Integration of Transcriptome and Exome Genotyping Identifies Significant Variants with Autism Spectrum Disorder

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Abstract: Autism is a complex disease with genetic predisposition factors. Real factors for treatment and early diagnosis are yet to be defined. This study integrated transcriptome and exome genotyping for identifying functional variants associated with autism spectrum disorder and their impact on gene expression to find significant variations. More than 1800 patients were screened, and 70 (47 male/23 female) with an average age of 7.56 ± 3.68 years fulfilled the DSM-5 criteria for autism. Analysis revealed 682 SNPs of 589 genes significantly (p < 0.001) associated with autism among the putative functional exonic variants (n = 243,345) studied. Olfactory receptor genes on chromosome 6 were significant after Bonferroni correction (α = 0.05/243345 = 2.05 × 10⁻⁹ with a high degree of linkage disequilibrium on 6p22.1 (p = 6.71 × 10⁻⁹). The differentially expressed gene analysis of autistic patients compared to controls in whole RNA sequencing identified significantly upregulated (foldchange ≥ 0.8 and p-value ≤ 0.05; n = 125) and downregulated (foldchange ≤ -0.8 and p-value ≤ 0.05; n = 117) genes. The integration of significantly up- and downregulated genes and genes of significant SNPs identified regulatory variants (rs6657480, rs3130780, and rs1940475) associated with the up- (ITGB3BP) and downregulation (DDRI and MMP8) of genes in autism spectrum disorder in people of Arab ancestries. The significant variants could be a biomarker of interest for identifying early autism among Arabs and helping to characterize the genes involved in the susceptibility mechanisms for autistic subjects.

Keywords: autism spectrum disorder; neurodevelopmental disorder; SNPs; regulatory variants; gene expression; RNA sequencing

1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that exhibits a wide range of symptoms of varying severity and has a high prevalence in the Gulf region [1]. Recent research suggests that gene variants, altered biological mechanisms, and the environment together influence the development of autism [2]. The most common symptoms of autism are impaired social skills, difficulty with communication,
and repetitive movements. Autism can be detected at the age of 3 years, and perhaps as early as 18 months [3]. ASD heritability has been estimated to be 40–90%, and a number of common variants contribute to the significant variability in the condition, in addition to epigenetic, environmental, and hormonal factors [4–6]. Moreover, the Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition includes atypical sensory processing, which has been reported in more than 70% of patients with ASD as a diagnostic criterion (DSM-5; American Psychiatric Association, 2013) [7–9].

Genes differentially expressed in autism are important for understanding the associated pathways [10–12]. This meta-analysis looked at ten Gene Expression Omnibus datasets, which included 364 cases and 248 controls. It discovered 3105 genes that were consistently expressed differently in autism (1680 downregulated genes and 1425 upregulated genes). PNPLA2, LYPLA2, LYPLA2P1, PLA2G4D, PLA2G6, PLA2G7, and PLA2G5 were also discovered to be related to phospholipase A2 (PLA2). The enriched gene ontology terms for molecular functions were involved in structural constituents of ribosomes and transcription regulator activity, while the enriched gene ontology terms for biological processes were involved in translational elongation and the response to cytokine stimuli, according to the study. The ribosome route was the most important pathway in our KEGG study. The meta-analysis discovered genes that were consistently differentially expressed in autism, as well as biological pathways linked to gene expression alterations [10]. Topological analysis of the autistic brain identified proteomic hub gene signatures: CDK2, BAG3, MYC, TP53, HDAC1, CDKN1A, EHZ2, GABARAPL1, TRAF1, and VIM [11]. The study also revealed the transcriptional regulating factors, such as YY1, FOXL1, USF2, FOXC1, GATA2, NFIC, E2F1, NFKB1, TFAP2A, and HINFP [11].

Despite rapid developments in genetic techniques, for diagnostic purposes, common variants of neuropsychiatric disorders are still lacking. An exome microarray provides a potential diagnostic method for many complex genetic diseases such as ASD. Unlike other genetic techniques, the exome microarray is effective for profiling variants in associated genes with >5% allele frequency [13]. In addition, it provides exceptional coverage of the whole exonic region delivered from over 12,000 individual exome sequences [14]. The present study conducted whole RNA sequencing and exome microarray analysis to identify the most prominent variants associated with autism in patients in Saudi Arabia.

2. Materials and Methods

2.1. Sample Collection

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Imam Abdulrahman Bin Faisal University. Cheek swab samples were collected for DNA and blood samples for RNA from children and adolescents up to 18 years of age from the Eastern Province of Saudi Arabia upon receiving signed informed consent from a parent and/or legal guardian for participation in the study. A total of 202 subjects of Arab ancestry (132 controls and 70 cases) provided cheek swab samples for genomic DNA extraction for exome genotyping (Table 1). A total of 1800 patients were evaluated in a pediatric neurology clinic and screened for autism from January 2018 to December 2018. Of these, 70 patients (47 males and 23 females) with an average age of 7.56 ± 3.68 years fulfilled the DSM-5 criteria for autism [9]. The clinical evaluation of the patients revealed an overall positive family history of 8.57% (n = 6). Patients with comorbidities were excluded from this study. Healthy subjects without familial autism were selected as controls.

2.2. DNA Extraction and Exome Array

The Gentra Puregene Buccal Cell Kit (Qiagen, Hilden, Germany) was used to extract DNA from the buccal cell samples. The Human Exome Bead Chip Kit v1.0 and v1.1 Illumina (San Diego, CA, USA), which consists of 243,345 putative functional exonic markers, was utilized with Illumina iScan for the microarray. All DNA samples were processed according to the manufacturer’s protocol, and iScan control software (Illumina) was used to acquire
data. DNA extraction and the microarray genotyping and analysis were performed at the genetics research laboratory of the Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. Genotyping was carried out in this lab from 2016 to 2018 using the same chip and procedures.

Table 1. Clinical characteristics of patients with autism and controls from Eastern Province, Saudi Arabia.

| Parameter       | Control Group $n = 132$ | Autism Group $n = 70$ | p-Value     |
|-----------------|-------------------------|-----------------------|-------------|
| Age (year)      | 8.04 ± 3.01             | 7.56 ± 3.68           | 0.1955      |
| Gender          | F = 71; M = 61          | F = 23; M = 47        | -           |
| Weight (kg)     | 30.34 ± 13.36           | 26.40 ± 12.04         | 0.0582      |
| Height (cm)     | 121.59 ± 22.01          | 121.68 ± 21.58        | 0.4913      |
| Body mass index | 19.93 ± 4.53            | 16.80 ± 1.78          | <0.00001*   |

The data are presented as the mean values ± standard deviations. * $p \leq 0.05$ was considered statistically significant.

