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Candidate Genes Related to Spiritual Mediumship: A Whole Exome Sequencing Analysis of Highly Gifted Mediums

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ABSTRACT

Objective: There has been a call for neuroscientific studies of spiritual experiences due to their global prevalence, significant impact, and importance for understanding the mind-brain problem. Mediumship is a spiritual experience where individuals claim to communicate with or be influenced by deceased persons or non-material entities. We assessed whether mediums possess specific genetic alterations.

Methods: We selected highly gifted mediums (n = 54) with over 10 years of

experience who engaged in mediumistic work without material benefits, analyzed whole exome sequencing, and compared them to non-medium first-degree relatives (n = 53).

Results: We identified 15,669 variants exclusively found in mediums, likely to impact the function of 7,269 genes. Thirty-three of these genes were altered in at least one-third of all mediums but in none of their relatives. The inflammatory pathway was the most frequently affected (43.9%) with the translocation of ZAP-70 to the immunological synapse being particularly prominent.

Conclusion: This is the first exome-wide investigation of genes possibly related to mediumistic experiences. We identified gene variants that are presented in mediums but not in their first-degree non-medium relatives. These genes emerge as possible candidates for further investigations of the biological underpinnings that allow spiritual experiences such as mediumship.

Keywords: **e**xome; gene; mediumship; anomalous experiences; spiritual experiences

INTRODUCTION

Spiritual experiences are very prevalent worldwide, have a strong impact on those experiencing them and have received increased attention from the scientific community. A recent paper at the *Nature* made an emphatic call for neuroscientific studies of spiritual experiences as “crucial to understanding the human brain — and human life” (p.27)[1]. A spiritual experience often reported by the general population and in sacred texts is the claim of being in communication with, or be influenced by, deceased persons or non-material entities.

An increasing body of research highlights the prevalence of these occurrences within the general population. Recent national surveys in the US and Brazil found that more than half of the general population reported having felt “the presence of the dead” or “that a family member who is dead has visited them”[2,3]. A British study revealed that over 20% of individuals reported having seen deceased individuals, whereas more than 15% claimed to have heard voices that others could not[4]. It is worth mentioning that these experiences cut across religious affiliations, extending to individuals who identify as atheists or agnostics. So, what is the significance of these widespread experiences?

Individuals who claim the ability to perceive, communicate with, or be influenced by

deceased individuals are often called mediums[5]. Mediumship has a long documentation history and has been the subject of thorough investigation, with rigorous scientific studies dating back to the latter half of the 19th century[6]. Remarkably, the examination of such phenomena has played a substantial role in shaping concepts like dissociation and the subconscious mind, providing foundational support for psychological and psychiatric development[7–9].

Mediumship very often manifest as unshared sensory experiences (hallucinations, e.g., seeing and hearing) that may be symptoms of a psychotic or dissociative disorder[10]. Nevertheless, a robust body of research indicates that most individuals presenting such phenomena do not exhibit signs of mental disorders[11]. Remarkably, certain individuals with high levels of these experiences, such as spiritual mediums, demonstrate levels of health on par with or even exceeding those without such experiences [12]. So, studies do not support the hypothesis that most of these phenomena are merely manifestations of mental disorders.

Because most mediums are mentally healthy, and their so-called hallucinations are not symptoms of mental disorders, some authors have proposed another denomination for these non-pathological unshared experiences[13]. For more than a century, there have been scientific investigations on whether these mediumistic experiences are perceptual errors or if they can provide accurate and veridical information that is not obtained by regular means (e.g., ordinary senses and reasoning).[14,15]

Some studies have investigated the neurophysiology of mediumship, including functional neuroimaging[16,17] and EEG[18]. However, we are not aware of any studies on genetics of mediumship. A broad genomics assessment, focused on DNA variants that result in high-impact alterations in the encoded genes, may support the hypothesis that specific individuals possess enabling biological foundations that a allow a different perception of reality. For this reason, exome sequencing emerges as a highly useful tool for identifying genetic variants associated with complex traits such as mediumship, providing insights into their genetic basis. In alignment with this proposition, this study's primary objective is to identify nucleotide variants associated with spiritual non-pathological experiences in mediums through whole exome sequencing, while concurrently comparing these variants to those found in their first-degree non-medium relatives, followed by a validation in an independent cohort. Our hypothesis posits that specific gene variants in pathways responsible for

information processing will exhibit a higher prevalence among medium individuals when compared to their first-degree non-medium relatives.

