



Sex Influences Genetic Susceptibility to Depression-Like Behaviors in Chronic Unpredictable Mild Stress-Exposed Wistar Rats

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Abstract

Depression is one of the most common mood disorders among psychiatric diseases. It affects about 10% of the adult population. However, its etiopathogenesis remains poorly understood. Exploring the dynamics of stress-susceptibility and resilience will help in understanding the molecular and biological mechanisms underlying the etiopathogenesis of depression. This study aimed to determine the differences and/or similarities in factors responsible for susceptibility to depression-like behaviors in male and female Wistar rats subjected to chronic unpredictable mild stress (CUMS). Sixty Wistar rats (30 male and 30 female) weighing between 120 and 150 g were used for this study. The rats were divided into two sub-groups: control (10) and test (20) groups. Rats in the test groups were subjected to CUMS. Depression-like behaviors were assessed using light–dark box, sucrose preference, and tail suspension tests. Rats that showed depression-like behaviors following the behavioral tests (CUMS-susceptible group) were sacrificed, and their hippocampi were excised. Genomic deoxyribonucleic acid (gDNA) was purified from the hippocampal samples. Purified gDNA was subjected to whole genome sequencing (WGS). Base-calling of sequence reads from raw sequencing signal (FAST5) files was carried out, and variants were called from alignment BAM files. The corresponding VCF files generated from the variant calling experiment were filtered. Genes were identified, their impacts estimated, and variants annotated. Functional enrichment analysis was then carried out. Approximately 41% of the male and 49% of the female rats subjected to CUMS showed significant ($p < 0.05$) depression-like behaviors following assessment on behavioral tests. WGS of the hippocampal DNA revealed 289,839 single nucleotide polymorphisms variant types, 7002 insertions, and 34,459 deletions in males, and 1,570,186 single nucleotide polymorphisms variant types, 109,860 insertions, and 597,241 deletions in female Wistar rats. Three genes with high-impact variants were identified in male and 22 in female Wistar rats, respectively. In conclusion, female Wistar rats are more susceptible to depression-like behaviors after exposure to CUMS than males. They also have more gene variants (especially high-impact variants) than male Wistar rats.

Keywords Chronic unpredictable mild stress · Gene variants · Single nucleotide polymorphisms · Susceptibility · Whole genome sequencing

Introduction

More than 350 million people are affected by depressive disorders worldwide (WHO, 2017). One out of every five of these patients suffers from its most prevalent form, major depressive disorder (MDD), throughout their lifetime [1]. Major depressive disorder is a long-term, recurring neuropsychiatric disorder that is characterized by constant low mood, social isolation, recurrent thought of death and suicide, a feeling of worthlessness, and anhedonia. It

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is twice as common in women than in men, and the associated symptoms in them vary.

An estimated 4–5% of adolescents in mid- to late adolescence suffer from unipolar depressive disorder [2], a frequent mental health issue in teenagers around the world (Lopez, 2006). The prevalence of depression increases significantly during adolescence [3]. Since adolescence is a developmental stage marked by significant biological and social changes, a variety of causes could account for the observed post-pubertal surge in prevalence [4]. Adolescent depression is a significant risk factor for suicide, which ranks between the second and third highest cause of death in this age range [5]. Over 50% of adolescent suicide victims had a depressive illness at the time of their deaths [6].

The etiopathogenesis of depression remains poorly understood. Several factors such as genetic, environmental, and epigenetic factors have been implicated. Its main environmental risk factor is chronic stress, and women report more stress in day-to-day activities than men [1]. However, not all individuals who are stressed develop depression. This underscores the need to explore the underlying mechanisms of vulnerability to stress. The inability of the body to resolve physiological response upon cessation of acute stressful stimuli can lead to harmful conditions which place a burden of adaptation on the brain and body [7]. This leads to susceptibility to stress and increased risk of neuropsychiatric disorders such as depression [8].

There are notable individual differences in how people react to stress: most people exposed to stress maintain normal psychological functioning (resilience to stress), but a small percentage of people (10–20%) who experience prolonged stress go on to develop stress-related psychiatric disorders, including depression (susceptibility to stress) [9]. Research on the neurological underpinnings of depression resilience vs susceptibility has become more and more prevalent. The genetic, epigenetic, and neurophysiological factors behind stress vulnerability as well as the active mechanisms underlying the resilience phenotype are becoming increasingly clear from the mounting body of information [9]. Evidence suggests a 30–50% contribution of genetic heritability to depression. This underscores the importance of investigating the specific genetic factors that may confer susceptibility to depression [10]. Furthermore, even though there is not a single gene that causes depression exclusively, GWAS research has found many genetic variations linked to a higher chance of getting the illness [11]. Multiple genetic variants across the genome collectively contribute to an individual's susceptibility, underscoring the complexity of depression. The diversity in the appearance and severity of depressive symptoms among individuals who share a genetic susceptibility can be explained by the polygenic character of depression [12].

Animal models are used to aid the understanding of its pathogenesis and search for therapies. In these models, several tests are used to assess depression-like behaviors in rodents. Some of the models of stress generate animals that are susceptible to stress, while others are resilient. The chronic mild stress model in rats shows that biochemical and molecular changes are dynamic and are more pronounced in stress-resilient than stress-susceptible rats [13]. Exploring the dynamics of stress-susceptibility and resilience will help in understanding the molecular and biological mechanisms underlying the etiopathogenesis of depression. Despite having important genetic elements, no specific genomic changes or polymorphisms can be directly linked to the etiopathology of depression [13]. Whole genome sequencing (WGS) can provide information regarding all genetic variants—rare variants, loss of function (LOF), functional intronic variants, and structural variants. Significant outcomes from studies on these variants can provide useful information to pinpoint causal genes and their associated pathways [14]. When exposed to CUMS, animals may exhibit depressive-like behavior (show susceptibility) or not (be resilient), depending on how sensitive they are to stressors at different phases of their postnatal development.