2.3. Genotyping and Functional Analysis

GenomeStudio 2.0 Data Analysis software (Illumina) was used for the initial quality check of the call rate. A total of 21 controls and 2 patients with autism were excluded from the analysis due to a call rate <0.975%, and the results were thus re-clustered. The Hardy–Weinberg equilibrium (HWE) was tested separately among the case and control groups with a 1 degree of freedom (df) genotypic chi-square test. Differences in clinical characteristics between cases and controls were calculated by the two-sample $t$-test or the $\chi^2$ test, as appropriate, using IBM SPSS Statistics version 23 software (IBM Co., Armonk, NY, USA). Kaviar [15] and SNP-Nexus [16] were used to confirm variants reported at a base pair position on the respective chromosome as per Genome Reference Consortium Human Build 37 (GRCh37.p13.). Case–control association analyses, odds ratios, and 95% confidence intervals were calculated to evaluate the effects of different alleles and haplotypes using Haploview version 4.2 [17] and gPLINK version 2.050 [18]. Bonferroni corrections or false discovery rate corrections were applied to correct the $p$-values of the 243,345 single nucleotide polymorphisms (SNPs) (corrected $\alpha = 0.05/243,345 = 2.05 \times 10^{-7}$) to control inflation of the type I error rate. A $p$-value < 0.05 was considered significant. The highly significant ($p < 1 \times 10^{-5}$) genes were annotated for functional implications using DAVID 6.7 [19], STRING 10.5 [20], SNP-Nexus [16], Expression Atlas [21], Reactome [22], FunRich [23], Toppgene [24], and Enrichr [25].

2.4. Whole RNA Sequencing and Differentially Expressed Genes

Total ribonucleic acid (RNA) was extracted from blood (30 controls and 30 patients with autism) using the RNeasy plus micro kit (Qiagen, Hilden, Germany) following the manufacturer’s guidelines. Total RNA was quantitated using the Qubit RNA HS assay kit (Life Technologies, Eugene, OR, USA), and the quality was assessed by an Agilent 2100 (Agilent, Santa Clara, CA, USA). High-quality RNA was used to construct the cDNA library. Twenty milligrams of total RNA from each sample was treated separately with oligo (dT) for mRNA enrichment. N6 random primers were used for reverse-transcribing the target RNA fragments into double-strand cDNA. End repair was performed on double-strand cDNA fragments, and adaptors were ligated to the double-strand cDNA. All the ligated double-strand cDNA were PCR-amplified, denatured by heat into single-strand DNA and quantified, and subjected to DNA nanoball synthesis and sequencing on the DNBseq platform. Transcriptome assembly was carried out after subjecting the raw reads to quality control. Reads of adaptors and unknown bases, as well as low-quality reads, were discarded. The raw RNA sequencing data reads with high quality were mapped using the tophat RNA Sequence mapping tool. The aligned sequence data were analyzed for the differential expression between the autistic and the control subjects by considering the foldchange $\geq 0.8$ and $p$-value $\leq 0.05$ for upregulated genes and the foldchange $\leq -0.8$
and \( p \)-value \( \leq 0.05 \) for downregulated genes. All the expressed genes from the autistic and control samples with a quality Phred score \( \geq 30 \) were only subjected to the differential expressed gene analysis. The highly significantly up- and downregulated genes were annotated for pathway enrichment analysis using DAVID 6.7 [19], STRING 10.5 [20], Expression Atlas [21], Reactome [22], FunRich [23], Toppgene [24], and Enrichr [25].

3. Results

The study revealed the significant regulatory variants associated with the development of autism in people with Arab ancestries using multiomic analysis (Figure 1).

![Figure 1. Integration of whole RNA sequencing-based differentially expressed gene analysis (up-regulated: foldchange \( \geq 0.8 \) and \( p \)-value \( \leq 0.05 \), \( n = 125 \); downregulated: foldchange \( \leq -0.8 \) and \( p \)-value \( \leq 0.05 \), \( n = 117 \)) of patients with autism in relation to the healthy controls with genes of significant genotypes. Venn diagram analysis of the curated data identifies three (rs6657480, rs3130780, and rs1940475) regulatory variants in the up- (ITGB3BP) and downregulated (DDR1 and MMP8) genes in autistic patients. * List of the fivefold downregulated and upregulated genes can be considered for the early identification of autism patients among Saudis.]

\[\text{Patients with Autism} \quad \text{Healthy Control}\]

Exome microarray

Genes with significant SNPs

\[\text{SNPs} \quad 586 \quad 0 \quad 124 \quad 0 \quad 115\]

\[\text{UpReg} \quad \text{DownReg}\]

Down regulation of MMP8 and DDR1 & Upregulation of ITGB3BP genes

Associated with Autism in Saudis
A total of 13 SNPs were significantly \((p < 2.05 \times 10^{-7})\) associated with ASD even after Bonferroni or false discovery rate corrections (corrected \(\alpha = 0.05/243,345 = 2.05 \times 10^{-7}\)) among the 243,345 SNPs screened, and they obeyed the HWE. Table 2 summarizes the most significant SNPs that were associated with autism in patients of Arab ancestry. Common variants in olfactory receptor family 12 subfamily D member 2 \((OR12D2)\) and olfactory receptor family 5 subfamily V member 1 \((OR5V1)\) showed the strongest signal in Arab patients with autism. The top 10 most significantly \((p < 2.05 \times 10^{-7})\) associated SNPs were in the region of the olfactory receptor genes \(OR12D2\) and \(OR5V1\) on chromosome 6p22.1 (Figure 2). Additional information about the significant SNPs \((p < 0.0001)\) in autism patients is provided in Table S1. The results suggest that a single gene, \(OR12D2\) (including up and downstream; NC_000006.11:g.29360183–g.29369519), has 10 common SNPs with large effects on risk \((rs9257819, rs2022077, rs9257834, rs4987411, rs2073154, rs2073153, rs2073151, rs2073149, rs1028411, and rs2394607)\), including five SNPs in the coding region \((rs9257834, rs4987411, rs2073154, rs2073153, and rs2073151)\).

