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The Role of Metabotropic Glutamate Receptor Genes in Schizophrenia

Carlo Maj[1,4], Alessandra Minelli[1], Edoardo Giacopuzzi[1], Emilio Sacchetti[2,3] and Massimo Gennarelli[1,4,*]

[1]*Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy;* [2]*Psychiatric Unit, University of Brescia, School of Medicine, Brescia, Italy;* [3]*Department of Mental Health, Spedali Civili Brescia, Brescia, Italy;* [4]*Genetics Unit, I.R.C.C.S. "San Giovanni di Dio" - Fatebenefratelli, Brescia, Italy*

Massimo Gennarelli

Abstract: Genomic studies revealed two main components in the genetic architecture of schizophrenia, one constituted by common variants determining a distributed polygenic effect and one represented by a large number of heterogeneous rare and highly disruptive mutations. These gene modifications often affect neural transmission and different studies proved an involvement of metabotropic glutamate receptors in schizophrenia phenotype. Through the combination of literature information with genomic data from public repositories, we analyzed the current knowledge on the involvement of genetic variations of the human metabotropic glutamate receptors in schizophrenia and related endophenotypes. Despite the analysis did not reveal a definitive connection, different suggestive associations have been identified and in particular a relevant role has emerged for GRM3 in affecting specific schizophrenia endophenotypes. This supports the hypothesis that these receptors are directly involved in schizophrenia disorder.

Keyword: Metabotropic glutamate receptors, schizophrenia, antipsychotic drugs, gene variants, omics data, bioinformatics.

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INTRODUCTION

Schizophrenia (SCZ) is a debilitating psychiatric disorder that affects more than one percent of the population. There are several forms of SCZ, classified on the basis of the most prominent symptoms that can vary among individuals. Symptoms can be categorized in three overlapping clusters: (i) positive symptoms, including hallucinations, paranoid delusions, and distorted perceptions, beliefs, and behaviours; (ii) negative symptoms, indicating a loss or a decrease in the ability to initiate plans, speak, express emotion, or find pleasure; (iii) cognitive symptoms, such as disordered thought processing and disruptions in working memory [1].

Moreover, SCZ is characterized by specific measurable endophenotypes as structural and functional abnormalities in superior temporal gyrus volume, sensory gating deficits, neuromotor abnormalities, and neuropsychological alterations [2]. The investigation of endophenotypes represents a useful strategy to dissect the biological mechanisms underlying a complex disorder as SCZ [3, 4].

Although antipsychotic drugs are often very effective in treating certain symptoms of SCZ, particularly hallucinations and delusions, current treatments are limited to alleviate other symptoms, such as reduced motivation and cognitive impairment. Furthermore, many patients discontinue or switch drug regimens because of treatment-emergent side effects. As a consequence, the search for alternative biological targets is an active research field, and in this regard recent studies support a potential role of Glutamate Metabotropic Receptors (GRMs) as drug targets [5].

This concept was developed originally on the observation that antagonist on other glutamate receptors (NMDA), upon which *GRMs* act as modulators, can induce psychotic symptoms and cognitive deficits closely resembling those seen in SCZ [6, 7].

At biochemical level, *GRMs* are members of the G-protein-coupled receptor (GPCR) superfamily, the most abundant receptor gene family in the human genome [8]. There exist eight human GRM proteins, labeled from mGluR1 to mGluR8 and encoded by genes *GRM1* to *GRM8* and they are classified into three groups based on sequence homology, G-protein coupling, and ligand selectivity. The group I includes mGluR1 and mGluR5 receptors, encoded by *GRM1* and *GRM5* human genes localized to chromosomes 6 (6q24) and 11 (11q14.3), respectively. The group II includes mGluR2 and mGluR3, encoded by *GRM2* and *GRM3* human genes localized to chromosomes 3 (p21.1) and 7 (7q21.1-q21.2) respectively. Finally, the group III includes mGluR4, mGluR6, mGluR7 and mGluR8 encoded by *GRM4* and *GRM6* to *GRM8* human genes are localized in chromosomes 6 (6p21.3), 5 (5q35), 3 (3p26-p25) and 7 (7q31.3-q32.1) respectively [8].

