposition to suffer from schizophrenia disclosed in the present invention, may further comprise the following SNPs:

- (a) rs9960721;

(b) rs685975; and

(c) rs7233574.

The specific alleles indicating predisposition or risk of manifestation of schizophrenia may further be selected from the group of:

5 (a) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;

(b) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
and

(c) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574.

10 In an embodiment of the present invention, the presence of any SNP of the present invention is determined in relation to the adjacent nucleotide sequence upstream and downstream from any of the polymorphic site disclosed herein, as a non-limiting example, about 14 nucleotides upstream and about 14 nucleotides downstream of the polymorphic site.

15 It should be understood that the present invention further contemplates that the presence of a specific haplotype or genotype may be determined in relation to a nucleotide sequence comprising 17, 24, 28, even 40 or more nucleotides upstream, or indeed any number within these ranges, and 17, 24, 28, even 40 or more nucleotides downstream or any number within these ranges, with respect to a polymorphic site disclosed herein.

20 Figure 1 schematically illustrates the respective loci of rs721286, rs2008323, rs12458282, rs2013963, rs470549, and rs7234883 at the MBP gene. Top figure 1 is a schematic illustration of the genomic structure of the *MBP* gene. Exons colored in black are *golli-MBP* exons and those colored in white are *classic-MBP* exons. Location of the 26 studied markers is shown; SNPs colored in black had significantly ($P < 0.05$) different genomic/allele distribution between patients and healthy controls. Markers marked with
25 “*” are of haplotype rs721286-rs2008323-rs12458282 that was genotyped in the second cohort. Linkage disequilibrium plot of the gene in the CEU population from the HapMap database (www.hapmap.org), adapted from Haploview (www.broad.mit.edu/mpg/haploview) was prepared.

30 Three SNPs of the present invention, namely, rs721286- rs2008323- rs12458282 are located approximately 4 kb downstream from the *golli-MBP* third exon. Interestingly, these SNPs represent a linkage disequilibrium (LD) block. The LD block with the associated haplotypes is marked at the bottom of Figure 1.

Although this strongly associated block encompasses an exon, its SNPs are intronic. Without being bound by theory, it is possible that these SNPs affect the regulation of the gene, by creating alternative secondary structures or by influencing interactions with the transcriptional machinery.

5 The respective location of each SNP in the sequence of the gene is shown in figures 2-10.

 The SNPs of the invention were identified in the Ashkenazi Jewish population. The Ashkenazi Jewish population is closely related to the general Caucasian population in terms of allele frequencies and linkage disequilibrium (Silberberg G et al. 2006).
10 Therefore, the SNPs and all other aspects of the invention can be used in Caucasian populations as well. Moreover, the use of the SNPs of the invention should not be limited to the Ashkenazi Jewish population or to Caucasian populations. The same SNPs may be schizophrenia-associated in other ethnic groups as well.

 In one embodiment of the invention, DNA is obtained from a subject. The
15 subject's DNA is then used to test for the presence of one or more of the schizophrenia-associated SNPs of the present invention. If the subject has been identified as carrying one or more of the SNPs then the subject is diagnosed as having a predisposition for schizophrenia.

 Therefore, in one aspect the present invention provides a method of diagnosing
20 a predisposition of a subject to schizophrenia which comprises testing a sample obtained from the subject for the presence of an allele or haplotype of the *golli-MBP* region indicative that the subject is susceptible to schizophrenia, wherein at least one haplotype is selected from the group consisting of:

- (a) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- 25 (b) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (c) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (d) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (e) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (f) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- 30 (g) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (h) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- (i) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and

(j) any combination thereof;

In other embodiments, the present invention provides a method of diagnosing a predisposition of a subject to schizophrenia which comprises testing a sample obtained
5 from the subject for the presence of an allele or haplotype of the *golli-MBP* region indicative that the subject is susceptible to schizophrenia, wherein said allele or haplotype is an SNP in complete LD with any of the group consisting of: the single nucleotide polymorphisms (SNPs) rs721286, rs2008323 or rs12458282; wherein the presence of each of said single allele or haplotype is indicative of a predisposition of the
10 subject to schizophrenia. In a specific embodiment, the presence of each of said single allele or haplotype is indicative of a predisposition to having an aberrant brain morphology or microstructure.

In a specific embodiment, where two or more SNPs associated with having a genetic predisposition to suffer from schizophrenia are detected within a sample
15 obtained from a subject then the confidence of the diagnostic result increases.

Therefore, in one embodiment, the haplotype may comprise allele C of the C/T polymorphism rs12458282, and allele T of the C/T polymorphism rs721286. In another embodiment, the haplotype comprises allele C of the C/T polymorphism rs12458282, and allele A of the A/G polymorphism rs2008323. Additionally, the haplotype may
20 comprise allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286. The haplotype may also comprise allele C of the C/T polymorphism rs12458282, allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286. The haplotype may also comprise allele C of the C/A single nucleotide polymorphism (SNP) rs9960721, allele A of the C/A single
25 nucleotide polymorphism (SNP) rs685975, and allele G of the A/G single nucleotide polymorphism (SNP) rs7233574.

Additionally, the SNPs identified herein can be used in combination with additional predictive tests including, but not limited to, additional SNPs, mutations, and clinical tests.

30 In another aspect, the present invention provides a method of diagnosing a subject as having low predisposition to schizophrenia, wherein said method comprising testing a sample obtained from the subject for the presence of at least one protective haplotype of the *golli-MBP* region.

The protective haplotype of the present invention may comprise allele T of the C/T polymorphism rs12458282, and allele C of the C/T polymorphism rs721286. Additionally, the protective haplotype may comprise allele T of the C/T polymorphism rs12458282, and allele G of the A/G polymorphism rs2008323. The protective
5 haplotype may further comprise allele G of the A/G polymorphism rs2008323, and allele C of the C/T polymorphism rs721286. The protective haplotype may comprise allele T of the C/T polymorphism rs12458282, allele G of the A/G polymorphism rs2008323, and allele C of the C/T polymorphism rs721286. The protective haplotype may comprise allele A of the C/A single nucleotide polymorphism (SNP) rs9960721,
10 allele C of the C/A single nucleotide polymorphism (SNP) rs685975, and allele A of the A/G single nucleotide polymorphism (SNP) rs7233574.

In another aspect, the present invention provides a method of diagnosing a subject as having low predisposition to schizophrenia, wherein said method comprising testing a sample obtained from the subject for the presence of at least one genotype of
15 the *golli-MBP* region. In some embodiments, the protective genotype is a G/G genotype of a single nucleotide polymorphism (SNP) rs2013963. In other embodiments, the protective genotype is a G/G genotype of a single nucleotide polymorphism (SNP) rs470549.

20 In another aspect, the present invention provides a method of diagnosing a predisposition of a subject to schizophrenia, wherein said method comprising testing a sample obtained from the subject for the presence of at least one genotype of the *golli-MBP* region selected from the group consisting of:

- (i) T/T genotype of a single nucleotide polymorphism (SNP) rs721286;
- 25 (ii) A/A genotype of a single nucleotide polymorphism (SNP) rs2008323;
- (iii) C/C genotype of a single nucleotide polymorphism (SNP) rs12458282;
- (iv) A/A genotype of a single nucleotide polymorphism (SNP) rs2013963;
- (v) A/A genotype of a single nucleotide polymorphism (SNP) rs470549;
- (vi) A/A genotype of a single nucleotide polymorphism (SNP) rs7234883;
- 30 (vii) C/C genotype of a single nucleotide polymorphism (SNP) rs9960721;
- (viii) A/A genotype of a single nucleotide polymorphism (SNP) rs685975;
- (ix) G/G genotype of a single nucleotide polymorphism (SNP) rs7233574;

and

(x) any combination thereof;

wherein the presence of each of said genotype is indicative of a predisposition of the subject to schizophrenia.

In accordance with the invention the subject may be human. Additionally, the subject can be an embryo, a fetus, a child, or an adult. In some embodiments, the human is of Caucasian descent.

In one embodiment, the sample is a body fluid, preferably blood, saliva, cerebrospinal fluid, urine, or sperm.

In an embodiment, the testing for polymorphism is performed by SNP genotyping. In one embodiment, the SNP genotyping is performed by a method selected from the group consisting of (a) a primer extension assay; (b) PCR assay; (c) an allele-specific PCR assay; (d) a nucleic acid amplification assay; (e) a hybridization assay; (f) a mismatch-detection assay, (g) an enzymatic nucleic acid cleavage assay, and (h) a sequencing assay.

Other methods and means for determining whether a particular SNP or group of polymorphisms is present within a subject, as would be known to a person of skill in the art can be employed in the practice of the present invention. By way of non-limiting example, PCR using primers comprising fluorescent probes is one exemplary technique. In a specific embodiment, primers may be derived from the following sequences:

20

GCGAAGTCTTAGGTCTCCCTAAAT[C/T]ATACTAAGGGAGGAGAGGAGTGGC
C, (SEQ ID: 10, per rs721286)

ACGTCACCAGCCCGCGTCTGTGTTC[A/G]CTGCGTCCACTTCACAGGGCCAA
GT, (SEQ ID: 11, per rs2008323)

25 CTCCATTGTCTGAGCAATGAAGTTG[C/T]GAATGTTTTCTCATGACTCTTCA
T, (SEQ ID: 12, per rs12458282).

AGCAGAAACAGTGGGATGTAGTGCC[A/G]CCCGCAGCGCAGTTACTCAAAG
TGA, (SEQ ID: 13, per rs2013963).

30 TCTGCATGAGTGATGCACGTTTCTCATCCTAGCAC[A/G]GGGAGAGCACTGG
CCTTCCTCAGCC, (SEQ ID: 14, per rs470549).

CTTGTGTCACGGCGCGCTGTGCTTG[A/G]GGTCACCAGGGAGGGCAAAGGA
AAC, (SEQ ID: 15, per rs7234883).

ATGTAAAGTAAAAATAAAGGCCCTAG[A/C]CCTCCAGGCAAGACGGAATAG
ACTC, (SEQ ID: 16, per rs9960721).

35 AGCACATAGGCAGGACATCCTAGTAC[C/A]AATGATGAATGAGTGTTTATGG
CAG, (SEQ ID: 17, per rs685975).

CAGGTCCTCAGAGGCACATGAAAAAT[A/G]CTAATGAACGGCAGCACTCATT
ATT, (SEQ ID: 18, per rs7233574).

For the purpose of the present invention "*schizophrenia-identifying marker(s)*" shall mean schizophrenia-identifying markers, genotype(s) and/or haplotype(s) or single allele(s) of genomic variations. It is therefore contemplated that the above described methods of the invention may be utilized in combination with additional schizophrenia-identifying markers. The schizophrenia-identifying markers may include, but are not limited to, the following polymorphic variations, as disclosed in WO2006/072075 and WO2008/093343: allele A at rs9494332 in C6orf217 which is linked to the human abelson Helper Integration Site 1 gene (AHI1); rs6925684, rs6902485, rs6935033, rs7739635, rs9494332 and rs1475069 on chromosome 6q23; rs911507 and rs12211505 on chromosome 6q23; rs737734, rs136770, rs763126, rs738598, rs1573726, rs138844, rs738596, rs135819, rs1573726, rs1053744, rs153221, rs2269523, rs737734, rs134474, rs134454, rs848768, and rs2073224 located at chromosome 22. The schizophrenia-identifying markers may also include polymorphisms in the genes dysbindin (DTNBP1), D-amino acid oxidase (DAAO), neuregulin1 (NRG1), D-amino acid oxidase activator (DAOA), catechol-O-methyltransferase (COMT) disclosed in WO 03/070082), regulator of G-protein signaling 4 (RGS4) disrupted-in-schizophrenia-1 (DISC1), proline dehydrogenase PRODH, trace amine receptor (TRAR4) reported in WO 2006/023719, human G protein coupled receptor Seq-40 disclosed in U.S. Patent Application Publication No. 20040115699, SP4 gene; other polymorphism associated with schizophrenia and schizophrenia related disorders located at chromosome 22; and polymorphisms of the Sulfotransferase 4A1 (Sult4a1) gene.