All the association tests were screened using the frequency of minor alleles in controls, the \(p\)-value of the HWE, and the type I error rate to identify the strongest genetic findings, which were input for linkage disequilibrium in HapMap SNPs of selected regions on chromosome 6 (Figure 2). The variants in the region of the olfactory receptor genes \(OR12D2\) and \(OR5V1\) had a high degree of linkage disequilibrium \((p = 6.71 \times 10^{-9})\) (Figure 2). The haplotype associated with the most significant risk in patients of Arab ancestry with autism, AAGTCTGATT \((p = 6.7082 \times 10^{-9})\), consisted of the top 10 SNPs \((rs9257819A, rs2022077A, rs9257834G, rs4987411T, rs2073154C, rs2073153T, rs2073151G, rs2073149A, rs1028411T, and rs2394607T)\) (Table 3). The protective haplotype most significantly associated with the control subjects of Arab ancestry, CTTCGGATGC \((p = 2.43 \times 10^{-8})\), also consisted of the top 10 SNPs \((rs9257819C, rs2022077T, rs9257834T, rs4987411C, rs2073154G, rs2073153G, rs2073151A, rs2073149T, rs1028411G, and rs2394607C)\) with the alleles that were not associated with autism (Table 3).

### 3.1. RNA Sequencing and Differentially Expressed Genes

Blood samples were collected in the RNA protectant vacutainer with an RNA stabilizer to obtain more accurate results. Total RNA was isolated for the transcriptome profiling study using paired-end sequencing. Raw reads were checked for a quality Phred score \(\geq 30\). The qualifying reads were processed for mapping on the reference genome and expression profile preparation. The raw RNA sequencing data reads were trimmed for the adaptors and mapped by using the tophat RNA Sequence mapping tool with the reference RNA sequence. All RNA sequence data were subjected to complete differential expression analysis between the autistic and control subjects. The differential expression between the autistic and control subjects was calculated, revealing upregulated \((\text{foldchange} \geq 0.8\) and \(p\)-value \(\leq 0.05)\) and downregulated \((\text{foldchange} \leq -0.8\) and \(p\)-value \(\leq 0.05)\) genes. Autistic patients and healthy controls were considered two distinct groups for differential expression calculation using Cuffdiff [26]. Genes were filtered for differential expression through all significant DEGs based on a \(p\)-value \(\leq 0.05\). For upregulated genes, we used a cutoff \(p\)-value \(\leq 0.05\) and foldchange \(\geq 0.8\), and for downregulated genes, we used a cutoff \(p\)-value \(\leq 0.05\) and foldchange \(\leq -0.8\). All the expressed genes from the autistic and control samples with a quality Phred score \(\geq 30\) were subjected to differential expressed gene analysis, and the results are presented in Figure 3 as volcano plots. The significantly upregulated and downregulated genes were considered with foldchange \(\geq 0.8\) and \(p\)-value \(\leq 0.05\) and foldchange \(\leq -0.8\) and \(p\)-value \(\leq 0.05\), respectively.
Figure 2. A: Manhattan plot of putative functional exonic variants \( n = 243,345 \) in the molecular genetics of autism from the association study. The \( \log_{10}(p) \) values from the association are plotted according to variant position on each chromosome. Positions of candidate genes of functional exonic variants at olfactory receptor genes on chromosome 6p22.1 for autism are indicated by red arrows. The horizontal red line indicates the preset threshold of \( p = 4.00 \times 10^{-7} \). The horizontal pink line indicates the suggestive threshold of \( p = 1.00 \times 10^{-5} \). B: Genetic association of the most significant variants at the OR12D2 and OR5V1 genes in the chromosome 6p22.1 region and linkage disequilibrium results in subjects of Arab ancestry with autism. Block 1 indicates the most significant risk haplotype, AAGTCTGATT \( (p = 6.7082 \times 10^{-9}) \), associated with the Arab-ancestry autism subjects.
Table 2. List of the most significant SNPs associated with autistic patients.

| S.No | SNP ID   | CHR   | BP     | MA    | MAF   | CHISQ | P         | OR (L95–U95) | Gene | AA  | Case, Control Frequencies | HWpval |
|------|----------|-------|--------|-------|-------|--------|-----------|-------------|-------|-----|----------------------------|---------|
| 1    | rs2073149| 6     | 2936542| 0.5795| 33.14 | 5.87 × 10^-9 | 0.2367 (0.143–0.3918) | OR12D2 | A   | 0.754, 0.424 | 0.0563 |
| 2    | rs2073153| 6     | 2936303| 0.5465| 30.5  | 5.34 × 10^-9 | 0.2489 (0.195–0.4131) | OR12D2 | T   | 0.769, 0.456 | 0.0308 |
| 3    | rs2073151| 6     | 2936491| 0.5398| 30.1  | 4.10 × 10^-9 | 0.2498 (0.150–0.4145) | OR12D2 | T   | 0.773, 0.457 | 0.0504 |
| 4    | rs2394607| 6     | 2936519| 0.5682| 30.07 | 4.18 × 10^-9 | 0.2586 (0.157–0.4241) | OR5V1  | T   | 0.746, 0.438 | 0.1677 |
| 5    | rs9527819| 6     | 2936187| 0.5398| 29.53 | 5.46 × 10^-9 | 0.2558 (0.185–0.3751) | OR5V1  | T   | 0.746, 0.422 | 0.0394 |
| 6    | rs9527834| 6     | 2936415| 0.5398| 29.55 | 5.46 × 10^-9 | 0.2558 (0.185–0.3751) | OR5V1  | T   | 0.746, 0.422 | 0.0394 |
| 7    | rs9497413| 6     | 2936463| 0.5398| 29.55 | 5.46 × 10^-9 | 0.2558 (0.185–0.3751) | OR5V1  | T   | 0.746, 0.422 | 0.0394 |
| 8    | rs2073154| 6     | 2936485| 0.5398| 29.55 | 5.46 × 10^-9 | 0.2558 (0.185–0.3751) | OR5V1  | T   | 0.746, 0.422 | 0.0394 |
| 9    | rs1028411| 6     | 2936759| 0.5398| 29.55 | 5.46 × 10^-9 | 0.2558 (0.185–0.3751) | OR5V1  | T   | 0.746, 0.422 | 0.0394 |
| 10   | rs2022077| 6     | 2936112| 0.5398| 28.55 | 9.14 × 10^-8 | 0.261 (0.157–0.4325) | OR5V1  | T   | 0.766, 0.466 | 0.0475 |
| 11   | rs9383563| 6     | 150212003| 0.2988 | 27.09 | 1.94 × 10^-6 | 0.1995 (0.06093–0.3193) | LOC10053 | G | 0.946, 0.719 | 0.2827 |

CHR: chromosome; SNP ID: single nucleotide polymorphism ID; BP: base pair position at the respective chromosome as per GRCh37.p13; MA: minor allele name; MAF: frequency of minor allele in controls; CHISQ: basic allelic test chi-square; P: p-value; OR: odds ratio; SE: standard error; L95: lower bound of 95% confidence interval for odds ratio; U95: upper bound of 95% confidence interval for odds ratio. AA: associated allele; HWpval: p-value of Hardy–Weinberg equilibrium.