MATERIAL AND METHODS

Study Design and Participants

This case-control study was conducted between April 2020 and April 2021 and included individuals identified by their spiritual communities as highly gifted mediums across all regions of Brazil. For this study, mediums were defined as individuals who assert the ability to perceive (either by seeing or hearing) or to act under the direct and evident influence of a purported deceased personality[5].

To identify highly skilled mediums, our initial approach involved collaborating with religious groups where mediumistic activities are regularly practiced, mainly Spiritism, Umbanda (an Afro-Brazilian religion), and Spiritualism. Subsequently, mediums were considered eligible for inclusion in the study if they met all the following criteria: (a) a minimum of 10 years of experience in mediumship; (b) engaged in mediumistic practices at least once a week; (c) recognized by their peers as having a notably high level of mediumistic ability; (d) engaged in their mediumistic work voluntarily, without receiving any financial compensation or material benefits; (e) regarded by their peers as having a consistent track record of obtaining verifiable and accurate anomalous information, which is information allegedly not acquired through normal means or the conventional five senses[19].

To compose a non-medium control group with high genetic and sociocultural similarity, we selected mediums' first-degree relatives during the same time frame. Inclusion criteria for this group were as follows: (a) adults; (b) first-degree relatives of the mediums; (c) not exhibiting mediumship themselves. When enrolling relatives, we followed a preference order for recruitment, prioritizing: 1. siblings of the same sex as the medium, 2. siblings of different sex, 3. parents, 4. children, 5. half-siblings, either from the father or mother, of the mediums.

To implement a validation cohort sample, mediums without a paired relative were included in the study. Additionally, two of the participating mediums were monozygotic twins, strategically integrated into the research design to bolster the qualitative assessment and strengthen the reliability of our findings. Both mediums' twins were compared with a third non-medium control sibling. Both the pair of monozygotic twins and the sample of mediums without paired relatives were utilized

to validate our findings as well.

This study is part of a larger project conducted by the Instituto Nacional de Biomarcadores em Neuropsiquiatria (INBION) and received approval from the Ethics Committee of the Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo – HCFMUSP (approval number 465412/2014-9, CAAE #41849020.5.0000.0068). Before participating in the study, all individuals signed an informed consent form.

Procedures

Initially, we conducted training sessions for collaborators (usually health professionals) in the cities of the spiritual centers to ensure they were proficient in applying the criteria mentioned above to recruit mediums and their relatives. Subsequently, once this initial selection process was completed, we contacted the chosen participants and their respective controls. Our purpose was to secure their informed consent, facilitate their completion of self-rated questionnaires via an online platform, and facilitate the collection of saliva samples.

Non-stimulated saliva was used as the source of DNA for this study. Each participant provided ˜2.5 ml of saliva, which was combined with an equal volume of stabilization buffer: 100 mM NaCl, 10 mM Tris, and 10 mM EDTA, proteinase K (0.1 mg/ml), and sodium dodecyl sulfate (0.5%) in 15 mL tubes. Samples were stored at room temperature (RT) for a maximum of 7 days or refrigerated at 4°C for up to 3 months. Subsequently, the tubes containing samples and stabilization buffer were sent to the Laboratory of Neurosciences (LIM-27), Institute of Psychiatry, HCFMUSP, for subsequent DNA extraction. Downstream analyses are described below.

Assessments

Sociodemographic Data

Information encompassing ethnicity (self-declared), educational background, and age were collected.