Several experimental methods have been exploited in order to analyze the biological role of *GRMs* genetics in SCZ phenotype, starting from candidate gene approach studies passing through Genome Wide Association Studies (GWAS)

*Address correspondence to this author at the Department of Molecular and Translational Medicine, Biology and Genetic Division, University of Brescia, Viale Europa, 11 – 25123 Brescia, Italy; Tel: +39 030 3501596; Fax: +39 030 3533513; E-mail: massimo.gennarelli@unibs.it

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up to the recent researches based on the production of Next Generation Sequencing (NGS) data. A number of studies evaluate the genetic of SCZ also by considering specific endophenotypes, as neurophysiological data and drug response, that is evaluating measurable characteristics which can be directly associated with genetic causes [3, 4, 9]. Here, we aim to provide a comprehensive report of known *GRMs* genetic variability to better evaluate genotype-phenotype associations between *GRMs* variants and SCZ. In this perspective, we combined the relevant knowledge available in literature with the information retrievable from public high-throughput databases comprising GWAS and NGS datasets.

MAIN EXPERIMENTAL APPROACHES IN GENETIC STUDIES

There exist different analytical approaches through which investigate the genetic variability in association with a given phenotype. The experimental methods can be classified in three main categories: I) Candidate gene; II) GWAS); III) NGS approaches. Each approach has its own advantages and limitations (Table **1**).

Candidate gene approaches have been the first ones to appear and they are still the most economic alternative, given that they are usually based on the evaluation of few target genes chosen according to a specific biological rationale [10]. The main limitation of candidate gene approaches is the need of an *a-priori* hypothesis regarding the involvement of specific genes over a phenotype trait. Instead an advantage is that the analysis is targeted in evaluating a small number of genetic variants providing an increased statistical efficiency for association analysis with respect to genome-wide approaches (i.e., GWAS and NGS). Candidate gene studies are usually performed on a small number of potentially well characterized subjects. This on the one hand allows the selection of homogeneous and comparable groups but on the other hand can identify associations that stand only for the analyzed sample. Therefore, candidate gene approaches are prone to non-replication results when tested in different cohorts [10].

GWAS methods come out as alternative to candidate gene approach mainly to overcome the limitation of hypothesis driven research which has often failed at identifying the genetic causes of complex diseases. In fact, GWAS explore a large number of genetic variations, such as thousands of Single Nucleotide Polymorphisms (SNPs) across all genome, thus without any specific bias. This allows to exploit a data driven approach which potentially leads to more robust results [11]. GWAS use microarray platforms usually designed to evaluate a large group of common genetic variants with a Minor Allele Frequency (MAF) greater than 5%. The main limit of GWAS is a direct consequence of the large number of SNPs simultaneously tested which is prone to the generation of a high false positive rate. This in turn requires the exploitation of statistical correction for multiple testing which often minimizes if not nullify the number of genetic variants identified as significantly associated with the considered phenotype. As a consequence to retrieve useful insight from GWAS it is important to have large sample sizes from randomized population (to avoid the influence of covariates as ethnicity). Another possibility is performing meta-analysis by aggregating different datasets to improve the statistical power of the studies.

The latest and more innovative approach to appear is represented by NGS. With NGS it is possible to retrieve the complete sequence of the whole genome (with the exception of some highly repetitive regions) or of target regions [12]. Therefore, NGS is the approach that is able to provide the more comprehensive source of information on genetic variability. It can theoretically detect any type of genetic variations including rare variants which are not detectable by GWAS approach. As a consequence, NGS beside the characterization of common variants allows also to analyze the contribution of rare deleterious mutations in specific genes in association with a given phenotype. Different studies based on NGS in effect consider a family design to identify mutations segregating with the considered phenotype which therefore represent potential genetic variants to be involved in the analyzed trait. The main issue for the use of NGS approach is that data processing is particular complex and not completely standardised. In fact, the bioinformatics pipelines exploited in data analysis are still under development and the retrieved variants depend also from the setting of several algorithmic parameters [13]. Therefore the diffusion of NGS approaches is currently limited by the complexity of bioinformatics analysis in conjunction with the fact that despite the decreasing trend in

Table 1. Comparison of experimental approaches.