Table 3. Significant haplotypes of SNPs on chromosome 6 in autistic patients.

| Block   | Haplotype | Frequency | Case, Control Ratio Counts | Case, Control Frequencies | Chi-Square | p-Value |
|---------|-----------|-----------|---------------------------|---------------------------|------------|---------|
| **Block 1** | AAGTCTGATT | 0.547 | 97.0:33.0, 89.0:121.0 | 0.746, 0.424 | 33.618 | 6.71 × 10^-9 ** |
|         | CTTCGGATG | 0.421 | 30.0:100.0, 113.9:0.070 | 0.231, 0.538 | 31.12 | 2.43 × 10^-8 ** |
|         | AAGTCTGGTC | 0.024 | 3.0:127.0, 5.0:205.0 | 0.023, 0.024 | 0.002 | 0.9655 |
| **Block 2** | CG | 0.653 | 102.0:28.0, 120.0:90.0 | 0.785, 0.571 | 16.104 | 6.00 × 10^-5 ** |
|         | AT | 0.347 | 28.0:102.0, 90.0:120.0 | 0.215, 0.429 | 16.104 | 6.00 × 10^-5 ** |
| **Block 3** | TA | 0.541 | 88.0:42.0, 96.0:114.0 | 0.677, 0.457 | 15.62 | 7.74 × 10^-5 ** |
|         | CG | 0.459 | 42.0:88.0, 114.9:0.600 | 0.323, 0.543 | 15.62 | 7.74 × 10^-5 ** |

**Significant risk haplotypes associated with autistic patients. * Significant protective haplotypes associated with control subjects.**
A list of the top 20 upregulated genes with foldchange $\geq 1.98$, a $p$-value $\leq 0.0025$ and a $q$-value $\leq 0.05$ is presented in Table 4. A list of the top 20 downregulated genes with foldchange $\leq -3.5$, a $p$-value $\leq 0.00005$, and a $q$-value $\leq 0.0044$ is presented in Table 5. The results indicate that the fivefold downregulated genes (foldchange $\leq -5$, $p$-value $\leq 0.00005$ and $q$-value $\leq 0.0044$) such as MPO, TMCC3, MMP8, CA1, and ELF3 can be notable downregulation biomarkers. Similarly, a fivefold upregulated gene, MTRNR2L8 (foldchange $\geq 1.98$, $p$-value $\leq 0.00005$ and $q$-value $\leq 0.004$), is a considerable upregulation biomarker. The fivefold downregulated MPO, TMCC3, MMP8, CA1, and ELF3 genes and the fivefold upregulated MTRNR2L8 gene can be considered for the early identification of autistic patients among Saudis.

Figure 3. Volcano plots of the significantly upregulated genes (red colored) and downregulated genes (green colored) between autism and controls. Upregulated: foldchange $\geq 0.8$ and $p$-value $\leq 0.05$; downregulated: foldchange $\leq -0.8$ and $p$-value $\leq 0.05$.  

A list of the top 20 upregulated genes with foldchange $\geq 1.98$, a $p$-value $\leq 0.0025$ and a $q$-value $\leq 0.05$ is presented in Table 4. A list of the top 20 downregulated genes with foldchange $\leq -3.5$, a $p$-value $\leq 0.00005$, and a $q$-value $\leq 0.0044$ is presented in Table 5. The results indicate that the fivefold downregulated genes (foldchange $\leq -5$, $p$-value $\leq 0.00005$ and $q$-value $\leq 0.0044$) such as MPO, TMCC3, MMP8, CA1, and ELF3 can be notable downregulation biomarkers. Similarly, a fivefold upregulated gene, MTRNR2L8 (foldchange $\geq 1.98$, $p$-value $\leq 0.00005$ and $q$-value $\leq 0.004$), is a considerable upregulation biomarker. The fivefold downregulated MPO, TMCC3, MMP8, CA1, and ELF3 genes and the fivefold upregulated MTRNR2L8 gene can be considered for the early identification of autistic patients among Saudis.
Table 4. List of the top 20 upregulated genes with fold changes ≥ 1.98, p-values ≤ 0.0025 and q-values ≤ 0.05.