Hence, the aim of this study was to determine the differences and/or similarities in susceptibility to depression-like behaviors and gene variants during adulthood in male and female Wistar rats subjected to chronic unpredictable mild stress (CUMS) during adolescence.

Methods

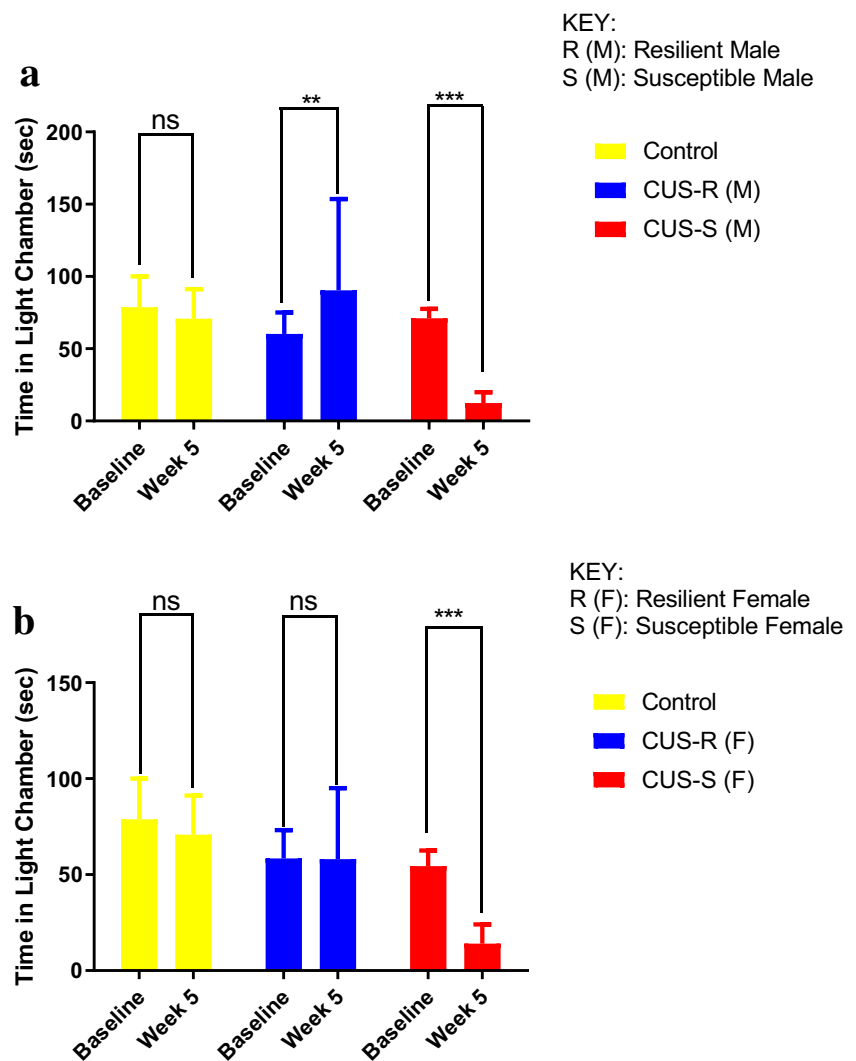
Animals and Animal Grouping

Sixty 6-week-old Wistar rats (30 males and 30 females) weighing between 120 and 150 g were used for this study. The animals were housed five per cage in plastic cages with mesh wire covers under a 12-h light/12-h dark cycle in the animal house of the College of Health Sciences, University of Ilorin, Ilorin, Nigeria. The animals were fed with rat feed pellets and distilled water *ad libitum*. The rats were acclimatized for 2 weeks, and then randomly grouped into the control group and the test group. The control groups consisted of 10 male and female rats each, while the test groups consisted of 20 male and female rats each. The control group was fed with feed pellets and distilled water *ad libitum* without exposure to any stressor. However, the rats in the test group were exposed to the chronic unpredictable mild stress.

Induction of Depression-Like Behaviors

The chronic unpredictable mild stress model (CUMS) was used to induce depression-like behaviors. Two of the

Fig. 1 a Time spent in the light chamber on light–dark box test by male Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post-hoc test. ns, not significant ($p>0.05$); ** $p<0.01$; *** $p<0.001$, respectively. **b** Time spent in the light chamber on light–dark box test in female Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post hoc test. ns, not significant ($p>0.05$); *** $p<0.001$, respectively



following stressors were randomly applied to the rats daily for five (5) weeks: food deprivation (18 h), water deprivation (18 h, followed by 1-h empty drinker), tilting of cages (45° vertical, 3 h), bed wetting (300 ml, 8 h), overcrowding (10 rats per cage for 24 h), and isolation (1 rat per cage for 24 h) [15].