	Candidate Gene	GWAS	NGS
Number of investigated variants	Low	High (fixed positions)	Maximum (entire sequence)
Screenable variants	Both common and rare	Only common variants	Both common and rare variants
Assumptions	Need of an a-priory hypothesis	No need of an a-priory hypothesis	No need of an a-priory hypothesis
Required sample size	From tens of subjects	From hundreds, usually thousands	From hundreds
Experimental design	Usually small case/control cohorts	Large case/control in randomized population	Family based and case/control
Reliability of results	Prone to sample specific biases	Robust if the sample is large enough	Potentially high but requiring complex data analysis
Cost per sample	Lowest	Medium	Highest

the cost, NGS methods are still the most expensive alternative to perform gene association analysis.

In the following paragraphs we will integrate the complementary information coming from the different experimental methods in order to provide the state of the art on the association between *GRM* genes and SCZ related phenotypes.

3. INSIGHTS FROM CANDIDATE GENE APPROACH STUDIES

The candidate gene approach focuses on association between genetic variability of specific genes and a given phenotype. A widely used approach is to genotype a number of subjects in a case-control design in which the presence of a statistically significant variation in the distribution of allele frequencies indicates that the tested variant is associated with the considered phenotype.

An identified association may be interpreted as a direct link, in which the genotyped SNP indeed is the true causal variant conferring disease susceptibility, or as an indirect association, in which the genotyped SNP is in proximity to another SNP being the true causal variant and there exist a linkage disequilibrium between the two SNPs [14]. Since the effect of a variant can arise in combination with the general genetic architecture, different candidate gene studies take also in consideration haplotype blocks to check if specific allele combinations occur together more frequently in one group [15]. Another possible experimental design is represented by family based approaches in which the aim is to search for genomic regions and variants specifically present in affected subjects and thus potentially associated with the disease [16]. The majority of the candidate gene studies on *GRM* genes and SCZ are based on the case/control design, while only a few of them evaluate segregation by exploiting a family based approach.

Indeed, on the basis of a hypothesized involvement of *GRMs* in SCZ, several gene association analyzes have been already carried out for all *GRM* genes except *GRM6* (Table **2**). In particular, different analyses estimate the effect of several SNPs within *GRM3* in different populations, considering both single alleles and haplotype combinations, leading overall to contrasting results [17-23]. Moreover, some associations that have been initially identified failed to be replicated when tested in a different cohort [17, 20]. Also, a large meta-analysis considering more than 3,000 cases and controls from different studies reveals that the data are not sufficient to assert a significant association between *GRM3* and SCZ [24].

A similar outcome emerged for the other *GRM* gene that has been more extensively studied in relation to SCZ, namely *GRM7*. In fact, multiple studies focused on gene association analysis between *GRM7* and SCZ considering different genetic markers providing both positive and negative results [25-30].

A few studies investigated the association of *GRM4* and *GRM8* with SCZ phenotype also leading to contrasting results. Regarding *GRM4* two studies evaluating a number of different SNPs both in intron and exon regions failed to detect any association between the analyzed variants and SCZ [27, 29] whereas in the family trios study by Fallin *et al.* a strong association was identified between one *GRM4* SNP and SCZ [19].

Concerning *GRM8,* Bolonna and colleagues failed in identifying association with a number of analyzed SNP [26]. Instead Takaki *et al.* in a later study performed in a different population revealed the presence of a significant single SNP association [31].