| Gene       | Locus                | FPKM Control | FPKM Case | log2 (Fold Change) | p-Value | q-Value | Ensemble Gene ID | Gene Description                                                                 |
|------------|----------------------|--------------|-----------|--------------------|---------|---------|-----------------|---------------------------------------------------------------------------------|
| RPS12      | 6:132814568–132817564| 325.7        | 2218.21   | 2.76778            | 0.00005 | 0.00441 | ENSG00000112306 | ribosomal protein S12                                                            |
| HNRNPH2    | X:101390823–101414133| 79.1408      | 1227.64   | 3.95532            | 0.00005 | 0.00441 | ENSG00000126945 | heterogeneous nuclear ribonucleoprotein H2                                      |
| KRT8       | 12:52897186–52952906 | 0.834316     | 9.49503   | 3.50851            | 0.00005 | 0.00441 | ENSG00000170421 | keratin 8                                                                       |
| RPL36A     | X:101390823–101414133| 79.1408      | 1227.64   | 3.95532            | 0.00005 | 0.00441 | ENSG00000241343 | ribosomal protein L36a                                                          |
| MTRNR2L8   | 11:10507893–10509186 | 4.3216       | 253.717   | 5.87551            | 0.00005 | 0.00441 | ENSG00000255823 | MT-RNR2 like 8                                                                 |
| RPL36A-HNRNPH2 | X:101390823–101414133| 79.1408      | 1227.64   | 3.95532            | 0.00005 | 0.00441 | ENSG00000257529 | RPL36A-HNRNPH2 readthrough                                                     |
| RPL26      | 17:8356901–8383213    | 88.4494      | 682.892   | 2.94873            | 0.00025 | 0.01343 | ENSG00000161970 | ribosomal protein L26                                                          |
| KRBA2      | 17:8356901–8383213    | 88.4494      | 682.892   | 2.94873            | 0.00025 | 0.01343 | ENSG00000184619 | KRAB-A domain containing 2                                                      |
| AC135178.3 | 17:8356901–8383213    | 88.4494      | 682.892   | 2.94873            | 0.00025 | 0.01343 | ENSG00000263809 | novel protein                                                                   |
| DBI        | 2:11936920–119372560  | 19.2423      | 82.5386   | 2.10079            | 0.0004  | 0.01764 | ENSG00000155368 | diazepam binding inhibitor, acyl-CoA binding protein                             |
| PROM1      | 4:1596244–16048378    | 29.4262      | 128.084   | 2.12192            | 0.0005  | 0.01954 | ENSG0000007062 | prominin 1                                                                      |
| FGFBP2     | 4:1596244–16048378    | 29.4262      | 128.084   | 2.12192            | 0.0005  | 0.01954 | ENSG00000137441 | fibroblast growth factor binding protein 2                                      |
| PPDPF      | 20:65207064–65222206  | 43.8331      | 377.291   | 3.10559            | 0.0008  | 0.02598 | ENSG00000125534 | pancreatic progenitor cell differentiation and proliferation factor             |
| IGHG3      | 14:105764502–105771405| 26.0445      | 179.176   | 2.78233            | 0.00085 | 0.02706 | ENSG00000211897 | immunoglobulin heavy constant gamma 3 (G3m marker)                              |
| CFD        | 19:859663–863641      | 57.7465      | 285.781   | 2.30711            | 0.00115 | 0.03281 | ENSG00000197766 | complement factor D                                                             |
| IMMP1L     | 11:31369839–31069645  | 3.40717      | 39.2517   | 3.52611            | 0.0015  | 0.03846 | ENSG00000148950 | inner mitochondrial membrane peptide subunit 1                                  |
| BAG1       | 9:33218364–33264270   | 91.759       | 363.23    | 1.98496            | 0.00155 | 0.03901 | ENSG00000107262 | BAG co-chaperone 1                                                              |
| COX5B      | 2:97646061–97645833   | 31.6214      | 128.267   | 2.02018            | 0.00165 | 0.04067 | ENSG00000135940 | cytochrome c oxidase subunit 5B                                                 |
| PDZK1IP1   | 1:47183581–47191044   | 41.5874      | 174.343   | 2.06771            | 0.0023  | 0.04607 | ENSG00000162366 | PDZK1 interacting protein 1                                                     |
| HBQ1       | 16:180458–181179      | 25.0227      | 198.741   | 2.98958            | 0.00245 | 0.04785 | ENSG00000086506 | hemoglobin subunit theta 1                                                      |
| UQCRB      | 8:96222946–96239149   | 13.8377      | 99.6279   | 2.84795            | 0.0025  | 0.04856 | ENSG00000156467 | ubiquinol-cytochrome c reductase binding protein                               |
Table 5. List of the top 20 downregulated genes with foldchange \( \leq -3.5 \), a \( p \)-value \( \leq 0.00005 \) and a \( q \)-value \( \leq 0.0044 \).

| Gene Locus            | FPKM Control | FPKM Case | log2 (Fold Change) | \( p \)-Value | \( q \)-Value | Ensemble Gene ID | Gene Description                                           |
|----------------------|--------------|-----------|--------------------|--------------|--------------|-----------------|------------------------------------------------------------|
| MPO                  | 17:58269854–58280935 | 74,2902 | 1.24471 | -5.89929 | 0.00005 | 0.0044 | ENSG00000005381 | myeloperoxidase                                            |
| TMCC3                | 12:9456712–94650557 | 377,371 | 6.56036 | -5.84606 | 0.00005 | 0.0044 | ENSG00000057704 | transmembrane and coiled-coil domain family 3             |
| CEACAM6              | 19:41708584–41786893 | 10,139  | 0.665306 | -3.92976 | 0.00005 | 0.0044 | ENSG00000086548 | CEA cell adhesion molecule 6                              |
| PLEKHG2              | 19:3942668–39428415 | 65,41  | 4.86538 | -3.74889 | 0.00005 | 0.0044 | ENSG00000090924 | pleckstrin homology and RhoGEF domain containing G2       |
| CEACAM5              | 19:41708584–41786893 | 10,139  | 0.665306 | -3.92976 | 0.00005 | 0.0044 | ENSG00000105388 | CEA cell adhesion molecule 5                              |
| GRB10                | 7:50590062–50793462 | 10,5363 | 0.725095 | -3.86106 | 0.00005 | 0.0044 | ENSG00000106700 | growth factor receptor bound protein 10                   |
| DDX5                 | 17:64498253–64662307 | 447,04  | 280,637 | -3.98607 | 0.00005 | 0.0044 | ENSG00000108654 | DEAD-box helicase 5                                        |
| CARS1                | 11:3000921–30434707 | 210,99  | 15,4503 | -3.77147 | 0.00005 | 0.0044 | ENSG00000110619 | cysteinyl-tRNA synthetase 1                                |
| MMP8                 | 11:102717795–102727050 | 25,7828 | 0.665084 | -5.27673 | 0.00005 | 0.0044 | ENSG00000181113 | matrix metallopeptidase 8                                 |
| AKAP1                | 17:57085091–57121346 | 53,8928 | 4.44175 | -3.60089 | 0.00005 | 0.0044 | ENSG00000121057 | A-kinase anchoring protein 1                               |
| TMCC2                | 1:205227945–205286532 | 96,9766 | 5.46508 | -4.14932 | 0.00005 | 0.0044 | ENSG00000133069 | transmembrane and coiled-coil domain family 2              |
| CA1                  | 8:85327607–85481493 | 521,489 | 11,6803 | -5.48049 | 0.00005 | 0.0044 | ENSG00000133742 | carbonic anhydrase 1                                       |
| ACE                  | 17:63477060–63521848 | 7,07334 | 0.510574 | -3.7922  | 0.00005 | 0.0044 | ENSG00000159640 | angiotensin I converting enzyme                            |
| ELF3                 | 1:20198237–202017183 | 9,77011 | 0.261935 | -5.22109 | 0.00005 | 0.0044 | ENSG00000163435 | E47 like ETS transcription factor 3                        |
| YPEL4                | 11:57638023–57661865 | 15,1177 | 0.851345 | -4.15035 | 0.00005 | 0.0044 | ENSG00000166793 | yippee like 4                                              |
| OLRI                 | 12:10158300–10191801 | 6,43025 | 0.410073 | -3.97092 | 0.00005 | 0.0044 | ENSG00000173391 | oxidized low-density lipoprotein receptor 1                |
| POLE                 | 12:132623752–132687376 | 46,5435 | 4.11377 | -3.5005 | 0.00005 | 0.0044 | ENSG00000177084 | DNA polymerase epsilon, catalytic subunit                   |
| DDX41                | 5:177511576–177515961 | 597,042 | 0.27217 | -4.14589 | 0.00005 | 0.0044 | ENSG00000183258 | DEAD-box helicase 4                                        |
| AC113554.1           | 17:63477060–63521848 | 7,07334 | 0.510574 | -3.7922  | 0.00005 | 0.0044 | ENSG00000264813 | novel protein                                              |
| AC243967.1           | 19:41708584–41786893 | 10,139  | 0.665306 | -3.92976 | 0.00005 | 0.0044 | ENSG00000267881 | novel protein, readthrough between CEACAM5-CEACAM6        |