Behavioral Tests

The following behavioral tests were used to assess depression-like behaviors in the experimental rats before and after the induction of depression-like behaviors. Rats were then assigned into CUMS-susceptible (those that show depression-like behaviors) and CUMS-resilient (those that did not show depression-like behaviors) groups.

i. Light–dark box test

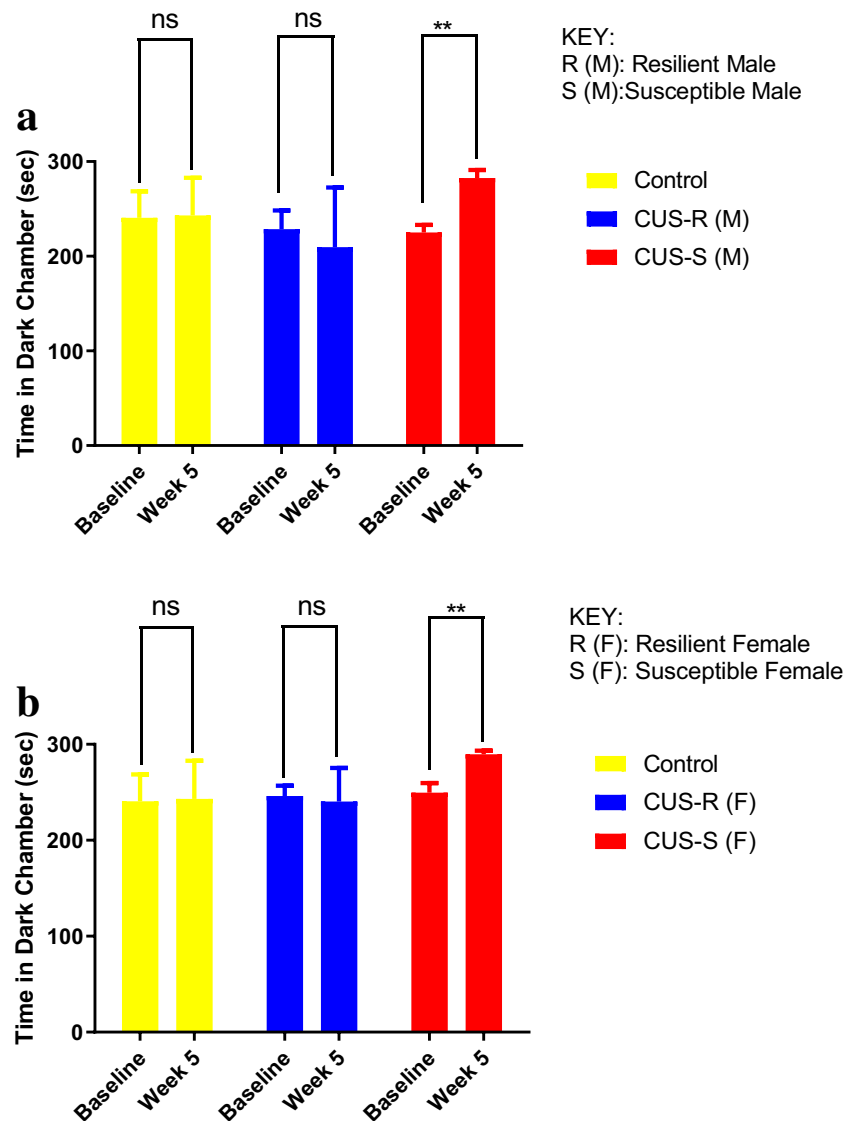
Each rat in the test group was placed in the dark chamber of the maze and allowed to explore the light and dark chambers for a period of 5 min and its move-

ment was recorded using a webcam. The time spent in the light and dark chambers, as well as the number of crosses, were measured.

ii. Sucrose preference test

Mice are acclimatized to having two drinking bottles in their home cage for at least 3 days before testing starts. The amount of water consumed was assessed every day in both bottles to look for potential side preferences in drinking behavior. Following their acclimatization, the rats were divided into single cages and given two drinking bottles to use in their home cage for 3 days: one with 2% sucrose and the other with water. The daily intake of water and sugar solution was determined by measuring the volume left in each bottle using a measuring cylinder. The positions of the two bottles were changed daily to lessen any confusion caused by a side bias. The percentage sucrose consumed was calculated as: % sucrose preference = sucrose intake / (sucrose intake + water intake) $\times$ 100%

Fig. 2 **a** Time spent in the dark chamber on light–dark box test in male Wistar rats. **b** Time spent in the dark chamber on light–dark box test in female Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post hoc tests. ns, not significant $p>0.05$; ** $p<0.01$, respectively



iii. Tail suspension test

The tail suspension test, which is not life-threatening and has a higher accuracy than the forced swim test [16], and no risk of hypothermia due to submersion in water [17], was used to assess depression-like behavior in the test rats. Briefly described, each rat in the test group was taped to a rod and suspended by its tail 1 cm away from the tip, in such a position that it could not escape nor hold on to nearby surfaces. In the test, which lasted for 6 min [18], the first 1 min was considered the habituation (training) period, and the last 5 min were used to measure the immobility of the rats [19]. The immobility time (the time the rat hung passively without any attempt to escape from suspension,) was measured via a sideview recording using a webcam.