A couple of candidate gene studies investigated a potential association between *GRM2* and SCZ in both cases failing in identifying any association [32, 33].

Only one candidate gene study tested association with SCZ for each *GRM1* and *GRM5* genes. In the *GRM1* study it was compared the number of non-synonymous SNPs between patients and controls revealing no overall significant difference in the two groups. However, the variants present in patients are enriched in deleterious mutations suggesting a contribution at functional level exerted by *GRM1* on SCZ phenotype [34].

With respect to *GRM5* a study identified a novel intragenic microsatellite affecting the intron-exon structure and noteworthy the frequency of such genetic marker is significantly different between cases and controls [37].

Interestingly, in regards to *GRM3*, the most promising *GRM* gene in association with SCZ, a number of extensive investigations on specific measurable endophenotypes are also available in literature. In particular, different *GRM3* SNPs have been found to be associated with a worse cognitive performance in SCZ patients related to prefrontal and hippocampal function [18]. A study showed that six SNPs in the *GRM3* gene could be useful predictors of negative symptoms [38]. Moreover, another study identified a *GRM3* SNP affecting the working memory tasks [39]. Moosner and colleagues investigated a functional SNP in association with results obtained in various cognitive tests assessed in 198 SCZ subjects and 206 controls, revealing an association between the performance and the genotype [22].

One study described an association of *GRM3* with white matter composition in SCZ [40]. In particular, the authors reported one SNP, located in an intronic region of the gene, to be significantly associated with white matter integrity. Recently a study on a Japanese cohort composed by 21 patients and 48 control analyzed 4 *GRM3* SNP and found one of them to be associated with prefrontal brain activity though only in the SCZ group [41].

Different studies focused also on the evaluation of *GRM3* variants in relation to SCZ treatment (i.e., antipsychotic response). In effect, despite the majority of SCZ drugs target affect the dopaminergic transmission, there are more and more evidences that the glutamate neural transmission may also directly or indirectly affect different aspects of SCZ phenotype [42]. In particular, GRM3 receptor modulates signalling through NMDA receptors which are a relevant contributor to cognitive and negative symptoms in SCZ [43]. For this reason the *GRM3* gene has been widely investigated for its putative role in antipsychotic response even if the

Table 2. Case-controls candidate gene studies between GRM and SCZ identified from literature.

Gene	Sample	Variants	SCZ Association	Comment	Article
GRM1	450/650 cases/controls	47 SNPs	+	Enrichment of SNP in 3'UTR	[34]
GRM2	213/220 cases/controls Japanese	13 SNPs	-	No significant frequencies variations	[32]
GRM2	738/802 cases/controls Japanese	rs3821829, rs1248797, rs4687771	-	No significant frequency variations	[33]
GRM3	1º 265/283 cases/controls 2º 228/162 cases/controls 3º 128 trios Germans	rs2228595	-	No frequencies variations	[35]
GRM3	100/100 cases/controls Japanese	rs1468412	+	Significant SNPs and haplotype difference	[17]
GRM3	217/136 cases/controls African americans, Caucasians	rs6465084	+	G allele exerts a dominant effect over the A allele	[18]
GRM3	274 trios Ashkenazi Jewish	10 SNPs	-	No frequency variations	[19]
GRM3	752/752 cases/controls Chinese	7 SNPs	+	Significant frequency variation	[20]
GRM3	674/716 cases/controls European, European Americans	7 SNPs	-	No single association and none haplotype association	[21]
GRM3	631/519 cases/controls	rs6465084	+	Increased frequency of A allele and AA genotype in patients	[22]
GRM3	1235/932 cases/controls	rs148754219	+	Significant frequency variation	[23]
GRM4	100/100 cases/controls	Exons SNPs	-	No frequency variations	[36]
GRM4	274 trios Ashkenazi Jewish	6 SNP	+	Significant frequency variations	[19]
GRM4	100/100 cases/controls Japanese	8 SNPs	-	No frequency variations	[29]
GRM5	231/421 cases/controls Scottish	G64931 sbSTS	+	novel intragenic microsatellite	[37]
GRM7	181/91 cases/controls British	rs2229902	-	No frequency variations	[26]
GRM7	2293/2382 Japanese	rs3749380	+	Significant frequency variations	[27]
GRM7	124 sib-pairs Indonesian	rs17031835	+	Significant SNPs and haplotype difference	[28]
GRM7	100/100 cases/controls	43 SNPs	+	rs12491620 and rs1450099 have significant frequency variations	[29]
GRM7	180/33 cases/controls Chinese	CNV	-	No significant copy number variations	[30]
GRM8	105/108 cases/controls British	2846-C/T	-	No significant frequency variation	[26]
GRM8	100/100 cases/controls	22 SNPs	+	rs2237748 and rs2299472 (without correction)	[31]