3.2. Pathway Enrichment Analysis

All the significantly upregulated and downregulated genes were searched for during the GO and pathway enrichment analysis. Significantly upregulated genes and their interactions (Figure 4) revealed highly significant protein–protein interactions based on the predictive model enrichment (\( p \)-value: \(<1.0 \times 10^{-16} \)). Downregulated genes (\( p \)-value \(< 0.001 \)) and their interactions (Figure 4) demonstrated significant PPI enrichment with a \( p \)-value = \(2.05 \times 10^{-6} \). Pathway enrichment by the significantly upregulated and downregulated genes is presented in Table 6. Ribosome, oxidative phosphorylation, protein export, Parkinson's disease, and Alzheimer's disease-associated pathways were significantly enriched by the upregulated genes (Table 6). The impact on the genetic information processing and translation was significantly enriched by the 17 upregulated genes through the ribosome pathway. The HIF-1 signaling pathway, pyruvate metabolism,
propanoate metabolism, metabolic pathways, the rap1 signaling pathway, the AMPK signaling pathway, the glucagon signaling pathway, proteoglycans in cancer, renal cell carcinoma, fatty acid biosynthesis, the insulin signaling pathway, and EGFR tyrosine kinase inhibitor resistance were significantly enriched by the downregulated genes (Table 6).

Figure 4. (A): Protein–protein interaction based on the predictive model and predicted functional partners of the OR12D2 and OR5V1 proteins using the STRING database, version 10. (B): Significantly upregulated genes and their interaction. Number of edges: 77 and the PPI enrichment p-value < 1.0 × 10^{-16}. (C): Significantly downregulated genes (p-value < 0.001) and their interaction. Number of edges: 420 and the PPI enrichment p-value = 2.05 × 10^{-06}.

3.3. Integration of Transcriptome and Exome Genotyping

Genotyping and exome array analysis among the 243,345 putative functional exonic variants revealed 589 genes significantly (p < 0.001) associated with autism, with 682 significant variants. Furthermore, the differentially expressed gene analysis based on transcriptome sequencing for the autistic patients compared to controls identified significantly upregulated (foldchange ≥ 0.8 and p-value ≤ 0.05; n = 125) and downregulated (foldchange ≤ −0.8 and p-value ≤ 0.05; n = 117) genes. The integration of significantly up- and downregulated genes and genes of significant SNPs using Venn diagram analysis identified three regulatory variants (rs6657480, rs3130780, and rs1940475) associated with upregulated (rs6657480, p = 0.000762, (NC_000001.10:g.63999868T > C) nearest upstream to the gene ITGB3BP) and downregulated (rs3130780, p = 3.12 × 10^{-5} (NC_000011:11:g.30874308T > G) nearest upstream gene, DDRI; and rs1940475, p = 0.0001807 (NC_000011:11:g.102593248T > C; NM_002424.3:c.259A > G; NP_002415.1:p.Lys87Glu, MMP8)) genes in autism spectrum disorder in patients of Arab ancestries (Figure 1).
Table 6. Associated KEGG pathway enrichments of the significantly up- and downregulated genes.

| KEGG Pathway ID | Term Description | OGC | BGC | Strength | False Discovery Rate | Matching Proteins in Your Network (Labels) |
|-----------------|------------------|-----|-----|----------|----------------------|----------------------------------------|
|                 |                  |     |     |          |                      | Upregulated genes                       |
| hsa03010        | Ribosome         | 17  | 130 | 1.37     | 3.53E-16             | RPS12, RPL35, RPL24D1, RPL13, RPL7, RPL21, RPS27, RPL11, RPL24, RPL34, RPL36A, RPS24, RPL29, RPL35A, RPL23, RPL26, RPS15 |
| hsa00190        | Oxidative        | 6   | 131 | 0.91     | 0.0041               | COX5B, ATP5G3, ATP6V1G1, ATP5G2, NDUFA11, UQCRB |
|                 | phosphorylation  |     |     |          |                      |                                        |
| hsa03060        | Protein export   | 3   | 23  | 1.37     | 0.0093               | SRP14, IMMP1L, SEC61G                   |
| hsa05012        | Parkinson’s       | 5   | 142 | 0.8      | 0.0242               | COX5B, ATP5G3, ATP5G2, NDUFA11, UQCRB   |
|                 | disease           |     |     |          |                      |                                        |
| hsa05010        | Alzheimer’s       | 5   | 168 | 0.73     | 0.0394               | COX5B, ATP5G3, ATP5G2, NDUFA11, UQCRB   |
|                 | disease           |     |     |          |                      |                                        |
|                 | Downregulated     |     |     |          |                      |                                        |
| hsa04066        | HIF-1 signaling   | 9   | 98  | 0.76     | 0.0115               | CREBBP, IGF1R, ARNT, TFRC, MKNK1, AKT2, SLC2A1, LDHA, VEGFA |
| hsa00620        | Pyruvate metabolism | 5 | 39  | 0.91     | 0.0243               | ACSS2, ACACB, ALDH3A2, LDHA, ACACA     |
| hsa00640        | Propanoate        | 5   | 32  | 0.99     | 0.0243               | ACSS2, ACACB, HIBCH, LDHA, ACACA       |
|                 | metabolism        |     |     |          |                      |                                        |
| hsa01100        | Metabolic pathways | 37 | 1250| 0.27     | 0.0243               | EXTL3, CYP27B1, GCLC, ACSS2, ACLY, AMPD2, POMT2, ATP6V0A1, CAD, POLG, AMT, ACAA8, GLB1, SGSH, POLE, GANC, DGKA, ALAS2, PLA2G6, ACACB, PFKM, ALDH3A2, PIGN, DNMT1, HIBCH, PHGDH, ACO3, BPGM, AMPD3, MTMR3, EARS2, PGM3, LDHA, MAN2A2, ALOX15, POLR2A, ACACA |
| hsa04015        | Rap1 signaling    | 11  | 203 | 0.53     | 0.0243               | SIPA1L3, FAR2, RAPGEF2, IGF1R, SRC, AKT2, ADCCY7, RASGRF3, FGFR1, DOCK4, VEGFA       |
| hsa04152        | AMPK signaling    | 9   | 120 | 0.67     | 0.0243               | TSC2, PFKFB4, IGF1R, TSC1, ACACB, PFKM, CRTC2, AKT2, ACACA                        |
| hsa04922        | Glucagon          | 8   | 100 | 0.7      | 0.0243               | CREBBP, ACACB, CRTC2, ITPR2, AKT2, SLC2A1, LDHA, ACACA                       |
|                 | signaling         |     |     |          |                      |                                        |
| hsa05205        | Proteoglycans in  | 11  | 195 | 0.55     | 0.0243               | DDX5, GAB1, IGF1R, ANK3, PTCH1, SRC, ITPR2, AKT2, FGFR1, PPP1R12B, VEGFA       |
|                 | cancer            |     |     |          |                      |                                        |
| hsa05211        | Renal cell        | 6   | 68  | 0.74     | 0.0289               | CREBBP, GAB1, ARNT, AKT2, SLC2A1, VEGFA                                       |
|                 | carcinoma          |     |     |          |                      |                                        |
| hsa00061        | Fatty acid        | 3   | 12  | 1.2      | 0.0368               | ACACB, ACO3, ACACA                   |
|                 | biosynthesis      |     |     |          |                      |                                        |
| hsa04910        | Insulin signaling | 8   | 134 | 0.57     | 0.0401               | TSC2, INPPL1, TSC1, ACACB, MKNK1, AKT2, IKBKB, ACACA                         |
| hsa01521        | EGFR tyrosine      | 6   | 78  | 0.68     | 0.0417               | GAB1, IGF1R, SRC, AKT2, NRG1, VEGFA                                              |
|                 | kinase inhibitor  |     |     |          |                      |                                        |
|                 | resistance        |     |     |          |                      |                                        |