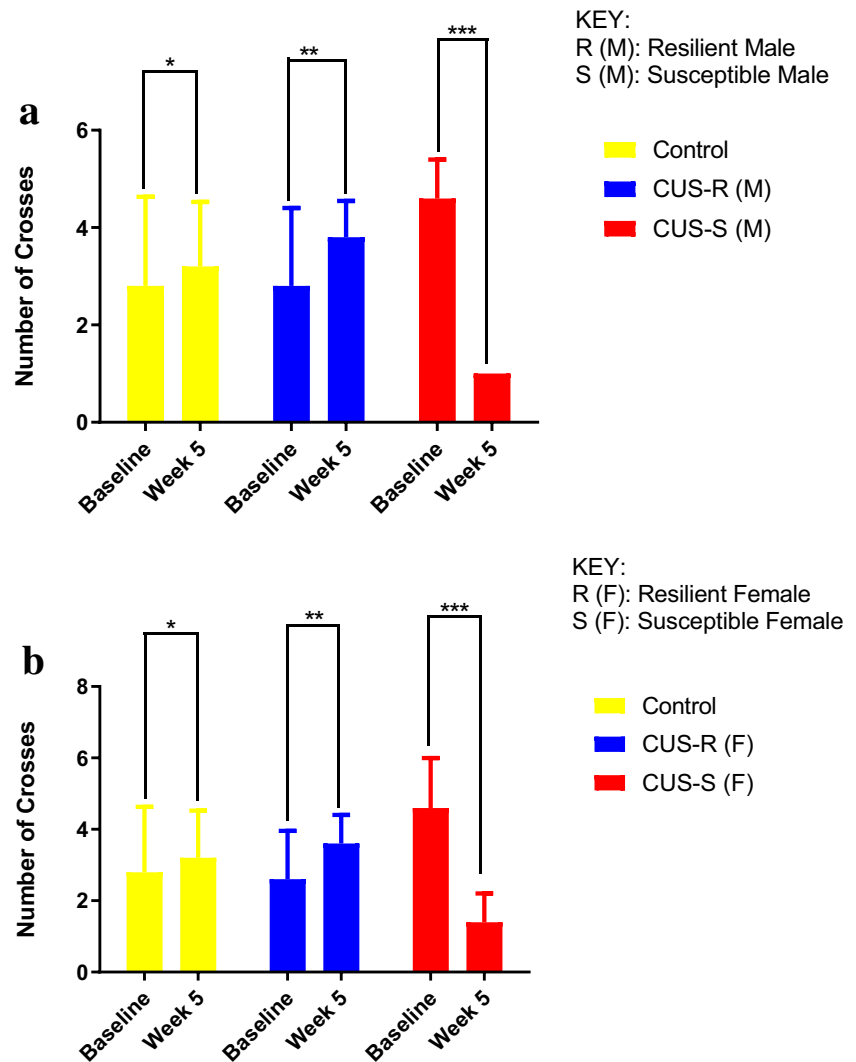
Hippocampal Sample Collection

Twenty-four hours after the behavioral tests, the rats were euthanized with an intraperitoneal injection of ketamine/xylazine 87.5/12.5 mg/kg b.w. The hippocampi of male and female rats in the CUMS-susceptible group were collected on ice under sterile conditions, placed in a plain bottle, and stored at -80°C until genomic DNA (gDNA) was extracted.

gDNA Extraction

Within 12 h of collection, hippocampus tissues were thawed at room temperature, 25 mg of hippocampal tissue was excised, and gDNA was extracted using Zymo Quick-DNA High molecular weight (~ 150 KB) MagBead Kit (cat No. D6060) as described. Briefly, gDNA was extracted from the

Fig. 3 **a** Number of crosses on light–dark box test in male Wistar rats. **b** Number of crosses on light–dark box test in female Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post-hoc test. * $p<0.05$; ** $p<0.01$; *** $p<0.001$, respectively



tissue sample by incubating it in a biofluid and solid tissue buffer alongside proteinase K at 55 °C for 24 h. The extracted DNA was extracted using Quick-DNA MagBinding Buffer and MagBinding Beads. The bound beads were washed using DNA pre-wash buffer and gDNA wash buffer. Finally, the purified DNA was eluted in 50 μ l of DNA elution Buffer (TE buffer).

Whole Genome Sequencing

Nanopore MinION Sequencer (Flowcell FLO-MINSP6) was used for the whole-genome sequencing (WGS) experiment. The rapid sequencing kit (SQK-RAD004) was used for sequencing library generation as described. The kit uses a transposase which simultaneously cleaves template molecules and attaches tags to the cleaved ends. Rapid Sequencing Adapters are then added to the tagged ends and the library was loaded onto the flowcell. The sequencing

experiment ran for 24 h per sample. Each run generated ~15–20 GB of raw sequence signal data (FAST5).