reliability of such studies is not particularly meaningful due to limited sample sizes and time depth.

A clinical trial considering an exploratory cohort of 78 African American and a validation cohort of 65 European American patients receiving risperidone (i.e., the leading antipsychotic used in SCZ treatment) at the dose of 2-6mg per day over 2-12 weeks identified a correlation between an intronic *GRM3* SNP (rs724226 G/A) and the treatment response. Specifically, the Positive and Negative Syndrome Scale (PANSS) increases as the number of G alleles increases following an additive model [44]. Noteworthy the SNP is characterized by a different allele frequency in the two populations as reported also in pharmacogenomics knowledgebase [45].

Another study considering 61 untreated first-episode SCZ patients who were assessed before and after six weeks of antipsychotic pharmacotherapy, mainly with risperidone, identified an association between another SNP of *GRM3* and negative symptoms improvement though it relies only on a two time points data (i.e. base point and after six weeks) and with patients stratified in small groups (4-50 subjects per group) [46]. This corroborates the hypothesis that disorders in glutamatergic transmission are mainly related to negative symptoms in early phase of SCZ [47].

Taken as a whole, the results of candidate gene studies provide only weak evidences that variants in *GRM* genes are an important genetic risk factor for SCZ. Nevertheless several studies indicate that among these receptors, *GRM3* is significantly associated with a number of SCZ related endophenotypes.

However, several limitations characterize candidate gene association studies preventing the possibility to achieve solid information and partially explaining the presence of contradictory results. Factors such as small samples size and differences in ethnicity could explain the difficulties in replicating the significance of putative polymorphisms associations. Therefore, in order to achieve a more robust knowledge on the role of *GRM* genes in SCZ it is essential to integrate candidate gene approach with the information retrievable from large-scale studies.

INSIGHTS FROM GENOME WIDE APPROACH

GWAS analyse simultaneously thousands of genetic markers, usually SNPs, to investigate if there are significant associations between common variants and a given trait [48]. GWAS offer several advantages with respect to candidate gene approach. The data produced can be easily aggregated in large meta-analysis. This allows the detection of minor effects and the simultaneous characterization of several markers allows to evaluate polygenic models considering a large number of common alleles with additive interactions. Noteworthy, the presence of a polygenic component was found to significantly contribute to SCZ phenotype [49].

In addition, GWAS can easily detect the presence of Copy Number Variation (CNV), a type of genetic variant particularly relevant in SCZ which is indeed often characterized by deletion and duplication of large genomic regions [50].

Whereas pure case-control GWAS in SCZ have failed in detecting significant associations, meta-analysis improving the statistical power by aggregating different datasets, along with the study of SCZ measurable endophenotypes, and the evaluation of CNVs distribution were able to identify relevant gene associations within SCZ phenotype [4, 51].

A major study focused on a Swedish cohort of 4,719 cases and 5,917 controls identified a significant increase of large CNVs (>500kb) in SCZ subjects compared to controls. Interestingly, CNVs are located in regions enriched in genes involved in different neural functions, including glutamatergic transmission including *GRM5* [52].