The methods of the present invention can further comprise measuring a clinical symptom of the subject. Clinical symptoms may be those known by physicians from literature or diagnosis manuals such as Diagnostic and Statistical Manual of Mental Disorders DSM-IV (American Psychiatric Association, 1994). The clinical symptoms may be used for increasing the confidence level results of the methods of the present invention.

The methods of the present invention optionally further comprise diagnosis of a schizophrenia risk factor. In one embodiment, the subject diagnosed has at least one additional risk factor associated with schizophrenia. The risk factor may typically comprise:

(1) the existence of a genetically based phenotypic trait associated with risk for schizophrenia;

(2) the subject has a family relative being selected from the group: a parent, uncle, aunt, grandparent, sibling, or child diagnosed as schizophrenic;

5 (3) mixed-handedness; and

(4) eye tracking dysfunction. Additionally, the present invention provides means for minimizing the effect of schizophrenia. The present invention can be used to identify, or diagnose and indeed allow early treatment of the prodromal phase of schizophrenia (pre-onset). This is achieved by early diagnosis of predisposition to
10 schizophrenia in combination with identification of other schizophrenia associated symptoms and risk factors characterizing pre-onset of the illness. As a mere non-limiting example, pre-onset of the illness is characterized by negative symptom of schizophrenia in which the individual lacks interest and drive i.e. avolition, or impairment of social behavior.

15 The present invention further provides a kit useful for identifying polymorphisms associated with predisposition to schizophrenia. For example, the kit of the invention can comprise one or more oligonucleotides designed for identifying both alleles for each SNP in the set of one or more SNPs of the present invention. In another embodiment, the kit further comprises a manual with instructions for performing one or
20 more reactions on a human nucleic acid sample to identify the allele or alleles present in the subject at each PS in the set of one or more SNPs.

The oligonucleotides in a kit of the invention may also be immobilized on or synthesized on a solid surface such as a microchip, bead, or glass slide (see, e.g., WO 98/20020 and WO 98/20019). Such immobilized oligonucleotides may be used in a
25 variety of polymorphism detection assays, including but not limited to probe hybridization and polymerase extension assays.

Kits of the invention may also contain other components such as hybridization buffer (e.g., where the oligonucleotides are to be used as allele-specific probes) or dideoxynucleotide triphosphates (ddNTPs; e.g., where the alleles at the polymorphic
30 sites are to be detected by primer extension). In a one embodiment, the set of oligonucleotides consists of primer-extension oligonucleotides. The kit may also contain a polymerase and a reaction buffer optimized for primer-extension mediated by the polymerase. Preferred kits may also include detection reagents, such as biotin- or

fluorescent-tagged oligonucleotides or ddNTPs and/or an enzyme-labeled antibody and one or more substrates that generate a *detectable* signal when acted on by the enzyme. It will be understood by the skilled artisan that the set of oligonucleotides and reagents for performing the genotyping or haplotyping assay will be provided in separate receptacles placed in the container if appropriate to preserve biological or chemical activity and enable proper use in the assay.

The present invention further provides a nucleic acid probe comprising a nucleotide sequence which hybridizes with at least a portion of golli-MBP region and which allows detection of at least one polymorphism.

The nucleic acid probe can be used for diagnosing schizophrenia, or for diagnosing a predisposition for schizophrenia. The nucleic acid probe can further be used in correlating at least one polymorphism of golli-MBP region with the outcome of a schizophrenia treatment.

EXAMPLES

The present invention focuses on the *golli-MBP* gene, by analyzing it in two large Jewish-Ashkenazi cohorts.

In particular, a case-control association analysis of *golli-MBP* in two separate Jewish-Ashkenazi cohorts was performed (cohort I: 120 patients, 236 controls; cohort II: 379 patients, 380 controls). In addition, an expression analysis of *golli-MBP* mRNA in postmortem dorsolateral prefrontal cortex (DLPFC) samples of schizophrenia patients, and matched controls was performed.

In the first cohort association between 6 (out of 26 tested) single nucleotide polymorphisms (SNPs) and the disease ($P < 0.05$) was observed. Out of these, 3 are from one linkage disequilibrium (LD) block which contains a CCCTC-binding factor (CTCF) binding region. As demonstrated below, haplotype analysis revealed significant risk- and protective-haplotypes (strongest $P = 0.005$, each) for schizophrenia. The 3 SNPs (rs12458282, rs2008323, rs721286) were then genotyped in the second cohort. The combined results showed strong effects, both in the single marker (strongest $OR = 1.77$, $P = 0.0005$) and in the haplotype analyses (strongest $OR = 1.61$, $P = 0.00001$). As demonstrated below, the present invention discloses that single nucleotide polymorphisms (SNPs) of the *golli-MBP* are susceptibility indicators for schizophrenia.

In addition, brain morphology and microstructure analyses using fMRI was performed in healthy subjects carrying the risk-haplotype: allele T of the C/T single nucleotide polymorphism (SNP) rs721286; allele A of the A/G single nucleotide polymorphism (SNP) rs2008323; and allele C of the C/T single nucleotide polymorphism (SNP) rs12458282 (referred to herein as the "TAC risk haplotype").

TAC risk-haplotype carriers showed significant gray matter volume reduction, as compared to non-risk-haplotype carriers, in several brain regions: left medial frontal gyrus, left middle temporal gyrus, the left insula, left superior temporal gyrus, and the anterior cingulate cortex. These brain regions have been previously reported to manifest gray matter deficits in schizophrenia (For example, see Wilke et al., 2001; Shenton et al., 2001; and Kubicki et al., 2002).

TAC risk-haplotype carriers also showed gray matter volume increase in the left side of the amygdala, claustrum and para-hippocampal gyrus. Increases of bilateral para-hippocampal gyrus volume have been reported in unaffected relatives of schizophrenia patients (Goghari et al., 2007).

Maps of Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) can be produced from the image analysis of the fMRI data.

A decrease in FA was found in the right hemisphere frontal and temporal gyrus and the anterior cingulate cortex, while an increase in FA was observed in the Insula, left superior frontal gyrus and Claustrum of TAC risk-haplotype carriers.

TAC risk-haplotype carriers also showed a wide-spread increase in the ADC in both hemispheres, including the anterior cingulate cortex, right middle temporal gyrus, left post central gyrus, cerebellum, post-central lobule, precuneus and posterior cingulate cortex.

The results showed herein indicate that the TAC risk haplotype is associated with morphological changes in specific brain regions.

At least some of these changes are similar to brain morphology changes observed in schizophrenia. These results provide further support for the association between the risk-haplotype and the schizophrenia.

MATERIALS AND METHODS

DNA Polymorphism in *golli-MBP*

Subjects The study enrolled two case/control groups. The first cohort included
5 120 unrelated Ashkenazi-Jewish schizophrenia patients (86 males, 34 females) and 236
unrelated control (143 males, 93 females) individuals. The patients included in this
group were 18–65 years old (average=38.2, SD=8.5) and met the DSM-IV criteria
(American Psychiatric Association, 1994) for schizophrenia. The patients were recruited
in Abarbanel hospital, Bat-Yam Israel, and signed informed consent forms. The control
10 group included non-clinical 22–54 years old (average=32.4, SD=5.8) ethnically-
matched subjects who approached the molecular genetics laboratory at Meir Hospital,
Kfar-Sava, Israel (run by R. Navon, 1990–2000). The second cohort included 379 (235
males, 144 females) unrelated Ashkenazi-Jewish schizophrenia patients which were
selected randomly from a larger cohort studied previously (Shifman et al. 2002). The
15 patients included in this group were diagnosed according to the DSM-IV, and were 18–
83 years old (average=46.5, SD=13.6). Control samples were acquired from 380 (319
males, 61 females) healthy 19–68 years old (average=39.6, SD=13.7) Ashkenazi-Jewish
individuals using blood bank volunteers. Only individuals who reported having 4
Ashkenazi-Jewish grandparents were included in the study. Controls were asked to
20 report on cases of psychiatric disorders in their families, and those who did were
excluded from the study. For patients in both cohorts, all diagnoses and exclusion
criteria were assigned by a standard procedure. This procedure included a direct
interview using the structured clinical interview for personality disorders (SCID), a
questionnaire with inclusion and exclusion criteria and cross-references to medical
25 records (for further detailing see Shifman et al. 2002). All samples were collected with
appropriate IRB (Internal Review Board) approvals and the participants voluntarily
signed an informed consent form prior to joining.

SNP selection SNPs included in the association analysis were selected from the
CEU (Caucasian European Utah) population in the HapMap project database
30 (<http://www.hapmap.com>), which highly resembles the Ashkenazi-Jewish population as
previously discussed [Korostishevsky et al. 2004]. Twenty six tagging SNP markers
(the minimal group of SNPs required to define all haplotypes in a given linkage
disequilibrium (LD) block), along the 123.5 kb DNA stretch of the two *golli-MBP*

transcripts, were selected for genotyping using the Haploview software, version 3.32 (Broad Institute of MIT and Harvard, Cambridge, MA, USA) (Figure 1). Screening criteria applied were: minor allele frequency > 0.1, minimum haplotype frequency > 0.05 and Hardy–Weinberg cutoff = 0.05.

5 **SNP genotyping** Genomic DNA from patients and controls was isolated from peripheral blood using the PureGene kit (Gentra, MN) according to the manufacturer's protocol and was second-handed for genotyping. SNP genotyping was performed by the high-throughput system of chip-based mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight; MALDI-TOF) (Sequenom, Inc., San Diego, CA,
10 <http://www.sequenom.com/>) using the iPLEX™ chemistry. All assays for the PCR and associated extension reactions were designed by the SpectroDESIGNER software (Sequenom) and primers were synthesized by Integrated DNA Technologies (Coralville, Iowa). The reaction volume of 5 µL contained 7.5 ng of genomic DNA, 100 nM each of
15 its 5' end, 0.5 mM of each deoxynucleoside triphosphate, 1.625 mM MgCl₂, reaction buffer, and 0.5 units of HotStarTaq DNA polymerase (Qiagen, Germany). PCR conditions were as follows: an initial denaturation step for 15 min at 94°C, followed by 45 cycles of 20 sec at 94°C, 30 sec at 56°C, 1 min at 72°C, and a final extension step for 3 min at 72°C. Allele-specific primer extensions were conducted with the Mass
20 EXTEND Reagents Kit containing di-deoxy nucleotides (Sequenom). The high-throughput liquid handling was performed with the aid of a MULTIMEK 96 automated 96-channel robot (Beckman Coulter, Fullerton, California). Primer extension products were loaded onto a 384-element chip (SpectroCHIP; Sequenom) by nanoliter pipetting robot (SpectroPOINT, Sequenom) and analyzed with a MassARRAY mass
25 spectrometer (Bruker Daltonik, Bremen, Germany). The resulting mass spectra were processed and analyzed for peak identification and allele determination with the MassARRAY 3.0 software (Sequenom). All SNPs were re-genotyped in 22 randomly-selected DNA samples, and 8-12 spots on each chip were loaded with water as negative controls. All replicates were in agreement with each other and no signal was observed in
30 the negative control wells.

Golli-MBP mRNA Quantification

Brain samples Samples from DLPFC (Brodmann area 46) were obtained from the Stanley Foundation Brain Collection and Neuropathology Consortium, Bethesda,

Maryland. The collection is well-matched for age, pH and gender (Torrey et al. 2000). Schizophrenic patients were diagnosed according to DSM-III-R (American Psychiatric Association, 1987) or DSM-IV (American Psychiatric Association, 1994) criteria.