Mediumship Activity Questionnaire

Mediumistic abilities were assessed using an instrument developed by our research team[20]. The instrument assesses a spectrum of phenomena, including psychophony (speaking under the influence of spirits), projection (out-of-body experiences),

psychography (writing under the influence of spirits), clairvoyance (seeing spirits), clairaudience (hearing spirits), incorporation (full trance or “embodiment”), physical effects, healing, and mediumistic painting.

WHOQOL-BREF

WHOQOL-Bref is a 26-tem self-rated scale developed to measure the quality of life and its four domains: physical, psychological, social relations, and the environment[21]. The total score ranges from 0 to 100. We used a validated version for the Brazilian population[21].

DNA extraction

The DNA extraction protocol was based on Goode et al (2014)[22]. Briefly, 250 µl of saliva samples was added to 500 µl of Cell Lysis Solution (Qiagen 158908). The mixture was incubated at RT for 30 min. RNA was removed by adding 4 µl of RNase A (100 mg/ml), incubated at 37 °C for 15 min, followed by Proteinase K digestion and protein precipitation (Qiagen 158912). After centrifugation supernatants were transferred into new tubes for DNA precipitation using isopropanol and glycogen. The recovered DNA was washed in 70% ethanol, dried, and resuspended in 60 µl of Tris-EDTA. Gel and spectrophotometry analysis indicated enough integrity, purity, and concentration adequate for exome sequencing.

Whole-exome sequencing (WES) analysis

The whole exome was captured (SureSelect V6, Illumina) and sequenced using the Illumina NovaSeq platform (Macrogen, Seoul. Korea). Germline variants were determined according to the best practices of the Genome Analysis Toolkit (GATK)[23,24]. A series of steps were taken to streamline the alignment, preprocessing, and variant calling (Supplemental Fig. 1). Briefly, sequencing reads (FastQ files) were mapped to the reference human genome sequence (GRCh37/hg19) using BWA-MEM (v0.7.17-r1188) and the default parameters[25] except for the number of threads which was set to 8. The aligned files (BAMs generated by the mapping) were pre-processed by GATK version 4.2, using the MarkDuplicates, BaseRecalibrator, and PrintReads tools with the default parameters, again with the number of threads set to 8.

The variant call was made by HaplotypeCaller with the –ERC GVCF parameters for each sample, then the CombineGVCs and GenotypeGVCFs steps were performed, characterizing the identification of variants in the 'joint analysis' format. The identified variants were annotated with the snpEff and SnpSift software using

several DNA polymorphism databases, described below. These variants were then filtered as follows: (a) Filter applying GATK's Variant Recalibration (VQSR) method with parameters HapMap, dbSNP and tranches 100.0, 99.90, 99.0 and 90.0; (b) Hard Filter with parameters recommended by The Genome Analysis Toolkit (GATK) Best Practices[26]. Common variants (>1% VAF - Variant Allele Frequency) that were also found in the public databases [such as dbSNP[27]), 1000 genomes[28], gnomAD[29] and a database of variants found in Brazilian subjects, ABRaOM[30]], which are less likely correlated with the phenotypes observed here, were identified and removed. . The remaining variants were annotated by Funcotator and a MAF file was generated. Silent variants – which lead to no amino acid alterations - were not considered for further analysis. Next, we selected variants that had enough vertical coverage (a sequencing depth of at least 8 sequencing reads covering a variant base) and a variant allele frequency of at least 30% (more likely compatible with heterozygous or homozygous alleles).

The resulting variants were retained for further analyses if they passed our criteria for 1) Potential functional impact – (a) non-synonymous variants or (b) variants leading to premature stop-codons or (c) indels that disrupt the reading frame or were located in splicing sites and 2) Specificity to the medium group - absent in paired controls (close biological relatives of medium subjects) and with very low frequencies (<1%) in the other control databases. Finally, to identify the genes that are more likely to be associated with mediums, we selected a subset of genes that have shown alterations in at least one-third of mediums, as well as those that also had high-impact variants in mediums from the validation cohort (unpaired mediums)

We utilized the Human Allen Brain Atlas Project (HABAP) in our investigation. This extensive multimodal atlas integrates gene expression data with anatomical insights to formulate a gene set relevant to pineal gland function[31]. To delineate this gene set, we compiled genes exhibiting significant upregulation (with a fold change of 10×) within the pineal gland compared to their expression levels across the entire brain, as indicated by at least one gene probe[32]. A total of 89 protein-coding genes were encompassed within this gene-set framework (Supplemental Table 2).