BGC: background gene count; OGC: observed gene count.
4. Discussion

This study performed a comprehensive investigation of the genetic architecture of ASD using an exome microarray analysis and RNA sequencing. We identified 155 significantly ($p < 0.0001$) associated SNPs, and the most strongly ($p < 2.05 \times 10^{-7}$) associated 13 SNPs were investigated further. None of the 13 SNPs had previously been reported in Saudis with autism [27]. The OR12D2 olfactory receptor gene, which codes for the G-protein coupled receptor (GPCR), carries the signal to sensory neurons to trigger smell and has 10 common variants and large effects on risk, was associated with autism in Arab-ancestry subjects and showed a high degree of linkage disequilibrium ($p = 6.71 \times 10^{-9}$) on the significant risk haplotype AAGTCTGATT. The OR12D2 gene (gene expression for OR12D2: ENSG00000168787.4) was overexpressed in the brain (hypothalamus $\times 13.9$, caudate $\times 10.7$, hippocampus $\times 8.2$, nucleus accumbens $\times 5.0$ and cervix-endocervix $\times 4.6$) [21,28].

Some studies have proposed unusual sensory processing, particularly olfactory processing, in a number of neurodevelopmental disorders, including autism [29,30]. Berko et al. [31] showed that the OR2L13 G-protein, joined by the olfactory receptor, which is involved in resetting the neuronal response to smells, is particularly prevalent in autism in terms of DNA methylation and expression, suggesting a possible rationale for olfactory dysfunction in ASD. The olfactory receptor genes OR12D2 and OR5V1, which are involved in the olfactory signaling pathway, G alpha (s) signaling events, GPCR downstream signaling, and GPCR signaling and signal transduction together regulate the overall olfactory growth, behavior, and impairment associated with autism (Figure 4) [32]. A functional analysis of the genes is presented in Table S2. The significant genes in this study included integrin subunit alpha 11 (ITGA11), which is associated with attention deficit hyperactivity disorder and attention deficit disorder with hyperactivity; MHC class I polypeptide-related sequence A (MICA), associated with symptoms of inflammatory bowel disease, chronic ulcerative colitis, and Crohn’s disease; bromodomain-containing 2 (BRD2), which has been linked to Alzheimer’s disease and myoclonic epilepsy; cell division cycle 14C pseudogene (CDC14C), which is associated with Parkinson’s disease and sleep; methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like (MTHFD1L), which is associated with stroke and Alzheimer’s disease; myosin IXB (MYO9B), associated with symptoms of celiac disease, inflammatory bowel disease, chronic ulcerative colitis, multiple sclerosis, and schizophrenia; and ring finger protein 144A (RNF144A), which has been associated with depressive disorder, schizophrenia, and stroke (Table S2) [33–45]. For example, SNP rs2772943 is significantly correlated with ASD and has been reported in bipolar cases as an intergenic variant that occurs between the BIG-2 and CNTN6 genes, both of which produce a neuronal membrane protein that forms the axonal connections in the developing nervous system [4].

Unlike previous results, our study identified a single gene, OR12D2 (including up and downstream; NC_000006.11:g.29360183-g.29369519), with 10 common SNPs and large effects on risk in patients with autism. Most previous results have suggested that no single gene has such large effects on risk ($p = 6.71 \times 10^{-9}$) [4]. A comprehensive review on the genetics of ASD described the role of various genes and their clinical relevance to autism. Here, we describe an additional gene, OR12D2, that should be considered for molecular pathway studies in the future [4]. The enrichment analysis of the genes/nearst upstream/nearst downstream genes of the highly associated SNPs using Enrichr in GWAS Catalogue 2019 revealed significant diseases/drug terms associated with ASD or schizophrenia ($p = 0.001107$; adjusted $p = 0.0321$) on eight genes, namely SFTA2, BRD2, HCP5, OR5V1, HLA-DMA, ZKSCAN8, OR12D2, and MICA [25]. Four genes (TMEM185B, MTHFD1L, QPCT, and H2AFV, $p = 0.0098$) were downregulated relative to their median expression in the ventral juxtaommissural pretectal nucleus among the genes/nearst upstream/nearst downstream genes of highly associated SNPs using Enrichr [25]. Children with systemic lupus erythematosus (SLE) have a high risk of developing autism. In the present study, seven genes (BRD2, OR12D2, CR2, OR5V1, SFTA2, HCP5, and HLA-DMA) were highly significant for SLE ($p = 0.000029$; Bonferroni $= 0.0041$) using DAVID [19,46].

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The risk haplotype of the *OR12D2* gene in autism subjects and the overexpression of *OR12D2* in the brain, dysfunction in neurodegenerative processes, olfactory impairment, and the overuse of aroma compounds or essential oils, flowers, aromatic wood, herbs, frankincense, and perfumes among the Arab population provide clues that olfactory sensory deficits and overexpression of *OR12D2* should be studied in detail among the Arab population to reveal the underlying etiology of autism [28–31,47,48].