RNA isolation and *golli-MBP* expression RNA isolation and cDNA synthesis procedures were performed as previously described (Korostishevsky et al. 2004). *Golli-MBP* mRNA expression was quantified in DLPFC brain samples obtained from 32 schizophrenia patients and 34 controls by real-time PCR using Absolute SYBR Green Mix (ABgene, Epsom, UK) on an ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, US). Amplification conditions were: 50°C for 2 min, 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. Real-time primers were designed using PrimerExpress software, with a specified amplicon length between 80 and 150 bp. Primer sequences for the *golli-MBP* gene were: forward, 5'-GAGAAGGCCAGTACGAATAG-3' (SEQ ID: 19) and reverse, 5'-GAACACTTCGTTGTCCTCTG-3' (SEQ ID: 20). Primer sequences for the reference gene TFRC (Transferrin receptor) were: forward, 5'-TGGCTACTTGGGCTATTGTAAACG -3' (SEQ ID: 21) and reverse, 5'-GGTGGTTCTGTTCCCTCTATCTCC -3' (SEQ ID: 22). TFRC gene was analyzed in our lab along with 15 other reference genes using a micro fluidic card (TaqMan® Gene Expression Assays) and was found to be the most stably expressed in the examined tissue (unpublished data). *Golli-MBP* mRNA quantity was expressed as the amount of target, normalized to an endogenous reference and relative to a calibrator according to the formula $2^{-\Delta\Delta C_t}$, where C_t is the threshold cycle, $\Delta C_t = C_t(\text{target}) - C_t(\text{reference})$, and $\Delta\Delta C_t = \Delta C_t(\text{sample}) - \Delta C_t(\text{calibrator sample})$. Real-time SYBR-green dissociation curves showed one species of amplicon for each primer combination (data not shown).

Genome analysis

The region surrounding the LD block with the associated SNPs was investigated for the presence of regulatory elements, transcription factor binding sites (TFBS) and conserved regions using the University of California, Santa Cruz (UCSC) Genome Browser (<http://genome.ucsc.edu>). The conservation among species, ESPERR (Evolutionary and Sequence Pattern Extraction through Reduced Representations) regulatory potential ("7x Reg potential" track), TFBS conservation and the ORegAnno (Open Regulatory Annotation) tracks were examined.

Polymerase chain reaction and DNA direct sequencing

Polymerase chain reaction (PCR) was used to amplify exon 3 and the CTCF-binding site region of *golli-MBP*. PCR was performed in a 50 µl reaction volume containing 100 ng genomic DNA, 0.2 µM dNTP mix, 10x reaction buffer, 5x enhancer solution, 10 µM of each primer and 2 units of Taq DNA polymerase (all reagents are from PeqLab, Erlangen, Germany). After DNA denaturation at 94°C for 3 minutes, PCR was performed in 36 cycles of 94°C for 30 seconds, 62°C (*golli-MBP* exon 3) or 56°C (*golli-MBP* CTCF) for 45 seconds, 72°C for 45 seconds, with a final extension step at 72°C for 10 minutes. The primer sequences were (amplicon length is in parentheses): *golli-MBP* exon 3: forward 5'-GTGCATGCCACCACACCT-3' (SEQ ID: 23) and reverse 5'-TGCTTAGGTGCTTAGGCAGA-3' (SEQ ID: 24) (597 bp); *golli-MBP* CTCF region: forward 5'-AAAAATCAGGGGAATATTATTACAAG-3' (SEQ ID: 25) and reverse 5'-CAGTTATACTCCATTACAGGATGCTT-3' (SEQ ID: 26) (818 bp). PCR products were purified using High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions, and visualized on 2% agarose gels. Direct sequencing was carried out by using the ABI PRISM Big Dye Terminator Cycle Sequencing kit and the ABI PRISM 3100 automated sequencer (Applied Biosystems).

Statistical analysis

Allele, genotype and haplotype frequencies were compared between patients and controls using the chi-square test. Simulated *P*-values (10,000 iterations for single markers and haplotypes) were calculated using the Haplo.Stats package in the "R" software (www.R-project.org). SNPs rs2013963 and rs470549 simulated *P*-values were calculated using two-tailed Fisher exact test. The Haploview program was used to estimate pairwise LD between SNP markers and to detect departure from Hardy-Weinberg equilibrium (HWE). Odds ratios were calculated according to Mantel-Haenszel method using the StatCalc program (Epi Info 2002; Centers for Disease Control and Prevention, Atlanta, GA). Mantel-Haenszel *P*-value of genotypic association (2 by 3 by *K* tables) was calculated using Cochran-Mantel-Haenszel Chi-Squared test in "R". Gene expression levels and correlations with the "risk"/"protective" haplotypes were compared by the appropriate tests using SPSS

v14.0 (SPSS, Chicago, IL). In the expression by haplotype analysis, phase resolutions for each individual were calculated by the *em.haplotype* function (DGCgenetics: R package version 1.0) in the “R” programming environment. Power analysis was carried out using the Power Calculator for Two Stage Association Studies (CaTS) (http://www.sph.umich.edu/csg/abecasis/CaTS/). Our study design had more than 90% power to detect a relative risk of 1.5 with a disease allele frequency above 0.15 and an estimated disease prevalence of 0.01, in a multiplicative genetic model.

Association of DNA Polymorphism in *golli-MBP* with brain morphology

10 *Subjects*

Blood samples were acquired from 100 healthy Jewish Ashkenazi volunteers, who were required to have no history of any familial psychiatric disorders. Written informed consent was obtained from all study participants. The study was approved by the ethics committee of the Health Ministry of Israel. DNA was isolated from blood samples and genotyping was preformed for the *golli-MBP* 3-SNPs haplotype (rs12458282, rs2008323, rs721286).

According to their genotyping, 26 subjects (Table 4) were asked to undergo an MRI scan and signed informed consent forms of the of Tel-Aviv Sourasky Medical Center ethics committee. The genotyped subjects were: 8 individuals homozygous to the risk-haplotype (the TAC risk-haplotype), 6 individuals homozygous to the protective-haplotype and 12 individuals who carry a neutral-haplotype that is neither the defined risk/protective haplotypes. These subjects were divided and analyzed in two groups: individuals that carry the TAC risk-haplotype (“Risk-Haplotype carriers” group) and individuals that carry the ‘protective’ and ‘neutral’ haplotypes (“Non-carriers” group) (Table 4).

Table 4 provides demographic characteristics of the studied groups which participated in the association study of DNA polymorphism in *golli-MBP* and fMRI brain morphology. Subjects were divided into two groups (Risk-Haplotype carriers; Non-carriers) according to their *golli-MBP* 3-SNPs haplotype status. There were no significant group differences in age or sex ratios.

Table 4

Group	Risk-Haplotype carriers	Non-carriers
Subjects (n)	8	18
AGE (years)	27.5 ± 4.3	27.0 ± 3.4
Male, Female (n)	3, 5	8, 10

DNA isolation and Genotyping

Genomic DNA was isolated from peripheral blood using the ArchiveGene™ DNA Purification kit (5 PRIME, Gaithersburg, MD) according to the manufacturer's protocol. SNPs were genotyped by high-throughput chip-based mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight; MALDI-TOF) (Sequenom, Inc., San Diego, CA, <http://www.sequenom.com/>) system, using the iPLEX™ chemistry. All assays for the PCR and associated extension reactions were designed by the SpectroDESIGNER software (Sequenom) and primers were synthesized by Integrated DNA Technologies (Coralville, Iowa). The 5 µL reaction volume contained 7.5 ng of genomic DNA, 100 nM each of the forward and reverse sequence-specific primer that contained a universal sequence at its 5' end, 0.5 mM of each deoxynucleoside triphosphate, 1.625 mM MgCl₂, reaction buffer, and 0.5 units of HotStarTaq DNA polymerase (Qiagen, Germany). PCR conditions were as follows: an initial denaturation step for 15 min at 94°C, followed by 45 cycles of 20 sec at 94°C, 30 sec at 56°C, 1 min at 72°C, and a final extension step for 3 min at 72°C. Allele-specific primer extensions were conducted with the Mass EXTEND Reagents Kit containing di-deoxy nucleotides (Sequenom). The high-throughput liquid handling was performed with the aid of a MULTIMEK 96 automated 96-channel robot (Beckman Coulter, Fullerton, California). Primer extension products were loaded onto a 384-element chip (SpectroCHIP; Sequenom) by nanoliter pipetting robot (SpectroPOINT, Sequenom) and analyzed with a MassARRAY mass spectrometer (Bruker Daltonik, Bremen, Germany). The resulting mass spectra were processed and analyzed for peak identification and allele determination by the MassARRAY 3.0 software (Sequenom). All SNPs were re-genotyped in 6 randomly-selected DNA samples, and 8 spots were loaded with water as

negative controls. All replicates were in agreement with each other and no signal was observed in the negative control wells.

Image Acquisition

5 Magnetic resonance imaging was performed by a 3T (GE) MRI system at the Sourasky Medical Center (Tel Aviv, Israel). The MRI protocol included conventional anatomy sequences and DTI acquired with a standard head-coil.

Conventional anatomy sequences

10 T₁ weighted images: 3D spoiled gradient recalled echo (SPGR) sequence with the following parameters: 66-78 axial slices, TR/TE = 400/3.2 ms and resolution 1x1x2 mm³. T₂ weighted images: Fast spin echo T2 weighted sequence with 24 axial slices, TR/TE=4600/85ms, echo train length of 32 echos and resolution of 1x1x3 mm³. FLAIR images: Fluid-attenuated inversion recovery sequence with 24 axial slices, TR/TE/TI =
15 9000/140/2100 ms and resolution 1x1x4 mm³. While the T2 and FLAIR images were used for radiological assessment of the subjects, the 3D T1 weighted images were used for volumetric analysis and as reference to the DTI experiments.

DTI protocol

20 Spin-echo diffusion weighted echo planar imaging (DW-EPI) sequence was performed with 48 axial slices and resolution of 2.5x2.5x2.5 mm³. Diffusion parameters were: $\Delta/\delta=25/19$ ms, b value of 1000 s/mm² acquired with 19 gradient directions. The DW-EPI sequence was gated to the cardiac cycle with TR of 30 R-R intervals and TE of 88ms.

25

Image Analysis

Image analysis included processing of the DTI and SPGR data sets. From the DTI data sets, maps of the fractional anisotropy (FA) and apparent diffusion coefficient (ADC) indices were produced using in-house software written in Matlab 7.3.0
30 (mathworks, Natick, MA). Image artifacts, normalization, unwrapping and statistical analysis, were performed using the SPM software (version 2, UCL, London, UK). Correction of head motion was performed using a least squares algorithm and 6 parameter (rigid body) transformations. Spatial normalization was done using 12-

parameter affine non-linear transformation of the DTI's T2 (b=0) or SPGR images to the Montreal Neurological Institute (MNI) template (EPI or T1). The normalization variables that best fit the DTI's T2 image were calculated, and were applied on the FA and ADC maps. The normalized image resolution was set to $1.5 \times 1.5 \times 1.5 \text{ mm}^3$. The
5 normalized SPGR images were then segmented into gray matter, white matter and CSF probability maps. All normalized images (FA, ADC, gray matter and white matter probability maps (GMP and WMP)) were then smoothed with 8mm full width half maximum Gaussian kernel.

10 *Statistical Analysis*

Voxel based analysis (VBA) was used detect regionally specific differences in brain tissue composition on a voxel by voxel basis (Ashburner and Friston, 2000) for each of the indexed maps (FA, ADC, GMP and WMP) (Crum et al., 2003). In this analysis we used ANOVA tests to compare between the genetically defined groups. The
15 results of the VBA analysis are presented as statistical parametric t-maps superimposed on SPGR brain template with resolution of $1.5 \times 1.5 \times 1.5 \text{ mm}^3$ where colored regions represent areas passing the statistical threshold of $p < 0.005$ and the color-scale represents the t-values.

20 *ROI analysis*

Using the statistical parametric maps, post-hoc region of interest analysis was performed. In each cluster surpassing the statistical threshold, a small volume correction was performed, using a sphere with radius of 6mm around the peak voxel. Only significant clusters ($p < 0.05$, Family-wise error (FWE) corrected) and clusters larger
25 than 50 voxels are reported. The anatomic location was determined using the SPM Wake Forest University WFU pickatlas toolbox (Maldjian et al., 2003).