The meta-analysis conducted by the Psychiatric Genomic Consortium (PGC) which combined several independent GWAS datasets in the largest resource available to date with 36,989 cases and 113,075 controls, revealed the association between SCZ phenotype and a number of different loci containing genes involved in glutamatergic transmission [53]. Concerning *GRM* genes, the rs12704290 SNP, located in an intronic region of *GRM3* reaches a GWAS significant level of association.

In the clinical domain, probably the most relevant GWAS analysis focusing on specific endophenotypes is the Clinical Antipsychotic Trials for Intervention Effectiveness (CATIE) [54]. This study was aimed at examining fundamental issues about second-generation antipsychotic medications (i.e., olanzapine, risperidone, quetiapine, and ziprasidone) considering their relative effectiveness through the evaluation of different measurable phenotypes on genotyped patients [55]. The detailed analysis of CATIE data has led to the identification of several suggestive associations distributed across many genes which do not reach a GWAS statistically significant level but that can still express an association trend that could be subsequently tested through targeted biological experiments.

Notably, some of the most relevant identified association regard *GRM* genes, namely *GRM1*, *GRM5*, *GRM6*, *GRM7* and *GRM8* [51]. In detail, the three most significant SNPs in association with PANSS scale are in *GRM7*, *GRM8* and *GRM3*, while a lower but prominent effect was also found for single SNPs in *GRM5* and *GRM6*. An improvement in cognitive score was also associated with SNPs in *GRM8* and *GRM7* genes though, with a certain degree of imbalance among ethnicities.

As a whole these results are in line with the hypothesized role of glutamatergic transmission in SCZ and suggest that *GRMs* receptors are somehow associated with antipsychotic treatment despite the underlying biological mechanisms remain unclear.

In addition to literature research nowadays it is also possible to query public GWAS database to dissect potential associations that have been previously neglected. To this purpose we exploited the Ricopili tool (http://www.broadinstitute.org/mpg/ricopili/) to retrieve the *p*-values of association for SNPs genotyped within *GRM* genes in the case/control GWAS mega-analysis performed by the PGC consortium considering a discovery sample with 9,394 cases

and 12,462 controls and a replication sample with 8,442 cases and 21,397 controls [56]. Despite the presence across G*RMs* of several significant SNPs at nominal level (i.e., without multiple testing correction), which suggest a potential role of such genes, *GRM3* is the only one in which two variants (one in the promoter and one in an intron) are still significant also after multiple correction for the number of SNPs tested in the gene.

Overall, GWAS data reveals the presence of a distributed polygenic component in SCZ thus confirming the difficulties in finding any specific association within *GRM* genes, although, in line with candidate gene approach studies, a potential relevant role could be hypothesized for *GRM3*.

INSIGHTS FROM NEXT GENERATION SEQUENCING STUDIES

Despite SCZ is characterized by a level of hereditability estimated to be around 80%, common variants analyzed by candidate gene approach studies and GWAS represent only a part of the genetic architecture of SCZ [57]. Possible explanations for the missing hereditability include gene-environment interactions, epigenetic effects and undetected rare variants [58, 59].

NGS methods are a valuable tool to search for rare variants by providing the complete characterization of genomic sequence and different studies provided useful insights in the analysis of genetic associations underlying SCZ phenotype [60, 61].

To date, most of the published studies are based on Whole Exome Sequencing (WES), an approach which allows an unbiased search of functional variants in coding text regions while considering a family experimental design to identify *de novo* and/or recessively inherited variants in affected child from healthy parents [62].

Since 2011, family trios and quartets have been analyzed by WES approach in different studies mainly focused on *de novo* variants and the identification of Loss of Function (LoF) (i.e., nonsense splice disrupting variants frameshift indels or predicted deleterious missense) [63-70]. Despite these studies found about 400 genes harboring *de novo* mutations in SCZ probands, including genes involved in glutamatergic transmission, no mutations in *GRM* genes have been reported so far [66].