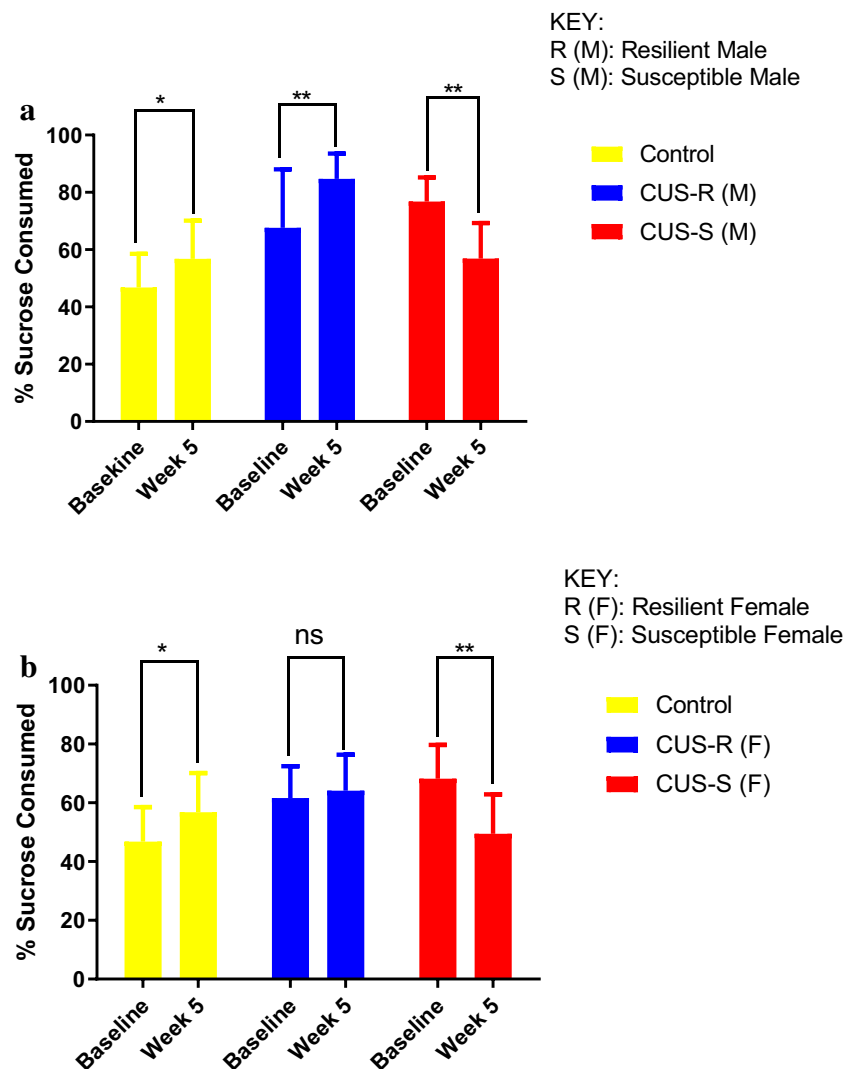
Sequence Base-Calling

Oxford Nanopore Guppy base caller (v5.0.16) was used for sequence reads base-calling from raw sequencing signal files (FAST5). Guppy dna_r9.4.1_450bps_hac.cfg configuration files were used for the base-calling experiment. The base-called sequences were improved using Medaka and Nanopolish [20] to correct base-called sequence errors.

Genome Mapping and Variant Calling

Sequence reads were mapped to the rat reference genome (rn6) using Minimap2 and variants called from alignment BAM files using the Clair3 [21] tool. The Clair3

Fig. 4 a Percentage of sucrose consumed on sucrose preference test in male Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post-hoc test. ns, not significant $p>0.05$; $**p<0.01$; $*p<0.05$, respectively. **b** Percentage of sucrose consumed on sucrose preference test in female Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post-hoc test. ns, not significant ($p>0.05$); $*p<0.05$; $**p<0.01$, respectively



r941_prom_hac_g360 pre-trained model was used for the variant calling experiments.

Variant Analysis

The corresponding VCF files generated from the variant calling experiment were filtered based on their quality score (>30) using SnpSift [22]. SnpEff (v5.0e) [23] was used for nearest gene identification, impact estimation, and annotation of the variants. G-Profiler (version e104_eg51_p15_3922dba) [24] was used for functional enrichment analysis, standard settings were used, and no background dataset was utilized. GeneCup [25] was used to conduct automated searches for genes associated with depression in previous studies.

Statistical Analysis

Data were analyzed using GraphPad version 8.02. Data were analyzed by two-way ANOVA followed by Tukey's multiple

post-hoc test. All values were expressed as Mean $\pm$ S.E.M. and the level of significance was set at $p<0.05$. Outliers were not included in the statistical analysis.

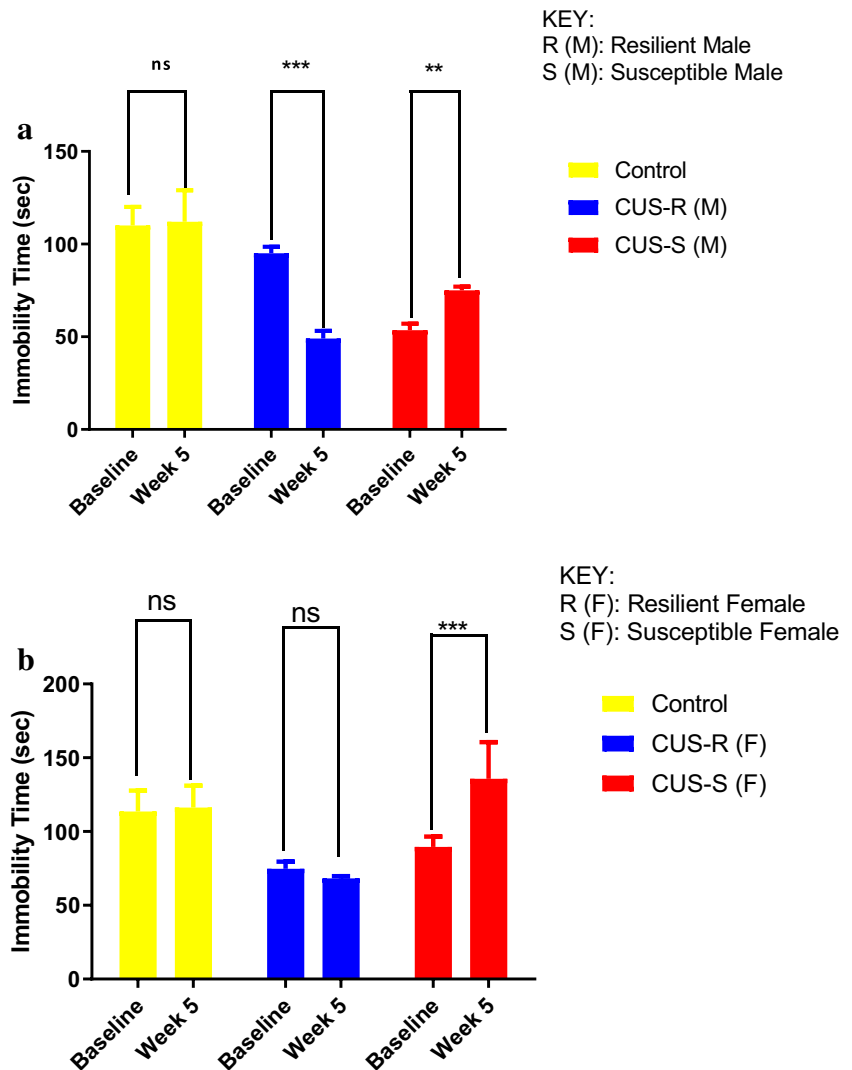
Results

Behavioral Tests

Light–Dark Box Test

Time Spent in the Light Chamber In male rats, there was no significant difference in the time spent in the light chamber at baseline and week 5 in the control group. However, at week 5 compared to baseline, there was a significant increase ($p<0.009$) in the time spent in the light chamber in the resilient group and a significant decrease ($p<0.0005$) in the time spent in the light chamber in the susceptible