RESULTS

30 *Golli-MBP association analysis*

Polymorphism analysis was performed in a two-stage design; In cohort I, a total of 26 SNPs were genotyped in 120 patients and 236 controls. A schematic representation of the *MBP* gene including the 26 SNPs and an LD map of the CEU

population are presented in Figure 1. Genotypes were obtained for most individuals with a mean completion rate of $95.2\% \pm 3.6\%$ for 25 SNPs, not including marker rs470291, which was successfully genotyped in only 25.7% of the samples and was excluded from the study.

5

Table 1 provides a list of genotype and allele frequencies pertaining to 6 SNPs significantly associated with schizophrenia of cohort I; OR, odds ratio for the risk genotype/allele, except of markers rs2013963 and rs470549 in which OR is for the protective genotype; 95%-CI, 95% confidence interval; *P*, Chi square *P*-value, except of markers rs2013963 and rs470549 that were calculated by Fisher exact test.

10

15

Table 1

Cohort I – Genotype and Allele frequencies of the SNPs associated with schizophrenia.

SNP ID and Location in chr18	Genotype Distribution (%)						Allele Distribution (%)					
	N	samples				P	OR [95%-CI]	samples				P
rs2013963 72913697			A/A	A/G	G/G			A	G			
	111	Patients	0	19.8	80.2	0.004	GG : 0.43 [0.22-0.86]	Patients	9.9	90.1	0.033	A : 1.90 [1.00-3.58]
228	Controls	1.3	8.3	90.4	Controls			5.5	94.5			
rs721286 72903128			C/C	C/T	T/T			C	T			
	111	Patients	10.8	40.5	48.6	0.020	TT : 1.87 [1.15-3.05]	Patients	31.1	68.9	0.010	T : 1.56 [1.09-2.22]
229	Controls	16.2	50.2	33.6	Controls			41.3	58.7			
rs2008323 72902924			A/A	A/G	G/G			A	G			
	114	Patients	37.7	50.9	11.4	0.074	AA : 1.52 [0.92-2.51]	Patients	63.2	36.8	0.028	A : 1.44 [1.02-2.02]
228	Controls	28.5	51.8	19.7	Controls			54.4	45.6			
rs12458282 72902841			C/C	C/T	T/T			C	T			
	107	Patients	6.5	53.3	40.2	0.015	CC : 1.52 [0.50-4.49]	Patients	33.2	66.8	0.008	C : 1.61 [1.11-2.34]
227	Controls	4.4	38.3	57.3	Controls			23.6	76.4			
rs470549 72876476			A/A	A/G	G/G			A	G			
	117	Patients	0	13.7	86.3	0.008	GG : 0.36 [0.16-0.82]	Patients	6.8	93.2	0.022	A : 2.31 [1.05-5.10]
227	Controls	0.4	5.3	94.3	Controls			3.1	96.9			
rs7234883 72873179			A/A	A/G	G/G			A	G			
	108	Patients	8.3	26.9	64.8	0.019	AA : 3.98 [1.18-14]	Patients	21.8	78.2	0.526	A : 1.14 [0.75-1.73]
224	Controls	2.2	34.8	62.9	Controls			19.6	80.4			

20

Allele and genotype frequencies of six SNPs (rs2013963, rs721286, rs2008323, rs12458282, rs470549 and rs7234883) were found to differ significantly between patients and controls (Table 1). Allele and genotype frequencies of both schizophrenia and control groups were within the Hardy-Weinberg equilibrium (HWE) except for

25

marker rs7233242 which departed from HWE for patients and controls combined ($P=0.0006$) and for patients alone ($P=0.0014$), but not for controls alone ($P=0.075$). The deviation in patients, however, is unlikely to be a consequence of a genetic effect since an excess of both homozygous genotypes was observed. Therefore, this SNP was excluded from our further analyses.

In the second stage, the study focused on 3 of the 6 SNPs, (rs721286, rs2008323, rs12458282), which formed one LD block with associated haplotypes that contains exon 3 of the gene, and genotyped them in cohort II. The other 3 SNPs which were associated with schizophrenia in Cohort I (rs2013963, rs470549 and rs7234883). Genotypes were obtained for most individuals with a mean completion rate of $96.4\% \pm 1.2\%$ and all SNPs were within Hardy-Weinberg equilibrium both in schizophrenia and control groups. No gender effect was observed on any of the markers in either cohort.

In cohort II, SNP rs12458282 showed both allelic and genotypic associations (Allele C P -value=0.003, OR[95%CI]=1.46[1.12-1.91]; Genotype CC P -value=0.018, OR[95%CI] =1.86[1.01-3.42]); SNPs rs721286 and rs2008323, however, exhibited trends in the same allelic directionality as in cohort I (P -value=0.07, OR[95%CI]=1.25[0.97-1.60] and P -value=0.1, OR[95%CI]=1.20 [0.96-1.50], respectively).

Table 2 provides a list of Genotype and allele frequencies pertaining to 3 SNPs of cohort II; OR, odds ratio for the risk genotype/allele; 95%-CI, 95% confidence interval.

Table 2

Table 2. Combined Mantel-Haenszel Analysis - Genotype and Allele frequencies.

SNP ID and Location in chr18	Genotype Distribution (%)							Allele Distribution (%)				
	N	samples				P	OR [95%-CI]	samples			P	OR [95%-CI]
rs721286 72903128			C/C	C/T	T/T				C	T		
	402	Patients	11.3	42.6	46	0.0156	TT : 1.42 [1.08-1.88]	Patients	32.6	67.4	0.004	T : 1.35 [1.10-1.65]
	508	Controls	16.1	43	40.9			Controls	37.6	62.4		
rs2008323 72902924			A/A	A/G	G/G				A	G		
	456	Patients	38	47.4	14.6	0.0323	AA : 1.31 [1.00-1.72]	Patients	61.7	38.3	0.012	A : 1.27 [1.05-1.53]
	549	Controls	33.3	48	18.7			Controls	57.3	42.7		
rs12458282 72902841			C/C	C/T	T/T				C	T		
	400	Patients	12.6	41	46.4	0.0005	CC : 1.77 [1.05-2.97]	Patients	33.1	66.9	0.0001	CC : 1.51 [1.22-1.87]
	504	Controls	7.2	36.1	56.7			Controls	25.3	74.7		

Table 3 provides a list of estimated haplotypes frequencies of cohort I, cohort II, and the combined data. Simulated P -values and OR were calculated using Mantel-

Haenszel Chi-Squared Test for the combined data of cohorts I and II with assigned covariates. The three most common haplotypes of each block are presented; OR, odds ratio; D', standardized LD coefficient.

Table 3

Table 3. Estimated haplotypes frequencies and association significance

Haplotype Block	D'	Cohort I				Cohort II				Cohort I+II			
		Frequency (%)		P	OR [95%-CI]	Frequency (%)		P	OR [95%-CI]	Frequency (%)		P	OR [95%-CI]
		Case	Control			Case	Control			Case	Control		
rs721286-rs2008323-rs12458282													
C-G-T		31.22	41.39	0.008	0.66 [0.46-0.93]	32.93	38.31	0.03	0.64 [0.49-0.85]	32.45	38.47	0.0009	0.74 [0.56-0.96]
T-A-C		26.27	19.01	0.005	1.51 [1.02-2.23]	28.4	19.01	0.0002	1.27 [1.00-1.63]	27.86	19.51	0.00005	1.6 [1.17-2.16]
T-A-T		36.18	35.13	0.72		32.6	36.1	0.33		33.57	36.11	0.43	
rs721286-rs2008323													
C-G	63	31.17	41.31	0.009	0.64 [0.45-0.91]	33.07	38.07	0.075	0.8 [0.63-1.02]	32.54	39.45	0.00249	0.75 [0.57-0.98]
T-A		62.46	54.15	0.031	1.4 [1.00-1.96]	60.98	55.84	0.065	1.27 [0.99-1.63]	61.36	55.16	0.00533	1.28 [1.00-1.67]
T-G		6.36	4.3	0.26		5.04	4.79	0.71		5.43	4.55	0.31	
rs2008323-rs12458282													
G-T		30.7	40.43	0.008	0.67 [0.47-0.95]	31.4	39.44	0.045	0.67 [0.51-0.89]	33.62	39.79	0.00205	0.75 [0.58-0.99]
A-C		27.05	18.31	0.002	1.66 [1.12-2.46]	28.64	20.36	0.001	1.24 [0.97-1.59]	28.41	19.49	0.00001	1.6 [1.18-2.17]
A-T		36.1	36.1	0.82		32.94	36.9	0.26		33.57	36.61	0.23	
rs721286-rs12458282													
C-T	97	32.81	41.41	0.005	0.66 [0.46-0.93]	32.11	37.96	0.032	0.66 [0.51-0.86]	31.87	39.51	0.00105	0.73 [0.56-0.96]
T-C		31.28	23.25	0.005	1.57 [1.09-2.27]	33.1	23.58	0.0003	1.28 [1.00-1.63]	33.01	23.44	0.00001	1.61 [1.20-2.14]
T-T		35.9	35.32	0.78		34.52	37.91	0.31		34.91	36.73	0.36	

The combined results for the 3 SNPs from cohort I and II were calculated using the Mantel-Haenszel method, revealing no confounding effects for any of the 3 SNPs (Table 2). The 3 SNPs formed an LD block with strongly associated risk and protective haplotypes, in cohorts I, II as well as in the combined data (table 3).

5 The strongest association effects were observed in the combined data analysis of the 3 SNPs - rs721286, rs2008323 and rs12458282 (Table 2), where rs12458282 had the strongest effect, both in genotypic ($P=0.0005$, OR[95%CI]=1.77[1.05-2.97] for the CC genotype) and allelic association ($P=0.0001$, OR[95%CI]=1.51[1.22-1.87] for the C allele). In addition, haplotypes constructed from the same three SNPs, were generally
10 more strongly associated with schizophrenia (global LD block P -value=0.0003) than the single-marker analysis (Table 3). In this block, haplotype C-G-T had a protective effect ($P = 0.0009$, OR [95%CI]=0.74[0.56-0.96]), whereas the T-A-C haplotype presented a risk effect ($P=0.00005$, OR[95%CI]=1.60[1.17-2.16]). Two-marker haplotype combinations within the block also showed significant effects, where the haplotypes
15 composed of the nonadjacent markers rs721286-rs12458282 (LD $D'=97$) had the strongest effect (global block P -value=0.00001). In this block, the C-T haplotype had a protective effect ($P = 0.00105$, OR[95%CI]=0.73[0.56-0.96]), whereas T-C showed the opposite effect ($P=0.00001$, OR[95%CI]= 1.61[1.20-2.14]).

20 **Conservation and regulatory elements analysis**

Conservation and regulatory elements analyses of the LD block with the associated haplotypes DNA region using the UCSC genome browser have yielded interesting findings. The LD block is predicted to be highly conserved by a gap scoring algorithm ("chain" tracks) with placental mammals. The associated haplotype lies in a
25 region that has a high regulatory potential (RP), which is conserved among 7 species ("7x Reg potential" track, predicted by the ESPERR algorithm; Max RP= 0.284, average RP = 0.108). Approximately 1.5 kb downstream of this haplotype, within the same LD block, there is a CCCTC-binding factor (CTCF) regulatory element (ORegAnno (Open Regulatory Annotation) database). When expanding the view, 3
30 more CTCF-binding sites were observed, 2 of which were downstream to the *classic-MBP* promoter.

Direct sequencing of *golli-MBP* exon 3 and CTCF-binding site regions

To examine the hypothesis that the associated markers/haplotypes were co-transmitted with a functional factor in the same LD block, *golli-MBP* exon 3 and regulatory CTCF-binding site regions were sequenced for 20 of the cohort I samples: 5 patients homozygote to the risk haplotype, 5 patients homozygote to the protective haplotype, 5 controls homozygote to the risk haplotype and 5 controls homozygote to the protective haplotype. While novel changes in the genomic sequence of the examined regions were not found, 3 SNPs in the CTCF-binding site region sequence (*rs9960721*, *rs685975*, *rs7233574*) were observed to be in complete LD with the associated haplotype. Namely, all subjects (patients and controls) who carry the associated risk haplotype were C-A-G homozygotes in the CTCF-binding site region SNPs, while all subjects (patients and controls) who carry the associated protective haplotype were A-C-A homozygotes in the CTCF-binding site region SNPs.