Noteworthy, case-control studies based on WES have suggested that in SCZ a high allelic heterogeneity is the most probable scenario for variants with moderate or high effect size and that these variants should present themselves as ultra rare [71], i.e. a large number of different alleles would be involved, but every one could affect only a single subject (or a small number). Therefore, it is possible that functional variants can be identified in the future also in *GRM* genes as more affected individual will be sequenced. A first evidence emerged in the study by Need *et al.* where the integration of both WES and GWAS data from 164 SCZ subjects and 307 controls and subsequent genotyping in a large independent cohort revealed the presence of two missense mutations, one in *GRM7* and one in *GRM1* which occur only in affected subjects [72].

To date, only one study on SCZ was carried out using a Whole Genome Sequencing approach and it concerns the sequencing of DNA extracted from postmortem brains of SCZ subjects [73]. The authors identified an increased content of specific genomic insertions in synaptic genes in affected subjects and three of these elements are placed in the intronic region of *GRM3*, *GRM5* and *GRM7*. Beside case-control experimental studies, NGS methods have been applied in SCZ also to analyze drug treatment response, identifying no connection between *GRMs* and antipsychotic effect [74].

Regarding the information available from repositories of NGS data we queried the web resource associated with the large case-control exome-sequencing study on SCZ carried out by Purcell *et al*. in a Sweden cohort concerning 2,536 patients and 2,542 controls [71]. Specifically, we retrieved the number of rare (<0.5%) nonsynonimous mutations in cases and controls within *GRM* genes. Despite an overall increase of such mutations is indeed present in cases with respect to controls, no final evidence for a role of *GRM* genes in SCZ is found, though a remarkable difference is present for *GRM1* (see Table **3**).

Table 3. Rare (<0.5%) disruptive mutations observed in SCZ, controls or both samples.

Gene	Only SCZ Patients	Only Controls	Both
GRM1	8	1	1
GRM2	2	0	2
GRM3	2	2	0
GRM4	0	0	0
GRM5	5	2	0
GRM6	1	1	0
GRM7	0	2	0
GRM8	2	3	1
Total	20	11	4

We also queried the database (http://exac.broadinstitute.org) provided by Exome Aggregation Consortium (ExAC) collecting the whole-exome sequencing data for a total of 61,486 unrelated individuals from both healthy cohorts and large genetic studies on complex diseases (including SCZ). The aim was to characterize the overall variability present in *GRM* exons.

We classified the retrieved SNPs in different functional classes according to their potential effect on receptor functionality, from putative regulatory variants in UTRs to highly disruptive mutations as frameshift indels or nonsense substitutions, identifying an heterogeneous distribution with

no particular enrichment in specific categories (see supplementary file 1).

In support to the hypothesis that *GRM* genes are essential genes with a low rate of functional alterations, the Residual Variation Intolerance Score (RVIS) computed by Slavé Petrovski *et al*. [75] representing the gene tolerance to mutations, shows that these receptors indeed are not prone to accumulate functional variations (Table **4**). Probably the mutation intolerance characterzing *GRMs* depends on their critical role in regulating neural transmission.

Table 4. Residual Variation Intolerance Score.

Gene	RVIS	RVIS Percentile
GRM1	-0.1451527877	42.338995046
GRM2	-0.3508434071	29.5411653692
GRM3	-1.2859333734	5.0837461665
GRM4	-2.4786730493	0.9613116301
GRM5	-1.2638839523	5.2606746874
GRM6	-1.3155643502	4.7947629158
GRM7	-1.6166924289	2.9370134466
GRM8	*-0.5694949983*	*19.0434064638*

The more negative RVIS value the fewer common functional mutations are reported in the gene (in human population). The percentiles with respect to the total RVIS distribution in human genes suggest a high level of mutation into lerance for most of GRM genes.

The evaluation of the large amount of NGS data allows also to assess the presence of un unbalanced distribution among population for specific variants [76]. Such variants could be of a particular interest since can also explain the heterogeneity in treatment response among ethnicities and suggest personalized treatment [77].