For genes with valid alterations, as reported here, an analysis of pathways more frequently altered was performed based on WebGestalt tool and the following parameters: *Homo sapiens* as the organism of interest, the Network Topology-based Analysis (NTA) as method of interest, and network/PPI BIOGRID as

functional database. For the network view, we used the program STRING[33] which summarizes the network of predicted associations for the remaining genes. Using the same tool, we performed KEGG analysis, where the p-values are corrected within each category using the Benjamini–Hochberg procedure.

Statistical Analyses

To analyze metabolic pathways, we conducted a parametric proportion test (prop.test - utilizing Pearson's chi-squared test statistic) to evaluate the significance of the proportion of genes exhibiting variants within a given pathway. Specifically, we assigned the count of genes with identified variants to parameter x, and the total number of genes within that metabolic pathway to parameter *n*. In instances where the requirements for prop.test (n > 30) were not satisfied, we opted for the non-parametric exact binomial test (binom.test).

Four mediumistic abilities were separately analyzed: (a) psychophony and/or incorporation; (b) clairvoyance; (c) clairaudience; and (d) the presence of five or more mediumistic abilities. The Fisher test was used to assess whether the variants present in specific genes are associated with these phenotypes, considering the presence of each mutated gene (yes or no) and the presence of mediumistic ability (yes or no) as variables.

RESULTS

Description of the Sample

The study enrolled 147 participants. Among these, ten individuals (5 mediums and 5 controls) did not provide salivary samples. Among the remaining 137 participants, the extraction of DNA material was unsuccessful in 14 cases due to inadequate sample quantity/quality (8 mediums and 6 controls). Consequently, DNA content was successfully assessed in 123 individuals, of whom 107 possessed a corresponding medium-relative control (54 medium and 53 controls). Notably, one relative (a sister) was a control for two mediums, resulting in an uneven distribution of mediums and controls. Of the 123 individuals, 12 were mediums without a matched control, and four were relatives without a matched medium. These 12 mediums were used in the subsequent analysis to validate the main findings.

From the whole sample of 123 volunteers, most were female (n= 68; 55.3%), white (n= 89; 72.4%), and had a college education or postgraduate degree (n= 92;

74.8%). The mean age was 53.4 years (SD = 17.2), and the average global WHOQOL score was 74.4 (SD = 17.6). Table 1 provides demographic information for mediums and their controls only included in the main analysis.

Table 1: Sociodemographic characteristics and quality of life of mediums and their controls

	Mediums (n=54)	Controls (n=53)
Female: n (%)	33 (62.26) n = 53	26 (52%) n = 50
Age: m (SD)	59.15 (12.79) n = 53	44.14 (18.57) n = 50
Education: n (%) Lower or Incomplete higher education Complete higher education Postgraduate	 6 (11) 20 (38) 27 (51) n = 53	 14 (28) 15 (30) 21 (42) n = 50
Race: n (%) White Black Brown Asian Other	 38 (71.70) 2 (3.77) 10 (18.87) 1 (1.89) 2 (3.77) n = 53	 40 (80) 3 (6) 6 (12) 1 (2) 0 n = 50
WHOQOL: m (SD)	76.65 (17) n = 53	71.74 (19.08) n = 46

Footnote: WHOQOL – quality of life measure.

The mediums' group without paired controls (n = 12) showed similar demographics. Most were female (56.3%, n = 7), white (83.3%, N=10), and achieved educational levels corresponding to complete higher education or postgraduate degree (58.3%, n = 7). The mean age within this subset was 65.92 years (SD = 8.64), and the average global WHOQOL score was 76.13 (SD = 15.26).