Expression analysis of *Golli-MBP* mRNA

To further investigate the link between *golli-MBP* and schizophrenia from another aspect, mRNA expression levels in postmortem DLPFC samples were examined. Real-time PCR analysis found no significant difference in the expression levels of the gene between schizophrenia patients and controls (Average relative quantification (RQ) values were 0.630 and 0.659 for schizophrenia patients and controls, respectively. $P = 0.15$). No correlation was observed between the gene expression levels and antipsychotic drug administration in patients, and therefore no correction for this variable was applied. The haplotypes found to be associated with the disease were examined for their affect mRNA expression levels. When the sample was divided into 3 groups (individuals that carry 1, 2 or 0 copies of the “risk”/”protective” haplotype (T-A-C and C-G-T, respectively) - no significant changes were observed. When the sample was divided into 2 groups (individuals that carry the “risk”/”protective” haplotype vs. those who do not) - no significant changes were observed.

This study demonstrated that SNPs in *golli-MBP* play a role in schizophrenia susceptibility.

Risk-haplotype for *Golli-MBP* correlates with changes in brain morphology

Table 5 provides for significant differences in gray matter probability between risk-haplotype carrier and non carriers. L – left, R – right, MFG – medial frontal gyrus, MTG – middle temporal gyrus, STG – superior temporal gyrus, SFG – superior frontal gyrus, IFG – inferior frontal gyrus, POG – post central gyrus, PCL – post-central lobule, ACC – anterior cingulate cortex, PHG – parahippocampal gyrus, Amg – amygdala. x,y,z (mm) represents the MNI atlas coordinate of the most significant voxel within the cluster, $P_{\text{corrected}}$ represents the p-value at the cluster level following small volume correction, K is the number of voxel of the cluster, T represents the t-value at the most significant voxel.

Voxel-based morphometry (VBM) of the gray matter probability maps revealed a significant decrease in gray matter volume of TAC risk-haplotype carriers compared to non-carriers in several brain regions, with strongest effects observed in the left medial frontal gyrus, left middle temporal gyrus, left insula and left superior temporal gyrus, Cerebellum, Lingual Gyrus, Right superior frontal gyrus, Left inferior frontal gyrus, Right medial frontal gyrus, Post central gyrus and the Post central lobule. Similar regions were found in the right hemisphere but with lower statistical significance (Table 5).

Figure 11 provides statistical parametric maps showing gray matter volume decrease (Left (A): focusing on the ACC gray matter reduction ($P_{\text{corrected}}=0.018$)) and increase (Right (B): focusing on the left Amygdala gray matter elevation ($P_{\text{corrected}}=0.005$)) in risk-haplotype carriers compared with non-carriers (at a threshold of $P_{\text{uncorrected}} < 0.05$). Threshold images have been transformed from MNI space into Talairach space and converted to T-scores, which were projected onto the pial surface of a representative standard surface; the range is shown in the corresponding color bars.

Figure 11 shows a significant gray matter decrease as was observed in the anterior cingulate cortex. Increase in gray matter volume was most pronounced in the left amygdala, left claustrum and left para-hippocampal gyrus.

Table 5

Gray matter decrease ($GMP_{non\ carriers} > GMP_{risk-haplotype\ carriers}$)				
Region	x, y, z (mm)	$P_{corrected}$	K	T
L MFG	-39, 41, 11	0.005	881	6.47
L MTG	-57, -5, -7	0.007	875	5.88
L Insula	-41, -12, -3	0.01	626	4.43
L STG	-38, 5, -33	0.012	382	4.34
Cerebellum	-38, -50, -39	0.01	1034	4.22
Lingual G.	11, -86, -25	0.005	2973	4.13
R SFG	6, 70, -10	0.005	1085	4.01
L IFG	-51, 21, -10	0.029	184	3.88
R MFG	36, -2, 41	0.019	295	3.83
POG	-45, -21, 38	0.013	512	3.58
PCL	-2, -43, 59	0.028	173	3.52
ACC	1, 5, 30	0.018	276	3.44
Gray matter increase ($GMP_{risk-haplotype\ carriers} > GMP_{non\ carriers}$)				
Region	x, y, z (mm)	$P_{corrected}$	K	T
L PHG	8, 0, -26	0.013	2557	5.37
L Claustrum	-20, 24, 16	0.006	1412	5.22
L Amg	-21, 1, -23	0.005	1049	4.30

5 FA changes

Table 6 provides for significant differences in FA between risk-haplotype carrier and non carriers. L – left, R – right, SLF – superior longitudinal fasc., MFG – medial

frontal gyrus, MTG – middle temporal gyrus, SFG – superior frontal gyrus, ACC – anterior cingulate cortex. x,y,z (mm) represents the MNI atlas coordinate of the most significant voxel within the cluster, $P_{\text{corrected}}$ represents the p value at the cluster level following small volume correction, K is the number of voxel of the cluster, T represents the t-value at the most significant voxel.

Voxel-wise analysis of the FA maps between the two genetic groups revealed significant FA reduction in the risk-haplotype carriers in several frontal and temporal regions of the right hemisphere (including the superior frontal gyrus, middle temporal gyrus and medial frontal gyrus) (Table 6).

Figure 12 provides statistical parametric maps showing FA decrease (Left (A): focusing on the ACC FA reduction ($P_{\text{corrected}}=0.02$)) and increase (Right (B): focusing on the left SFG FA elevation ($P_{\text{corrected}}=0.05$)) in risk-haplotype carriers compared with non-carriers (at a threshold of $P_{\text{uncorrected}}<0.005$). Threshold images have been transformed from MNI space into Talairach space and converted to T-scores, which were projected onto the pial surface of a representative standard surface; the range is shown in the corresponding color bars.

Figure 12 shows a significant FA reduction as found in the anterior cingulated cortex. Increase in FA values of the risk-haplotype group compared with the non-carriers group was found in small regions including the insula, left superior frontal gyrus and claustrum.

Table 6

FA decrease ($FA_{\text{non carriers}} > FA_{\text{risk-haplotype carriers}}$)				
Region	x, y, z (mm)	$P_{\text{corrected}}$	K	T
L SLF	23, -3, 33	0.009	155	4.43
R SFG	12, 6, 63	0.009	175	4.24
R MTG	60, -41, -9	0.01	225	4.03
R MFG	29, -9, 51	0.017	147	3.92
ACC	17, 24, 26	0.02	322	3.62
Cerebellum	39, -69, -37	0.001	138	3.56
FA increase ($FA_{\text{risk-haplotype carriers}} > FA_{\text{non carriers}}$)				
Region	x, y, z (mm)	$P_{\text{corrected}}$	K	T
L SFG	-8, -58, -7	0.005	277	4.33
L Insula	56, -33, 20	0.02	118	4.05
Clastrum	-36, -11, 8	0.012	119	3.75

5

ADC changes

The most significant changes were observed in the voxel- wise analysis of the ADC maps indicating a wide-spread increase in the ADC in both hemispheres. The strongest effects were in the right middle temporal gyrus, the left post central gyrus and the posterior *cingulated* cortex. ADC comparison results are summarized in Table 7 and are presented in Figure 13 for the cingulate cortex at a threshold of $P < 0.001$ in purpose of localizing the specific significantly differentiated areas.

10

Figure 13 provides statistical parametric map showing ADC elevation in risk-haplotype carriers compared with non-carriers (focusing on the ACC complex region at a threshold of $P_{\text{uncorrected}} < 0.01$). Threshold images have been transformed from MNI

15

space into Talairach space and converted to T-scores, which were projected onto the pial surface of a representative standard surface; the range is shown in the corresponding color bars.

5

Table 7

ADC increase ($ADC_{\text{risk-haplotype carriers}} > ADC_{\text{non carriers}}$)				
Region	x, y, z (mm)	$P_{\text{corrected}}$	K	T
R MTG	69, -44, -5	0.002	662	7.39
L POG	-30, -30, -54	0.007	502	7.30
Cerebellum	2, -60, -44	0.0001	1156	5.87
PCL	23, 47, 59	0.002	641	5.66
Precuneus	32, -83, 23	0.0001	2506	5.52
PCC	-3, -44, 30	0.006	527	4.71
ACC	3, 5, 27	0.02	316	5.35

CLAIMS

1. A method of diagnosing a predisposition of a subject to schizophrenia, said method comprising testing a sample obtained from the subject for the presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (v) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (vi) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (vii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (viii) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- (ix) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and
- (x) any combination thereof;

wherein presence of each of said allele or haplotype is indicative of a predisposition of the subject to schizophrenia.

2. A method according to claim 1, wherein said haplotype comprises allele C of the C/T polymorphism rs12458282, and allele T of the C/T polymorphism rs721286.

3. A method according to claim 1, wherein said haplotype comprises allele C of the C/T polymorphism rs12458282, and allele A of the A/G polymorphism rs2008323.

4. A method according to claim 1, wherein said haplotype comprise allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286.

5. A method according to claim 1, wherein said haplotype comprises allele C of the C/T polymorphism rs12458282, allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286.

6. A method according to claim 1, wherein said haplotype comprises allele C of the C/A polymorphism rs9960721, allele A of the C/A polymorphism rs685975, and allele G of the A/G polymorphism rs7233574.
- 5 7. A method according to claim 1, further comprising testing for the presence of at least one additional schizophrenia-identifying marker.
8. A method according to claims 1-7, wherein said subject is human.
- 10 9. A method according to claims 1-8, wherein said subject is an embryo, a fetus, a child, or an adult.
10. A method according to claims 1-9, wherein said sample is a body fluid.
- 15 11. A method according to claims 10, wherein said body fluid is selected from the group consisting of blood, saliva, cerebrospinal fluid, urine, and sperm.
12. A method according to claims 1-11, wherein said testing for polymorphism is performed by SNP genotyping.
- 20 13. A method according to claim 12, wherein said SNP genotyping is performed by a method selected from the group consisting of (a) a primer extension assay; (b) PCR assay; (c) an allele-specific PCR assay; (d) a nucleic acid amplification assay; (e) a hybridization assay; (f) a mismatch-detection assay, (g) an enzymatic nucleic acid
- 25 cleavage assay, and (h) a sequencing assay.
14. A method according to claims 1-13, further comprising measuring a clinical symptom of the subject.
- 30 15. A method according to claims 1-14, further comprising performing morphological analysis of the subject's brain for detecting aberrant brain morphology or microstructure.

16. A method according to claim 15 wherein said morphological analysis is performed using MRI or functional magnetic resonance imaging (fMRI).

17. A nucleic acid probe comprising a nucleotide sequence which hybridizes with at least a portion of golli-MBP region and which allows detection of at least one polymorphism therein for use in diagnosis of schizophrenia or predisposition to schizophrenia.

18. A nucleic acid probe of claims 17, wherein said polymorphism comprises at least one single nucleotide polymorphisms (SNP) selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele C of the C/T single nucleotide polymorphism (SNP) rs721286;
- 15 (v) allele G of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (vi) allele T of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (vii) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (viii) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (ix) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- 20 (x) allele G of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (xi) allele G of the A/G single nucleotide polymorphism (SNP) rs470549;
- (xii) allele G of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (xiii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xiv) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- 25 (xv) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- (xvi) allele A of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xvii) allele C of the C/A single nucleotide polymorphism (SNP) rs685975;
- (xviii) allele A of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and
- 30 (xix) any combination thereof.

19. A nucleic acid probe of claims 17 or 18, for use in combination with least one additional schizophrenia-identifying marker.

20. Use of a nucleic acid probe comprising a nucleotide sequence which hybridizes with at least a portion of golli-MBP region and which allows detection of at least one polymorphism in diagnosis of schizophrenia or predisposition to schizophrenia.