Fig. 5 a Immobility time on tail suspension test in male Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post-hoc test. ns, not significant ($p>0.05$); *** $p<0.001$; ** $p<0.01$, respectively. **b** Immobility time on tail suspension test in female Wistar rats. Expressed as mean $\pm$ SEM, $n=5$. Data were analyzed by one-way ANOVA followed by Tukey's multiple post-hoc test. ns, not significant $p>0.05$; *** $p<0.001$, respectively



group as shown in Fig. 1. In the female rats, there was no significant difference in the time spent in the light chamber at baseline and week 5 in the control and resilient groups. However, there was a significant decrease ($p<0.0009$) in the time spent in the light chamber at week 5 compared to the baseline in the susceptible group.

Time Spent in the Dark Chamber There was no significant difference in the time spent in the dark chamber at baseline and week 5 in the control and resilient groups but, there was a significant increase in the susceptible group in both male ($p<0.005$) and female ($p<0.07$) rats, as shown in Fig. 2a and b, respectively.

Number of Crosses There was a significant increase in the number of crosses at week 5 compared to baseline in the control and resilient groups in both male ($p<0.04$; 0.005)

and female ($p<0.04$; 0.007) rats respectively. However, there was a significant decrease in the number of crosses at week 5 compared to baseline in the susceptible group in both male ($p<0.0001$) and female ($p<0.0006$) rats (Fig. 3).

Sucrose Preference Test

In the male rats, there was a significant increase in the percentage of sucrose consumed in the control ($p<0.01$) and resilient ($p<0.001$) groups at week 5 compared to baseline. However, in the susceptible group, there was a significant decrease ($p<0.002$) in the percentage of sucrose consumed at week 5 compared to baseline. In the female rats, at week 5 compared to baseline, there was a significant increase in the percentage of sucrose consumed in the control group ($p<0.04$), no significant difference in the resilient group,

Table 1 Variant analysis

A.		Male		Female	
	SNP	289839		1570186	
	INS	7002		109860	
	DEL	34459		597241	
	MIXED	0		1	

B.		Male		Female	
	Type	Count	Percent	Count	Percent
	MISSENSE	2244	63.913%	16454	63.672%
	NONSENSE	117	3.332%	789	3.053%
	SILENT	1150	32.754%	8599	33.275%

C.		Male		Female	
	Type	Count	Percent	Count	Percent
	DOWNSTREAM	16491	4.26%	236264	8.456%
	EXON	3869	0.999%	46012	1.647%
	INTERGENIC	229423	59.261%	1638922	58.655%
	INTRON	116912	30.199%	737911	26.409%
	SPLICE_SITE_ACCEPTOR	84	0.022%	1266	0.045%
	SPLICE_SITE_DONOR	34	0.009%	351	0.013%
	SPLICE_SITE_REGION	282	0.073%	1992	0.071%
	UPSTREAM	18506	4.78%	120119	4.299%
	UTR_3_PRIME	1222	0.316%	8464	0.303%
	UTR_5_PRIME	317	0.082%	2834	0.101%

D.		Male		Female	
	Type	Count	Percent	Count	Percent
	HIGH	354	0.091%	21130	0.756%
	LOW	1493	0.386%	11233	0.402%
	MODERATE	2245	0.58%	16574	0.593%
	MODIFIER	383048	98.943%	2745214	98.249%

(A) The total number of single nucleotide polymorphism, insertions, deletions, and mixed variants. (B) The total variant types. (C) Variants per region of interest. (D) The impact of variants on the genome

and a significant decrease ($p < 0.001$) in the susceptible group (Fig. 4).

Tail Suspension Test

There was no statistically significant difference in the immobility time of the control group at baseline and week 5. However, there was a significant ($p < 0.0005$) decrease in the immobility time of the resilient group, and a significant increase in the immobility time in the susceptible group in the male ($p < 0.04$) (Fig. 5a) and female (Fig. 5b) rats, respectively.

Sequencing

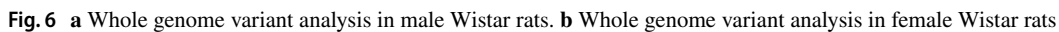
We carried out a low genome coverage sequencing of the brain samples (hippocampus) excised from male and female rats in the susceptible group. 3.2 million reads were generated with an average read length of 42 kb per sample, over

a sequencing run of 48 h. A 2-times coverage was achieved when mapped to the reference genome: at each point of the genome, there was 98–99% coverage, with 95–99% percentage accuracy (Table 1). A higher number of variants were observed in the female samples than in the male samples, 98% of variants had modifier impacts, majority of the variants were found in the intergenic and intron regions of the genome (Table 1 and Fig. 6a and b).

Gene Ontology (GO)-Term and Kyoto Encyclopedia of Genes and Genome (KEGG)-Term Enrichment Analysis

Twenty-five genes (22 in female, 3 in male) implicated in other depression studies were affected by high-impact variants in this study; stop gained and frameshift modifications are the variants that affected the genes (Fig. 6a and b, Table 2).

The image summarizes all bioinformatic results in this study. The image is a circular diagram consisting of tracks or rings. Each track represents a different set of data or analyses. From the outermost track (Track 1) we represent the



to have high-impact variants and have also been implicated in other depression studies.