Even though the olfactory receptor genes on chromosome 6 are significant after a Bonferroni correction ($\alpha = 0.05/243345 = 2.05 \times 10^{-7}$) with high linkage disequilibrium ($p = 6.71 \times 10^{-9}$ on 6p22.1), their significance on the transcriptome sequencing was lacking. Furthermore, the most significantly upregulated gene, *RPS12*, and the most significant downregulated gene, *MPO*, were observed with no significant variants in the exome array. This suggests that whole-genome sequencing on these subjects may reveal the significant genes with novel variants which are not covered in the exome array. Differentially expressed genes in autism are essential for the complete understanding of the associated functional and autism development pathways [10–12]. Earlier studies have revealed many significantly differentially expressed genes from various populations [10–12]. However, there were no studies on the differentially expressed genes in autism in Saudi Arabians. Hence, this study can be considered as a first of its kind study on autistic patients from Saudi Arabia. Additionally, the integrated analysis of exome array and transcriptome sequencing is an initial study on the Arab ancestries with autism. The upregulated integrin subunit Beta 3 binding protein (*ITGB3BP*) gene with rs6657480 ($p = 0.000762$) in the present study is in line with the previous studies on the genes predicted to affect risk for ASD [49]. The upregulated *ITGB3BP* gene in the present study is targeted as an autism-risk gene in human fetal brains [50], and also differential expression was observed in a mouse model [51]. Downregulation of *ITGB3BP* was observed in GABAergic neural development [52]. SNPs associated with schizophrenia risk were identified in the gene *ITGB3BP* [53].

SNP rs3130780 was observed in this study, which is located close to the downregulated discoidin domain receptor 1 (*DDR1*) gene. *DDR1* was reported earlier as a susceptibility gene for schizophrenia [54,55] and self-consciousness [56]. *DDR1*-mediated signaling systems were observed in *Drosophila* in the mushroom body encoding learning and memory [57,58]. *DDR1* plays an important role in myelination and neuropsychiatric diseases [59]. *DDR1* expression increases in parallel with neural myelination throughout mouse brain development, according to previous research [60]. The exonic variation of rs1940475 (NP_002415.1:p.Lys87Glu) in the *MMP8* gene was found to be a significant coding variant in the genotype association study, and it impacts the expression level of the *MMP8* gene. Pathway analysis and genome-wide association in major depressive disorder revealed a significant association with the *MMP8* gene [61]. rs1940475 in the *MMP8* gene was reported for its association with osteoarthritis [62], femoral head osteonecrosis [63], and bladder cancer in non-smokers [64]. On the other hand, a recent meta-analysis of the K87E (rs1940475) variant showed that this variant is not significantly associated with cancer susceptibility [65]. Overall, the literature on the *ITGB3BP*, *DDR1*, and *MMP8* genes indicates that the observation regarding the integration of transcriptome and exome genotyping in autism is a notable addition for the understanding of the genetics of autism. Defects in the SH3 and multiple ankyrin repeat domains 3 (Shank3) protein cause numerous synaptopathies, including autism [66–68]; upregulation of the *RPL36A* was reported in the striatal synaptosome of Shank3-overexpressing transgenic mice [66]. This is in line with the present observation on the upregulation of *RPL36A* in autistic patients and indicates that the upregulation of *RPL36A* can influence the development of autism through the dysfunction of SHANK3-associated synaptopathies [66,69]. The small number of subjects enrolled in this study was a notable limitation. However, the autistic patients were selected from 1800 patients visiting the neurology clinic. Currently, research guiding medication choice and dosing in patients with ASD is limited. Autism symptoms are usually managed with a variety of medications such as psychotropic (antipsychotics and antidepressants) drugs to manage behaviors such as mood symptoms, resulting in polypharmacy- and
drug-related adverse effects. Knowledge of the clinical and genetic predictors of drug response variability and the outcomes of personalized medicine can advance the clinical care of young patients with ASD. Pharmacogenomics is a method that can be used for medication choice that affects a person’s genetic information and clinical presentation to make informed choices, but no studies have specifically examined the outcomes of PGX for patients with ASD. We believe that we can use the result of our study to help in the diagnosis and treatment of patients with autism. Further studies are needed to provide further information on the genetic risk in relation to treatment effectiveness. Analysis of reactive oxygen species (ROS) generation and stress enzymes in the cases and controls may strengthen the observed findings.

5. Conclusions

The Saudi autistic patients are significantly different according to the gene expression profile. The fivefold downregulated MPO, TMCC3, MMP8, CA1, and ELF3 genes and the fivefold upregulated MTRNR2L8 gene can be considered for the early identification of autism patients among Saudis. This study identified the OR12D2 gene, including the 5’ and 3’ regions associated with 10 ASD SNPs on chromosome 6p22.1. The SNPs detected in patients with autism in the current study are variants in genes that either control fundamental cell survival processes or direct neurological functions that might contribute to the severity of ASD. Screening the molecular defects that are prevalent in Saudi autistic cases is a very important step to better understand the possible genes that are involved in the development of ASD. Regulatory variations (rs6657480, rs3130780, and rs1940475) identified the up- (ITGB3BP) and downregulation (DDR1 and MMP8) of genes in autism spectrum disorder in patients of Arab ancestry discovered through the integration of up- and downregulated genes and genes of significant genotypes. Further studies are needed to investigate the pathogenic pathways of the reported SNPs and genes to develop potential therapeutic targets in the future. The presence of a panel of the most prevalent variants in ASD cases will aid in enhancing our knowledge and understanding of the complexity behind the variability in ASD.

Supplementary Materials: The following are available online: https://www.mdpi.com/article/10.3390/ph15020158/s1, Table S1 (Supplementary Material) List of significant SNPs at \( p < 0.001 \); Table S2 (Supplementary Material) The functional annotations of the most significant genes (\( p < 0.0001 \)) based on the DAVID 6.7 and Reactome.

Author Contributions: The study was designed by N.B.A., A.A., M.A., F.A.A., S.A. (Sayed AbdulAzeez) and J.F.B. Data were collected by N.B.A., A.A., S.A. (Safah Alzahrani), A.A.-m., M.A., F.A.A., S.A. (Sayed AbdulAzeez) and J.F.B.; N.B.A., S.A.A., D.N.A., A.A.A., S.A. (Sayed AbdulAzeez), and J.F.B. wrote the manuscript. Data were analyzed by N.B.A., S.A.A., D.N.A., H.E.A., A.A.A., S.A. (Safah Alzahrani), A.A.-m., M.A., F.A.A., S.A. (Sayed AbdulAzeez) and J.F.B. All authors have read and agreed to the published version of the manuscript.

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