5

21. The use of claim 20, wherein said polymorphism comprises at least one single nucleotide polymorphisms (SNP) selected from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
 - (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
 - 10 (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
 - (iv) allele C of the C/T single nucleotide polymorphism (SNP) rs721286;
 - (v) allele G of the A/G single nucleotide polymorphism (SNP) rs2008323;
 - (vi) allele T of the C/T single nucleotide polymorphism (SNP) rs12458282;
 - (vii) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
 - 15 (viii) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
 - (ix) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
 - (x) allele G of the A/G single nucleotide polymorphism (SNP) rs2013963;
 - (xi) allele G of the A/G single nucleotide polymorphism (SNP) rs470549;
 - (xii) allele G of the A/G single nucleotide polymorphism (SNP) rs7234883;
 - 20 (xiii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
 - (xiv) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
 - (xv) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
 - (xvi) allele A of the C/A single nucleotide polymorphism (SNP) rs9960721;
 - (xvii) allele C of the C/A single nucleotide polymorphism (SNP) rs685975;
 - 25 (xviii) allele A of the A/G single nucleotide polymorphism (SNP) rs7233574;
- and
- (xix) any combination thereof.

22. An array comprising a substrate having a plurality of segments, wherein at least one of said segments comprises a probe which hybridizes with at least a portion of golli-MBP region and which allows detection of at least one polymorphism therein in diagnosis of schizophrenia or predisposition to schizophrenia.

30

23. The array of claim 22, wherein said polymorphism is at least one single nucleotide polymorphisms (SNP) from the group consisting of:

- (i) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;
- (ii) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;
- 5 (iii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (iv) allele C of the C/T single nucleotide polymorphism (SNP) rs721286;
- (v) allele G of the A/G single nucleotide polymorphism (SNP) rs2008323;
- (vi) allele T of the C/T single nucleotide polymorphism (SNP) rs12458282;
- (vii) allele A of the A/G single nucleotide polymorphism (SNP) rs2013963;
- 10 (viii) allele A of the A/G single nucleotide polymorphism (SNP) rs470549;
- (ix) allele A of the A/G single nucleotide polymorphism (SNP) rs7234883;
- (x) allele G of the A/G single nucleotide polymorphism (SNP) rs2013963;
- (xi) allele G of the A/G single nucleotide polymorphism (SNP) rs470549;
- (xii) allele G of the A/G single nucleotide polymorphism (SNP) rs7234883;
- 15 (xiii) allele C of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xiv) allele A of the C/A single nucleotide polymorphism (SNP) rs685975;
- (xv) allele G of the A/G single nucleotide polymorphism (SNP) rs7233574;
- (xvi) allele A of the C/A single nucleotide polymorphism (SNP) rs9960721;
- (xvii) allele C of the C/A single nucleotide polymorphism (SNP) rs685975;
- 20 (xviii) allele A of the A/G single nucleotide polymorphism (SNP) rs7233574;

and

- (xix) any combination thereof.

24. Use of an array comprising a substrate having a plurality of segments, wherein
25 at least one of said segment comprises a probe which hybridizes with at least a portion of golli-MBP region and allows detection of at least one polymorphism therein in diagnosis of schizophrenia or predisposition to schizophrenia.

25. A kit for carrying out the methods of any of claims 1-24.

26. The kit of claim 25, wherein said kit is compartmentalized to receive a reagent
30 for measuring single nucleotide polymorphism (SNP) in the *golli-MBP* region in a nucleic acid sample of a subject, said kit comprising at least one oligonucleotide that

interacts with a single nucleotide polymorphism (SNP) in *the golli-MBP* polynucleotide region wherein said SNP polymorphism is selected from the group consisting of rs12458282, rs2008323, rs721286, rs2013963, rs470549, rs7234883, rs9960721, rs685975, rs7233574 and any combination thereof.

5

27. The kit of claim 25 or 26, wherein said kit comprises at least one additional oligonucleotide that interacts with a single nucleotide polymorphism (SNP) of at least one additional schizophrenia-identifying marker.

10 28. A method of determining a predisposition of a subject to having an aberrant brain morphology or microstructure, said method comprising testing a sample obtained from the subject for the presence of at least one allele or haplotype of the *golli-MBP* region selected from the group consisting of:

(v) allele T of the C/T single nucleotide polymorphism (SNP) rs721286;

15 (vi) allele A of the A/G single nucleotide polymorphism (SNP) rs2008323;

(vii) allele C of the C/T single nucleotide polymorphism (SNP) rs12458282;

and

(viii) any combination thereof;

20 wherein presence of each of said allele or haplotype is indicative of a predisposition to having an aberrant brain morphology.

29. A method according to claim 28, wherein said haplotype comprises allele C of the C/T polymorphism rs12458282, allele A of the A/G polymorphism rs2008323, and allele T of the C/T polymorphism rs721286.

25

30. A method according to claim 15 or 28, wherein aberrant brain morphology is manifested by at least one parameter selected from the group consisting of:

(i) an increase in apparent diffusion coefficient (ADC);

(ii) a change in fractional anisotropy (FA); and

30 (iii) a change in gray matter volume.

31. A method according to claim 30, wherein the apparent diffusion coefficient (ADC) increase is observed in a brain region selected from the group consisting of:

- (a) Right – middle temporal gyrus;
- (b) Left - post central gyrus;
- (c) Cerebellum;
- (d) Post central lobule;
- (e) Precuneus;
- (f) Posterior cingulate cortex; and
- (g) anterior cingulated cortex ACC.

5

32. A method according to claim 30, wherein the fractional anisotropy (FA) change is observed as a decrease in FA in a brain region selected from the group consisting of:

10

- (a) Left – superior longitudinal fasciculus;
- (b) Right – superior frontal gyrus;
- (c) Right - middle temporal gyrus;
- (d) Right – medial frontal gyrus;
- (e) anterior cingulated cortex; and
- (f) Cerebellum.

15

33. A method according to claim 30, wherein the fractional anisotropy (FA) change is observed as an increase in FA in a brain region selected from the group consisting of:

20

- (a) Left – superior longitudinal fasciculus;
- (b) Left Insula; and
- (c) Claustrum.

34. A method according to claim 30, wherein the change in gray matter volume is observed as a gray matter volume decrease in a brain region selected from the group consisting of:

25

- (a) Left - medial frontal gyrus;
- (b) Left - middle temporal gyrus;
- (c) Left Insula;
- (d) Left – superior temporal gyrus;
- (e) Cerebellum;
- (f) Lingual Gyrus;
- (g) Right – superior frontal gyrus;

30

- (h) Left – inferior frontal gyrus;
- (i) Right - medial frontal gyrus;
- (j) post central gyrus;
- (k) Post central lobule; and
- (l) anterior cingulated cortex.

5

35. A method according to claim 30, wherein the change in gray matter volume is observed as a gray matter volume increase in a brain region selected from the group consisting of:

- (a) Left – parahippocampal gyrus;
- (b) Left - Claustrum; and
- (c) left amygdala.

10

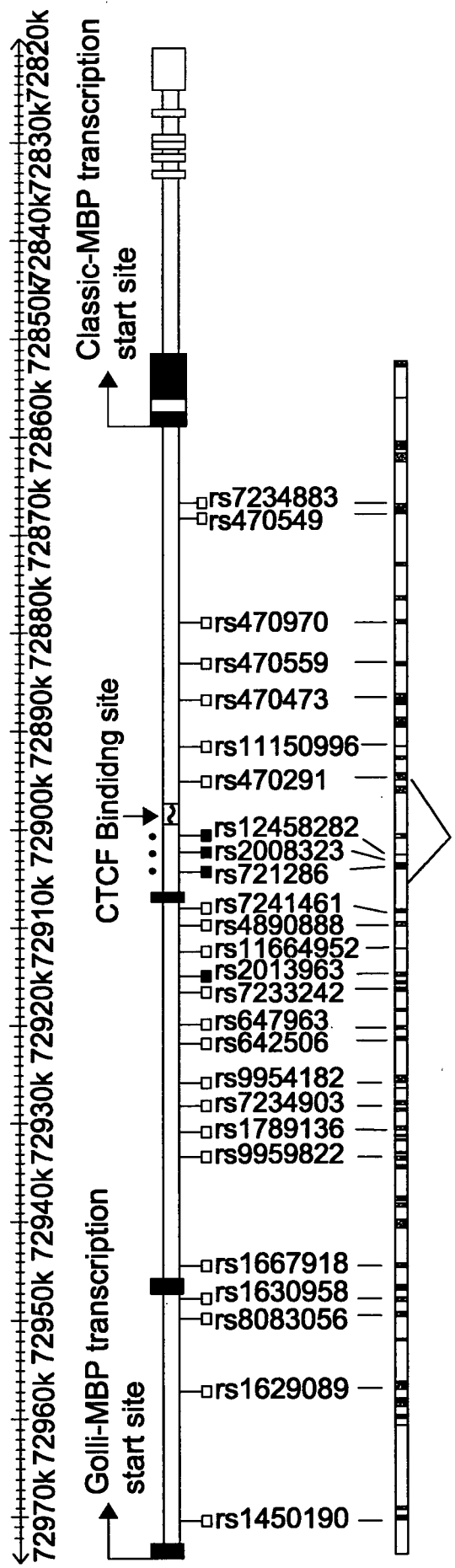


Fig. 1

Fig. 2

[SEQ ID NO: 1]

dbSNP: rs721286, C/T single nucleotide polymorphism (N)
 Position: chromosome 18 at pos 72903128
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs721286:
 chr18:72902899-72903523 (Strand: +)

```

CAAGCTTTGG GGAAGCAGGC CATTTTTCAG TAGACCAGTT GCTGCTAAGC GTTTTCTGAC
CCACACACCC ACAGGTAGTG CACACACTGT CCTCCTCCTT AGAAACCAGG CGGGAAGGAA
GGCAGGAGTC AGTCTCCACC TCTTCTCCCG CCAGAGTTGA CTAATCGTGG GGCTAAACTA
AAGGCAGGCA ATTCAGTTGA GGTGAGCGAA GTCTTAGGTC TCCCTAAAT
N
ATACTAAGGG AGGAGAGGAG TGGCCAAAAG AGGGCACTAC TGCCCCGGTG ACTGACTGTG
GTTCTCTCTCA GCCAGGACAG GCATCTCAAA GACAGGGCTG GAGGGATGTG GACTCCGTGT
CATGTGTAAG CTAGTTCACT CCACATGTGC CACAGAGGAT GGATGTCCTA CCGTGAAAAC
GTCACCAGCC CGCGTCTGTG TTCACTGCGT CCACTTCACA GGGCCAAGTC CTGTGTGCAC
AGCATGTAAG GGCAAACCCC CATGAAGAGT CATGAGGAAA ACATTCACAA CTTCATTGCT
CAGACAATGG AGATAGAAAT ATCTGTGTTT AATGTTTATT TAAAAAGTAT TCATCCCAGA
AACATTTAAG TACATATATT GGTGTGTGCG TGTTT

```

Fig. 3

[SEQ ID NO: 2]

dbSNP: rs2008323, A/G single nucleotide polymorphism (R)
 Position: chromosome 18 at pos 72902924
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs2008323:
 chr18:72902492 -72903288 (Strand: +)

```

GTCAAAGCTT GGGGAAGCAG CCATTTTTC A GTAGACCAGT TGCTGCTAAG CGTTTTCTGA
CCCACACACC CACAGGTAGT GCACACACTG TCCTCTTCTT AGAAACCAGG CGGGAAGGAA
GGCAGGAGTC AGTCTCCACT CTTCTCCCGC CAGAGTTGAC TAATCGTGGG GCTAAACTAA
AGGCAGGCAA TTCAGTTGAG GTGAGCGAAG TCTTAGGTCT CCCTAAATCA TACTAAGGGA
GGAGAGGAGT GGCCAAAAGA GGGCACTACT GCCCCGGTGA CTGACTGTGG TTCCTCTCAG
CCAGGACAGG CATCTCAAAG ACAGGGCTGG AGGGATGTGG ACTCCGTGTC ATGTGTAAGC
TAGTTCACTC CACATGTGCC ACAGAGGATG GATGTCCTAC CGTGAAAACG TCACCAGCCC
GCGTCTGTGT TC
R
CTGCGTCCAC TTCACAGGGC CAAGTCCTGT GTGCACAGCA TGTAAGGGCA AACCCCCATG
AAGAGTCATG AGGAAAACAT TCACAAC TTC TCACTCAGA CAATGGAGAT AGAAATATCT
GTGTTTAATG TTTATTTAAA AAGTATTCAT CCCAGAAACA TTTAAGTACA TATATTGGTG
TGTGCGTGTT TTTACTCTTC TTTATTTTAT AAAGCTTGAG TTAGCTGACA GTAGGAAAAA
ACACCAGGAA ACAGTAAAAT GTAAATAAAA TTGAGAAGTC TAGATGAAGT GAAGGAAGAC
CACAAACACA TCAACCATGT GACAATGGCT GCAGGGAGCC GCCCGTTTCA TCTGAGCTGC
CCAG