Looking at 1,000 Genome and ExAC NGS data, we investigated the presence in *GRM* genes of variants diffused only in one population or showing relevant differences in allele frequency among different populations (see supplementary file 2). Population specific variants occur in CDS of *GRM5* and *GRM6* genes and this data could be of interest given their role as drug targets [78]. In summary, what arises from NGS studies is the presence of a certain level of population specificity for *GRMs* variants and the overall intolerance to functional mutations of such receptors. This fact can potentially justify absence of highly damaging mutations associated with SCZ.

THE POTENTIAL PIVOTAL ROLE OF GRM3 IN SCHIZOPHRENIA

Although with heterogeneous results, candidate gene, GWAS and NGS studies converge in revealing associations between *GRM3* variants and SCZ related phenotypes.

In particular the presence of a number of associations with specific endophenotypes corroborates a potential role for *GRM3* as a pharmacological target. This is in line with the glutamate hypothesis of SCZ [79]. Glutamate theories on SCZ are based on the fact that glutamate receptors antagonists are able to normalize glutamatergic transmission, especially in prefrontal cortex. Therefore molecules acting as *GRM3* antagonistcan be an efficient alternative to the monoaminergic antipsychotic acting directly on NMDA [80]. In this regards *GRM3* has been already tested as an antipsychotic drug target. Patil ST *et al*. in a clinical trial using LY2140023, a selective agonist of *GRM3* (and *GRM2*) observed improvement for both positive and negative symptoms at week 4 without relevant side effects with respect to placebo [81]. However, a subsequent study on the same drug was not able to demonstrate an improvement in PNASS score compared to placebo while confirming the high tolerability of LY2140023 by showing no serious adverse effects [82]. Despite the inconclusive results in past clinical trials a recent article evaluating the translation from gene associations data to the treatment of SCZ confirms that GRM3 is among the most promising target [83]. In effect the variability of the study outcomes can be at least partially explained by the presence of specific *GRM3* variants [46]. Overall, these findings on the one hand represent an issue for the development of a generalized therapy but on the other hand indicate that *GRM3* can be a proper pharmacological target to consider for alternative SCZ treatment.

CONCLUSION

For psychiatric disorders, genetic studies have often failed in finding significant associations between genetic markers and the pathological phenotypes due to the underlying complex genetic architecture. This scenario is also verified for gene association analysis between *GRMs* and SCZ. In this context it is important to integrate all the available information arising from the different experimental approaches in order to have the highest degree of reliability in hypotheses testing evaluating the effect of both common and rare genetic variants. We combined the available information on *GRMs* in association with SCZ phenotype considering the data obtained by candidate gene analyses, GWAS, and NGS studies. The obtained results corroborate the hypothesis that *GRM* receptors are somehow related to SCZ phenotype by identifying a number of potential associations even though with a variable range of reliability. The analysis of NGS exome data indicate that *GRMs* genes are not tolerant to functional mutations, providing a possible explanation in the lack of highly penetrant *GRMs* variants for SCZ phenotype. Instead, the presence of a number of gene-level associations for non-coding SNPs seems to indicate a potential pivotal role of common regulatory variants distributed across *GRMs* genes even if it is not possible to clearly state a definitive association with SCZ. In conclusion, different experimental approaches converge to support the association of common regulatory variants within *GRM3* with SCZ and pathognomic measurable endophenotype. In the next future it is expected that NGS studies in large cohorts of patients will complete the analysis of the genetic variability of *GRM3* and the other GRM genes, providing a more comprehensive picture of the role of this receptor subgroup within the complex genetic architecture of SCZ and related endophenotypes.

SUPPLEMENTARY FILES

Supplementary file 1: Classification of *GRMs* variants from exome sequencing of ExAC consortium.

Supplementary file 2: List of *GRM* gene variants with unbalanced frequencies between different population from 1000K and ExAC datasets.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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