Several studies have shown that the CUMS model is capable of producing susceptible and resistant animals [26, 27]. Various hereditary, epigenetic, environmental, and

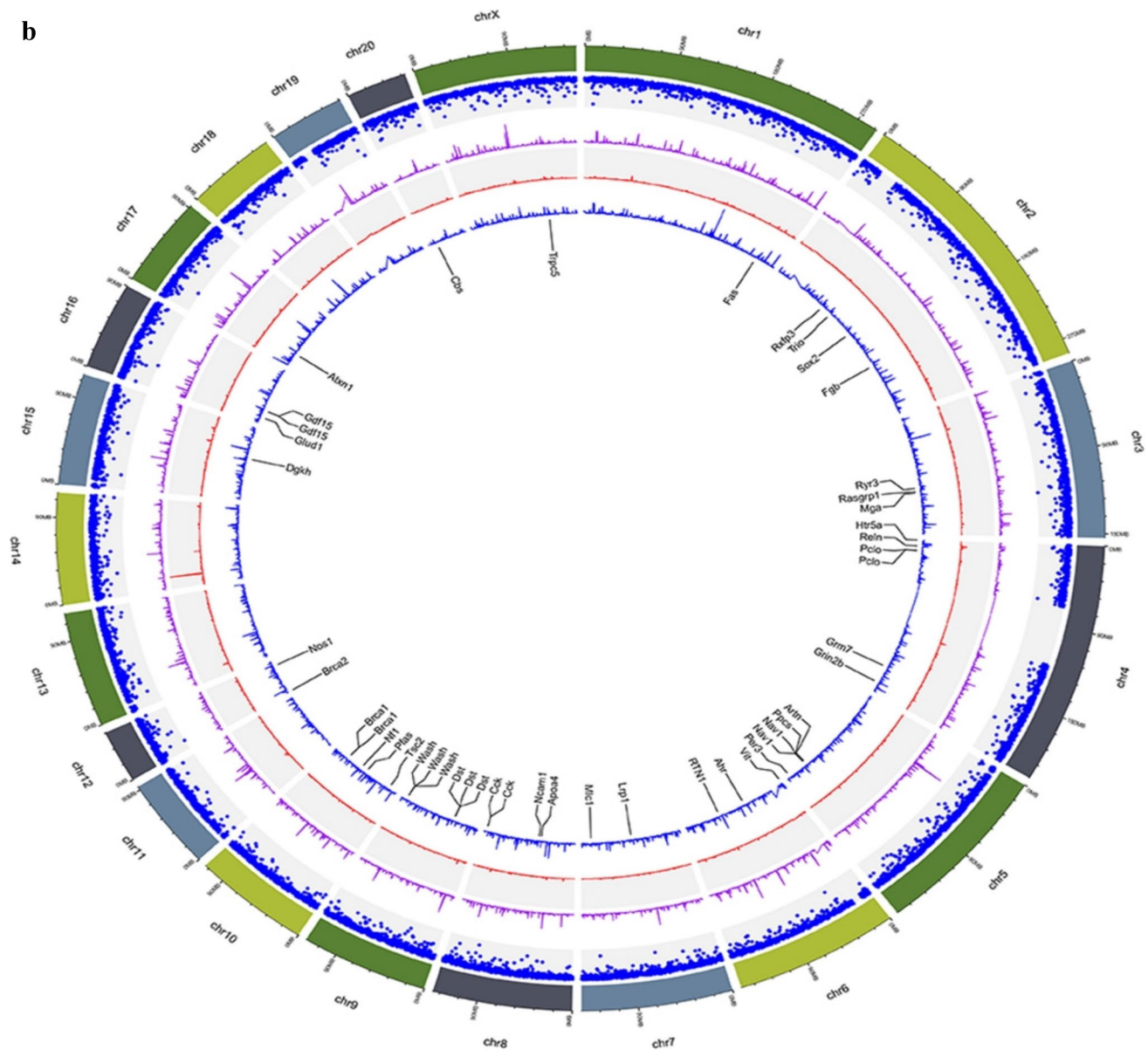


Fig. 6 (continued)

endocrine risk factors affect susceptibility to depression [28]. In this study, approximately 41% of the male rats and 49% of female rats subjected to CUMS showed significant depression-like behaviors following assessment on behavioral tests, while approximately 31% and 29% of the male and female rats, respectively, were resistant to CUMS. The percentage of susceptible male rats reported in this study is higher than those reported in a study conducted by Kotel'nikova et al. [29], where 37.5% of male albino rats subjected to learned helplessness showed depression-like behaviors.

The percentages of susceptible and resilient male Wistar rats in this study are slightly lesser (41%) and higher (31%)

respectively than those reported by Henningsen et al., [26], who showed that 43% of male Wistar rats subjected to CUMS were anhedonic, while 23% were resistant. These findings are in tandem with a higher incidence of depression in human females.

Furthermore, whole genome sequencing of gDNA from the rat hippocampi in this study revealed variations in the single nucleotide polymorphisms (SNPs), insertions, deletions, and high-impact variant genes in male and female Wistar rats. To the best of our knowledge, the authors believe that this study is the first to compare these variations (single nucleotide polymorphisms (SNPs), insertions, deletions, and high-impact variant genes) in male and female Wistar rats.