```

Fig. 4

[SEQ ID NO: 3]

dbSNP: rs12458282, C/T single nucleotide polymorphism (Y)
 Position: chromosome 18 at pos 72902841
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs12458282:
 chr18: 72902541 – 72903141 (Strand: +)

```

CCCCTTCCAC TTGGGCAGCT GGGCAGCTCA GAGTGAAACG GCGGGCTCCC TGCAGCCATT
GTCACATGGT TGATGTGTTT GTGGTCTTCC TTCACTTCAT CTAGACTTCT CAATTTTATT
TACATTTTAC TGTTTCCTGG TGTTTTTTCC TACTGTCAGC TAACTCAAGC TTTATAAAAT
AAAGAAGAGT GAAAACACGC ACACACCAAT ATATGTACTT AAATGTTTCT GGGATGAATA
CTTTTAAAT AACATTAAA CACAGATATT TCTATCTCCA TTGTCTGAGC AATGAAGTTG
Y
GAATGTTTTT CTCATGACTC TTCATGGGGG TTTGCCCTTA CATGCTGTGC ACACAGGACT
TGGCCCTGTG AAGTGGACGC AGTGAACACA GACGCGGGCT GGTGACGTTT TCACGGTAGG
ACATCCATCC TCTGTGGCAC ATGTGGAGTG AACTAGCTTA CACATGACAC GGAGTCCACA
TCCCTCCAGC CCTGTCTTTG AGATGCCTGT CCTGGCTGAG AGGAACCACA GTCAGTCACC
GGGGCAGTAG TGCCCTCTTT TGGCCACTCC TCTCCTCCCT TAGTATAATT TAGGGAGACC

```

Fig. 5

[SEQ ID NO: 4]

dbSNP: rs2013963, A/G single nucleotide polymorphism (R)
 Position: chromosome 18 at pos 72913697
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs2013963:

chr18: 72913402 – 72914036 (Strand: +)

```

CTGATTGCCT GGGTAACAGG GAAAATAACT GAGTTTTCTG TACCTGTTTG CTCAGCTTTA
AAATGAACAT TATAATCATA CTTTTCTATG TGGTCATCGT GAAGAGCATT CAATAAGTGG
AAACTGCTCT TTGCAGAAGC ACACAAAAC TTTGAAAATG CAGATCAGTA GCATCTTAGC
TTCACACTGT TATCAGAATA AGCCCTTATT TTTTCTCAGA ACCATCAAAG CCACTTCTCA
GGGGCTCTGG CCCAGGACTC CTGGTGTGGC AGCAGAAACA GTGGGATGTA GTGCC
R
CCCGCAGCGC AGTTACTCAA AGTGAGATCT TAAGCGAGTC AAGCTCCCTC CACCTCAGTA
TCCCCAGGAG GACAGTGGTC CCCATCTTGC GGCATCATTA TGAGGACTGC AAATGGCACA
TTTCTCTGGA GGAAAGCTCA CTGCTTTTAG GACGCCTGGC CTGTTCAGCC ATTGTCAGCA
ATGGTCAACC CTCCTTGCTG GCAGCAACGT GTTCTTTCTG AATCTTCGCC CACTGCCATT
GCCCTGTCTT AGATTCTCTT CCAATTAATC CACACCAGAG ATTTCTAGGA TTTCTAATGA
AACTTTAGTT GACAATGAAT ACTTCCAAAT ACTTTGTCA

```

Fig. 6

[SEQ ID NO: 5]

dbSNP: rs470549, A/G single nucleotide polymorphism (R)
 Position: chromosome 18 at pos 72876476
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs470549:

chr18: 72876176 - 72876776 (Strand: +)

```

TTGCAGGAGA GTCTTTCCTG GCAACGTGTT TGTTCTGAAA GGCCCTCCC TCAATATGAT
GACCATTTGC TTTGTGTAAA GTATTTTTC TCAGAAACAT AGGATTATAG ACTTAGCTCC
AGCAGTGTAT TTAATTTGTA TGAAAATAAG TCAGCCTCAT GGTCCCCCCC CCTTCACCTG
CAGCGTGACG TGTGGTGTCA CGGCATTGCA TTAGCGTCGT CTGCAGGGGC TGTGTTGTTG
CTGGTCAAGA CAGTTCACAG ACAGCTCTGC ATGAGTGATG CACGTTTCTC ATCCTAGCAC
R
GGGAGAGCAC TGGCCTTCCT CAGCCAAAGG CTGATTCCTA ATGGAGACGT GGACAGATGG
TTCGCATCTT TGA CTGAAGC ACATTTTGAA CTGAGAAAGA GAAAAACAGG ATAGATTGGT
AAAATTTAAC AGCCTTCTTT ACTCAGAAAT ATTTAGTGGA CATCTACTGG GCACACAACA
TTCCAATCAT AGAGAAAAAC TATTCCAAAG GCGGTACAGT GGTGATATGT GGGCAAAGAG
ACACTTAGAA TGCAGTGTGT ACGTCCCGGG ACACAGGTGG GTCCCAGTTA CTGTGAAAGC

```

Fig. 7

[SEQ ID NO: 6]

dbSNP: rs7234883, A/G single nucleotide polymorphism (R)
Position: chromosome 18 at pos 72873179
Band: 18q23

dbSNP flanking sequences and observed allele code for rs7234883:
chr18: 72872979 - 72873379 (Strand: +)

```
TAAAGGTGCA GAACCGAGCT TTAAGCTGTG GCCCCTTGTC CCACTGGAGA AGGCGCCGGG
GGCACGCAGG GCCTCCACTC TCCTGGCCCC TCTCAAGGAG GCCGAGCCCT GCAGAGACCA
TGGAATTTGG AAGTGTGGGC CAGGGCCAAA GAGGACGCTC CTTGTTTGTG GAATACTTGT
GTCACGGCGC GCTGTGCTTG
R
GGTCACCAGG GAGGGCAAAG GAAACAAAAA GGGCCATGGT GTGGCCTGTG GATTTGCGAT
GGCAGAAATT CTGCATCTAT GAGAGGCATG TGGCTGCCTG CCAGGCTTCC CGCAGAGATT
TCTTGAAAC TTTCCCACAA ACGAGGCTCA TCAACCCACC CATCTCACAC CAATGCCTCC
TTTGTGTGCC CAGAACATAT
```

Fig. 8**[SEQ ID NO: 7]**

dbSNP: rs9960721, A/C single nucleotide polymorphism (M)
 Position: chromosome 18 at pos 72900917
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs9960721:
 chr18: 72900717- 72901305 (Strand: +)

```

CCGCCTTCAA GAGGCTTGCA TTTGTGGAAG AAAAAACACA CCAAGTATGC AAATGCAATA
ACCAAAAGGC CAATGTGAAG GACATGAAAT AAGGCAGTGG GATAAAGAAG TGTGGCATGG
GAGACAGGCG GGCTTCTTGG TTAGATATGA CAGGAACAGC CTCTTAGAAA AGTGATGtaa
agtaaaaata aaggccctag
M
cctccaggca agacggaata gactctttgt ggcaataaga tagcaaatta tgggctgggc
gtggtggctc atgcctgtaa tccagcactt tgggaggctg aggcCCTCGT TCCATGTCCT
TCTATGTTAA ACCATCCCCC AAATGGTGTG AAGTCCCCAG AATGGCTGGC CCCGCCATTT
CTCTATTTTG TTTTCTCTAA CCTCCTGCCC TCTCTCTTCC ACCTCCAGTA TATGGCACGG
CACATTGGAG GTGCTCAAAA ATGATTTAAT AAATGAATGG AGAAAGCACA TAGGCAGGAC
ATCCTAGTAC CAATGATGAA TGAGTGTTTA TGGCAGATGT GACCCAGGTC CTCAGAGGCA
CATGAAAAAT GCTAATGAAC GGCAGCAC

```

Fig. 9**[SEQ ID NO: 8]**

dbSNP: rs685975, C/A single nucleotide polymorphism
Position: chromosome 18 at pos 72901228
Band: 18q23

dbSNP flanking sequences and observed allele code for rs685975:
chr18:72901201 - 72901252 (**Strand: +**)

AGCACATAGGCAGGACATCCTAGTAC[C/A]AATGATGAATGAGTGTTTATGGCAG

Fig. 10

[SEQ ID NO: 9]

dbSNP: rs7233574, A/G single nucleotide polymorphism (R)
 Position: chromosome 18 at pos 72901288
 Band: 18q23

dbSNP flanking sequences and observed allele code for rs7233574:
 chr18: 72900911 - 72901788 (Strand: +)

```

CCCTAGCCCT CCAGGCAAGA CGGAATAGAC TCTTTGTGGC AATAAGATAG CAAATTATGG
GCTGGGCGTG GTGGCTCATG CCTGTAATCC AGCACTTTGG GAGGCTGAGG CCCTCGTTCC
ATGTCCTTCT ATGTTAAACC ATCCCCCAA TGGTGTGAAG TCCCCAGAAT GGCTGGCCCC
GCCATTTCTC TATTTTGTTC TCTCTAACCT CCTGCCCTCT CTCTTCCACC TCCAGTATAT
GGCACGGCAC ATGGGAGGTG CTCAAAAATG ATTTAATAAA TGAATGGAGA AAGCACATAG
GCAGGACATC CTAGTACCAA TGATGAATGA GTGTTTATGG CAGATGTGAC CCAGGTCCTC
AGAGGCACAT GAAAAAT
R
CTAATGAACG GCAGCACTCA TTATTCACGT TATGAACGTG GCTTCAAGCA GTAGAGATGT
TAAAGCATCC TGTAATGGAG TATAACTGTT TTCTCTCTCT CTCCTTTTTT TTTTTTTTTT
TTTTATGAGG CGGAGTTTTG CTCTTGTTGC CCAGGCTGGA GTGCAGTGGT GTGATCTCAG
CTCACTGCAA CCTCTGCCTC CTGGGTTCAA GGAATTCTGC CTGAGCCTTC CAAGTAGCCA
GGATTACAGG TGTGCACTAC TACATCCAGC TAATTTTGTA TTTTGTGGTA GAGATGTGGT
TTCCCCGTGT TGGTCAGGCT GGTCTCGAAC TCCTGGCCTC AGGTGATCCA CCTGCCTCAG
CCTCCCAAAG TGCTGGGATT ATAGGCATGA GCCATCATAC CCGGCCAAAT TGTCTCTCA
ATGGGCCCTT GCAACAATTC TTGAGGGTCA ATAGGAAACC CTGTTTCTG GAGGAGGAAA
CAGGCACAGA GAGATGAAGT