In response to the Mediumship Activity Questionnaire, 53 mediums provided insights into their experiences. The findings revealed that 51 individuals (92.7%) acknowledged speaking under the influence of spirits, 39 (70.9%) expressed themselves in writing under such influence, 29 (52.7%) reported seeing spirits, 28 (50.9%) engaging in full trance, 26 (47.3%) out-of-body experiences, 23 (41.8%) auditory perceptions of spirits, 19 (34.5%) claimed healing abilities, seven (12.7%) reported physical effects, and four (7.3%) revealed painting activities.

Exome Sequencing

Successful whole exome sequencing was achieved for 123 subjects, including 66 individuals designated as mediums and 57 controls. Four of these controls were later removed, due to the low quality of the exome data obtained from their corresponding medium relatives. This leads us to 119 subjects, 66 mediums and 53 controls, divided into two groups: a) 54 mediums with their 53 first-degree relative controls and b) 12 mediums with no control, who composed the validation sample described below.

Coverage metrics showed that 60% of the exome had a minimum coverage of 10X for the mediums and 58% for controls. From the paired samples, the median per subject of observed variants was 487, with the majority being missense mutations (85.4%) resulting in amino acid alterations, followed by Insertions/Deletions (11.2%) that can disrupt reading frames, nonsense mutations (2.1%) causing premature stop codons, or variants located within splicing sites (1.2%). The predominant variants are transitions, specifically C>T or T>C mutations (Supplemental Fig. 2). Close biological controls were paired to most mediums (54/66 mediums - 81.8%). Following the stringent criteria adopted here, including sequencing coverage depth, high allelic variant frequency in the subjects, low frequency in public databases, absence in biological relatives, and presence in multiple mediums, a total of 15,669 variants with the potential to impact the function of 7,269 genes were found in mediums, whereas their control relatives did not show such variants (Supplemental Table 3). Among these, 33 were altered in at least 33% (18 out of 54) of mediums, while not displaying such changes in their respective control counterparts. These genes with more frequent mutations in mediums were often genes related to mucous protection of epithelial and exhibiting immunological functionalities (see Table 2 for the list of genes presenting mutations in at least half of the mediums and Supplemental Table 1 for the comprehensive list of genes showing mutations in at least 33% of the medium).

Table 2: Genes presenting mutations in at least one-third of mediums subjects of this study.

Gene Name	Higher expression tissue[a]	Function	Frequency n (%)				
			Mediums Sample (n = 54)	Twins[b] (n = 2)	Validation Sample[c] (n = 12)	Sensory System	Pineal Gland[d]
Mucin 19	Minor salivary gland	Mucous protection of epithelial tissues	47 (87.04%)	2 (100%)	11 (91.67%)	-	X

Mucin 3a	Small intestine	Mucous protection of epithelial tissues	36 (66.67%)	2 (100%)	11 (91.67%)	-	-
Mucin 4	Colon	Mucous protection of epithelial tissues	35 (64.81%)	2 (100%)	8 (66.67%)	-	-
Major histocompatibility complex class II DR Beta 5	Lung	Antigen presentation	34 (62.96%)	2 (100%)	9 (75%)	-	-
Zinc finger protein 717	Thyroid	Transcriptional regulation	31 (57.41%)	1 (50%)	8 (66.67%)	-	-
Major histocompatibility complex class II DQ Beta 1	Lung	Antigen presentation	31 (57.41%)	2 (100%)	8 (66.67%)	-	-
Major histocompatibility complex class II DR Beta 1	Lung	Antigen presentation	30 (55.56%)	2 (100%)	9 (75%)	-	-
Transmembrane phosphatase with Tensin homology	Testis	Signal transduction	28 (51,85%)	2 (100%)	11 (91.67%)	-	-
Notch receptor 4	Adipocytes	Regulates cell fate determination	27 (50.00%)	2 (100%)	5 (41.67%)	-	-
Ephrin type-B receptor 6	Brain cortex	Modulates cell adhesion and migration	27 (50.00%)	2 (100%)	10 (83.33%)	-	-
Fc gamma binding protein	Colon	Maintenance of mucosal structure	27 (50.00%)	2 (100%)	11 (91.67%)	-	-

Footnote: Gene name, tissue expression and function are given as provided by genecards.org.
[a]Tissue with higher expression according to RNA-Seq data, WBC: white blood cells, CNS: central nervous system, from Genecards; higher expression as determined by RNASeq experiments from (genecards.org).
[b]Twins: a pair of monozygotic twins presenting mediumship.
[c]Validation sample: 12 mediums without controls used for independent validation.
[d]In this column, "X" indicates that the gene is on the gene set related to pineal gland function.