Table 2 Overview of high-impact variants on depression-implicated genes

	Chr	Position	Mutation	Gene	Modification
Male	3	97924557	C > T	Traf6	Stop gained
Male	3	97924563	C > T	Traf6	Stop gained
Male	3	97924580	delTGCCA	Traf6	Frameshift variant
Male	14	37836294	delA,G > A,A > G	Tec	Frameshift variant
Male	14	37836337	G > A	Tec	Stop gained
Male	20	29242400	dupA	Tet1	Frameshift variant
Male	20	29242404	dupA	Tet1	Frameshift variant and stop gained
Male	20	29242410	dupG	Tet1	Frameshift variant
Female	1	259844521	A > T	Fas	Stop gained
Female	2	140811198	G > A	Sox2	Stop gained
Female	2	100260172	C > T	Trio	Stop gained
Female	3	118286397	dupG	Mga	Frameshift variant
Female	4	16622740	insAA	Pclo	Frameshift variant and stop gained
Female	4	9737399	dupGG	Reln	Frameshift variant
Female	1	171668688	C > T	Per3	Stop gained
Female	8	129318023	C > T	Cck	Stop gained
Female	8	129318092	C > T	Cck	Stop gained
Female	8	52499191	C > T	Ncam1	Stop gained
Female	8	49164970	A > T	Apoa4	Stop gained
Female	9	37725028	C > T	Dst	Stop gained
Female	9	37715667	C > T	Dst	Stop gained
Female	9	37715685	C > T	Dst	Stop gained
Female	9	113458846	C > T	Wash	Stop gained
Female	10	64739817	C > T	Nf1	Stop gained
Female	10	89206949	Del	Brca1	Frameshift variant
Female	10	55320860	dupC	Pfas	Frameshift variant
Female	10	13790466	G > A	Tsc2	Stop gained
Female	12	531527	insTACA	Brca2	Frameshift variant
Female	12	46173740	dupGT	Nos1	Frameshift variant
Female	16	20413223	dupGC	Gdf15	Frameshift variant
Female	16	9011268	C > T	Glud1	Stop gained
Female	20	12565369	dupG	Cbs	Frameshift variant
Female	X	114179074	C > T	Trpc5	Stop gained

In this study, CUS-susceptible female rats showed over five times the number of single nucleotide polymorphisms than CUS-susceptible male rats on whole genome sequencing. Numerous investigations have documented single-nucleotide polymorphisms in the clock genes of depressed patients. In a CMS-induced depressive state in rats, Christiansen et al. [30] reported a selective and region-specific alteration in the expression of clock genes, especially *Per2*, *Bmal1*, and *Per1* genes. Zhang et al. [31] reported that the SNPs at the 5-HTTLPR and *BDNF* gene loci and SNPs of the microRNA146a gene at rs2910164, the *ACE2* gene at rs2285666, and the *SGK1* gene at rs1743963 and rs1763509, are associated with the onset of comorbid depression in coronary heart disease. The majority of phenotypic and behavioral differences between individuals or strains are most likely caused by single nucleotide polymorphisms (SNPs),

which are the most frequent source of genetic diversity in populations [32]. The higher number of SNPs in CUS-susceptible female rats observed in this study suggests that a higher number of SNPs may be associated with increased susceptibility to depression-like behaviors in female rats as seen in this study.

Similarly, the whole genome sequencing of the hippocampal gDNA carried out in this study revealed a massive variation in insertions and deletions (indels): more than 15-fold increase in the number of insertions, and a greater than seventeen-times elevation in the number of deletions, in CUS-susceptible female rats compared to the CUS-susceptible male rats. Whole genome sequencing revealed more insertions and less deletions in depression and stress-reactivity genes in Wistar Kyoto less immobile (WLI) rats than the Wistar Kyoto more immobile (WMI)

rats [33]. However, it has been reported that male rodents are more likely than females to carry minor indel mutations and nucleotide alterations [34]. Higher frequencies of low-activity (short) alleles which increase susceptibility to depression are found in males with uni- and bi-polar depression [35]. The significantly higher number of indels in CUS-susceptible female rats revealed in this study may be evidence of the molecular mechanism underlying the increased incidence of depression in human females and depression-like behaviors in female rodents.

Whole genome sequencing is a valuable tool that is used to reduce the number of genetic variants to a manageable candidate list [36] through the identification of genes with high-impact variants. In our study, we observed genes with high-impact variants (3 in males: TRAF6, TEC & TET1; and 22 in females: FAS, SOX2, TRIO, MGA, PCLO, RELN, PER3, CCK, NCAM1, APOA4, DST, WASH, NF1, BRCA1, PFAS, TSC2, BRCA2, NOS1, GDF15, GLUD1, CBS, and TRPC5) in the groups that were susceptible to depression-like behaviors. The modifications in these genes are stop gained and frameshift variants, which were associated with increased susceptibility to depression-like behaviors.

Conclusion

This study revealed that a higher number of female Wistar rats showed depression-like behaviors following exposure to chronic unpredictable mild stress and have more single nucleotide polymorphisms, insertions, deletions, and high-impact variants than male Wistar rats. This suggests a genetic implication as to why major depressive disorder is more common in females than in males.

Recommendation

The high-impact variants found in this study should be further targeted as biomarkers for the risk, diagnosis, pharmacological treatment, and prognosis of major depression.

Limitation

This study did not consider the estrous cycle of the female Wistar rats. Although there have been contradictory reports on the effect of estrous cycle in female Wistar rats on susceptibility to depression, future research comparing depression-like behaviors in female Wistar rats at the different phases of the estrous cycle will further elucidate the role of estrous cycle in susceptibility to depression.

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Data Availability Data generated from this study are available upon request.

Declarations

Ethical Approval This study was approved by the University Ethical Review Committee (UREC), University of Ilorin Kwara state, with the approval number: UREC/ASN/2023/2426. All of the experimental procedures involving animals were conducted in accordance with the institutional guideline and regulations for care and use of laboratory animals were followed.

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