```

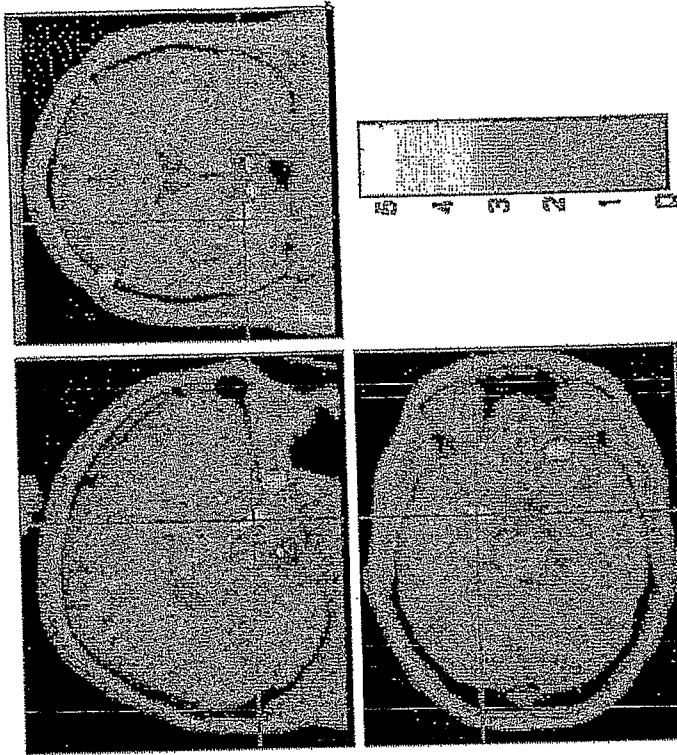


Fig. 11B

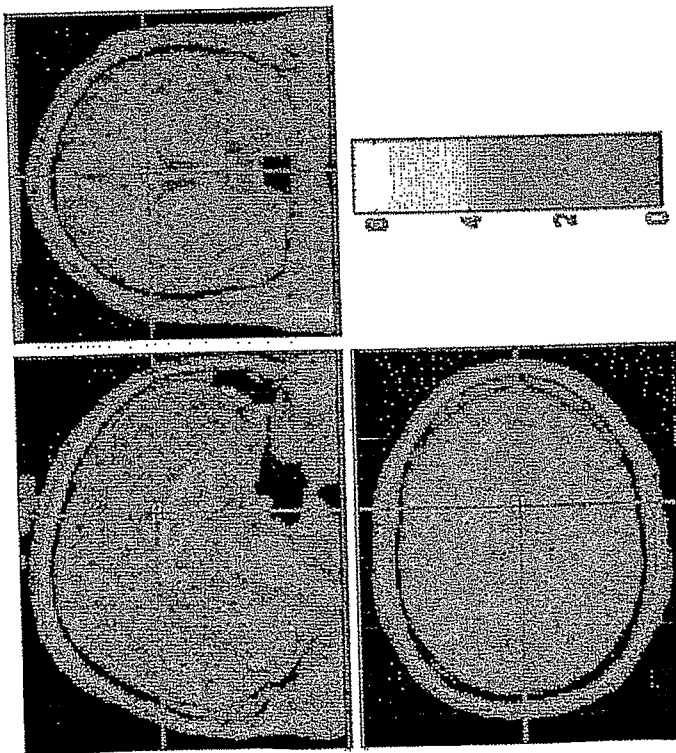


Fig. 11A

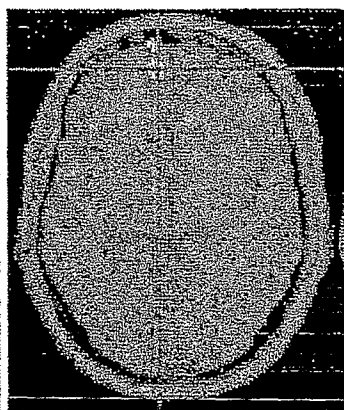
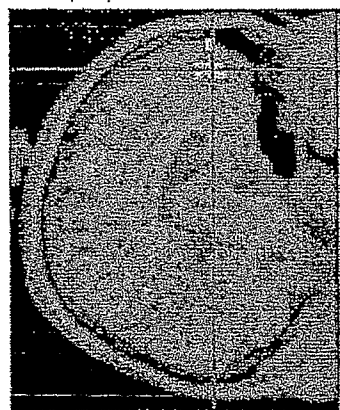
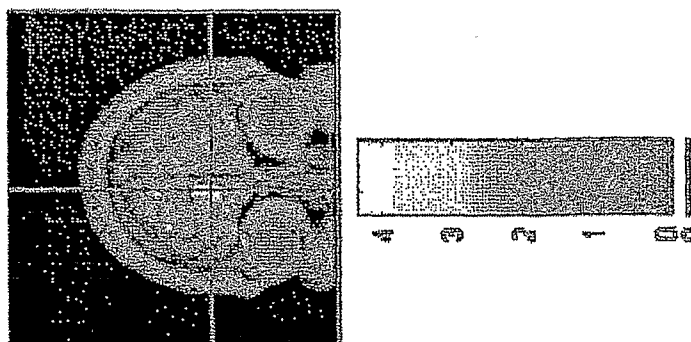


Fig. 12B

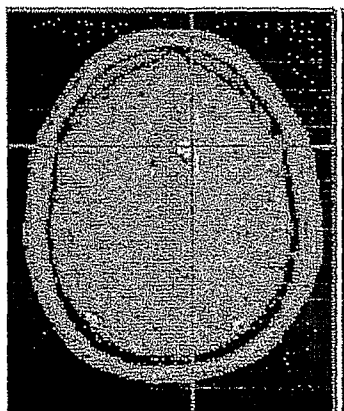
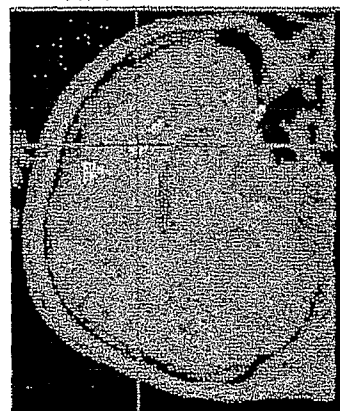
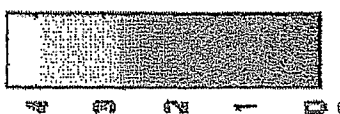
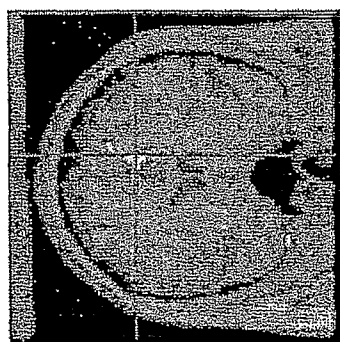


Fig. 12A

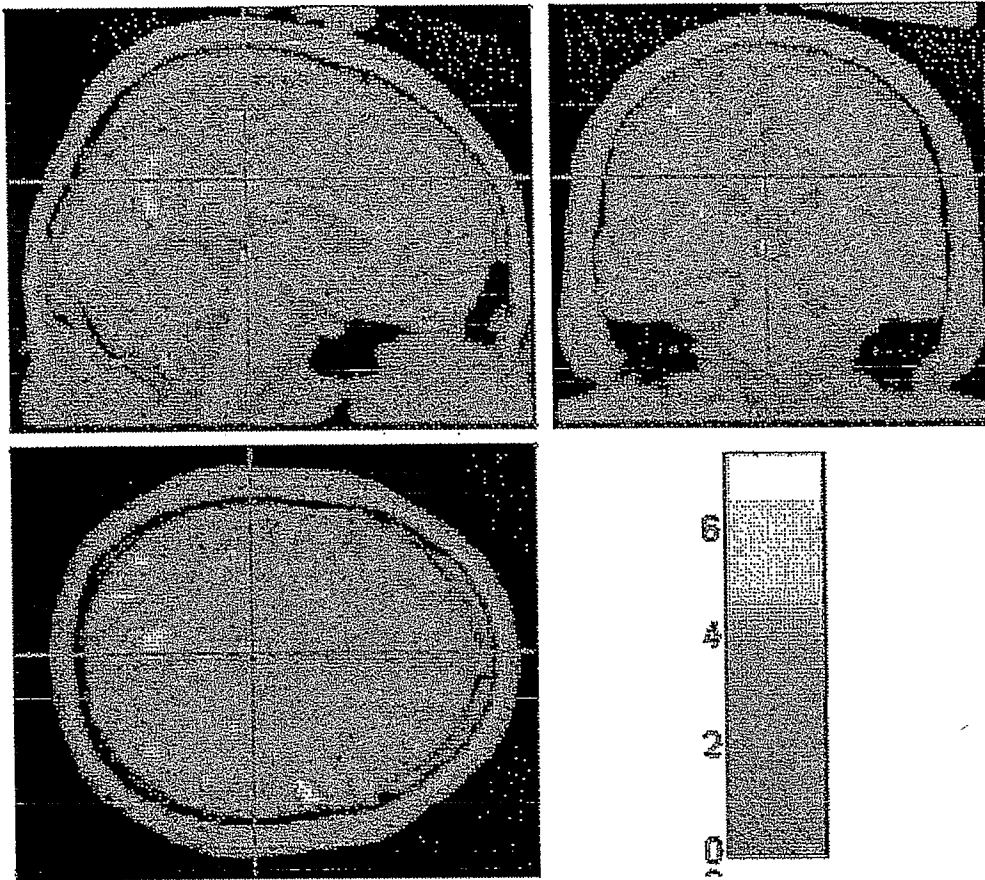


Fig. 13

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2009/001185

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE Probe [Online] 2005, "Bead microarray element (bead) probe for Homo sapiens variation rs721286. Has been used in the HapMap project for genotyping. Reagent is available from Illumina." XP002569073 retrieved from NCBI Database accession no. Pr864193.1 the whole document ----- -/--	17-19, 22-23, 25-27

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

17 February 2010

Date of mailing of the international search report

17/03/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Cornelis, Karen

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2009/001185

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE Probe 16 September 2005 (2005-09-16), "Bead microarray element (bead) probe for Homo sapiens variation rs2008323. Has been used in the HapMap project for genotyping. Reagent is available from Illumina." XP002569074 retrieved from NCBI Database accession no. Pr863453.1 the whole document	17-19, 22-23, 25-27
X	----- DATABASE Probe 2 March 2006 (2006-03-02), "Sequence-specific oligonucleotide (SSO) probe for Homo sapiens variation rs12458282. Has been used in the HapMap project for genotyping. Reagent is available from Perlegen." XP002569075 retrieved from NCBI Database accession no. Pr005575599.1 the whole document	17-19, 22-23, 25-27
X	----- DATABASE Geneseq [Online] 4 October 2007 (2007-10-04), "SNP found in human HindII fragment SEQ ID NO:22278." XP002569076 retrieved from EBI accession no. GSN:AFF22860 Database accession no. AFF22860 compound	17-19, 22-23, 25-27
A	----- WAN ET AL: "Polymorphisms of myelin-associated glycoprotein gene are associated with schizophrenia in the Chinese Han population" NEUROSCIENCE LETTERS, LIMERICK, IE, vol. 388, no. 3, 18 November 2005 (2005-11-18), pages 126-131, XP005046279 ISSN: 0304-3940 the whole document ----- -/--	1-35

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2009/001185

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WALSS-BASS CONSUELO ET AL: "Evidence of genetic overlap of schizophrenia and bipolar disorder: linkage disequilibrium analysis of chromosome 18 in the Costa Rican population." AMERICAN JOURNAL OF MEDICAL GENETICS. PART B, NEUROPSYCHIATRIC GENETICS : THE OFFICIAL PUBLICATION OF THE INTERNATIONAL SOCIETY OF PSYCHIATRIC GENETICS 5 NOV 2005, vol. 139B, no. 1, 5 November 2005 (2005-11-05), pages 54-60, XP002569077 ISSN: 1552-4841	1,20-21
A	the whole document	2-19, 22-35
X	----- MCINTOSH A M ET AL: "The effects of a neuregulin 1 variant on white matter density and integrity" MOLECULAR PSYCHIATRY, vol. 13, no. 11, November 2008 (2008-11), pages 1054-1059, XP002569078 ISSN: 1359-4184	28
A	the whole document	29-35
A	----- JUNGERIUS B J ET AL: "An association screen of myelin-related genes implicates the chromosome 22q11 PIK4CA gene in schizophrenia" MOLECULAR PSYCHIATRY, vol. 13, no. 11, November 2008 (2008-11), pages 1060-1068, XP002569079 ISSN: 1359-4184 the whole document -----	1-35