Table 3: Kegg pathway sub-categories related to the sensory system.

Sub-category (Kegg pathway)	Genes in the sub-category	Altered genes (%)	Total variants found (n)	Sample (n)	p-value	Adjusted p-value
Inflammatory (hsa04750)	98	43 (43.9%)	66	39	0.91	1.0
Taste (hsa04742)	86	33 (38.4%)	54	32	0.99	1.0
Olfactory (hsa04740)	439	156 (35.5%)	304	47	1.0	1.0
Phototransduction (hsa04744)	29	7 (24.1%)	13	9	1.0	1.0

Validation cohort

This cohort comprised 12 unrelated mediums and was used to verify if the initially identified variants would be again identified in an independent group of mediums. Remarkably, the genes most frequently exhibiting mutations in paired mediums were also highly frequent in this validation cohort (Table 2 and Supplemental Table 1). A total of 1,574 variants (834 genes) found in the paired medium group could be confirmed in most mediums (11/12) of the validation cohort. This validation cohort is

also composed of one pair of mediums who are identical twins. Four hundred thirty-four mutations (354 in both mediums) were identified in 230 genes (167 in both mediums) within the medium twins, which were absent in their non-medium relative (Supplemental Table 4). Among the 33 genes exhibiting the highest frequency of mutations among paired mediums, 15 genes (45%) also carried variants in both twins and were absent in their non-medium sibling (Table 2 and Supplemental Table 1).

Mediumistic Abilities and Sensory System

The Fisher test revealed no association between any of the 7,269 mutated genes in mediums and specific mediumistic abilities. (Supplemental Table 3).

Gene pathways analysis indicated a total of 611 genes included in one or more of the functional categories related to the "Sensory System", as classified by KEGG[34] (release 104.1, November 1, 2022). Among these, we observed 226 genes (37%) carrying 420 variants identified in 48 different subjects of the medium group. This functional cluster pathway includes 4 sub-categories, related to inflammatory processes, taste, olfactory and phototransduction sensing. As seen in Table 3, the inflammatory pathway was the category with the highest percentage of altered genes (43.9%). At the same time, the largest sub-category (olfactory) was also the one with the higher number of mediums showing alterations. No significant associations were found when proportion tests assessed whether metabolic pathways are significantly represented among genes with variants (Supplemental Table 5).

Pathways, Network and Gene Ontology

The STRING tool identified 12 overrepresented pathways within genes presenting mutations in at least one-third of mediums from our sample (Table 4). The pathway with the most significant strength is the translocation of ZAP-70 to immunological synapse (HSA-202430). Moreover, we have also performed a network analysis (Figure 1), which revealed the presence of two distinct clusters. The first cluster comprises the genes leukocyte immunoglobulin like receptor B1 (*LILRB1*), leukocyte immunoglobulin like receptor B3 (*LILRB3*), Human Leukocyte Antigen, class II, DR beta 1 (*HLA-DRB1*), Human Leukocyte Antigen, class II, DR beta 5 (*HLA-DRB5*), Human Leukocyte Antigen, class II, DQ beta 1 (*HLA-DQB1*), and notch receptor 4

(*NOTCH4*). The second cluster is composed of zinc finger protein 717 (*ZNF717*), mucin 4 (*MUC4*), mucin 3A (*MUC3A*), and mucin 19 (*MUC19*) genes. The enrichment analysis (Gene Ontology) identified 12 distinct significant pathways, as outlined in Table 5.

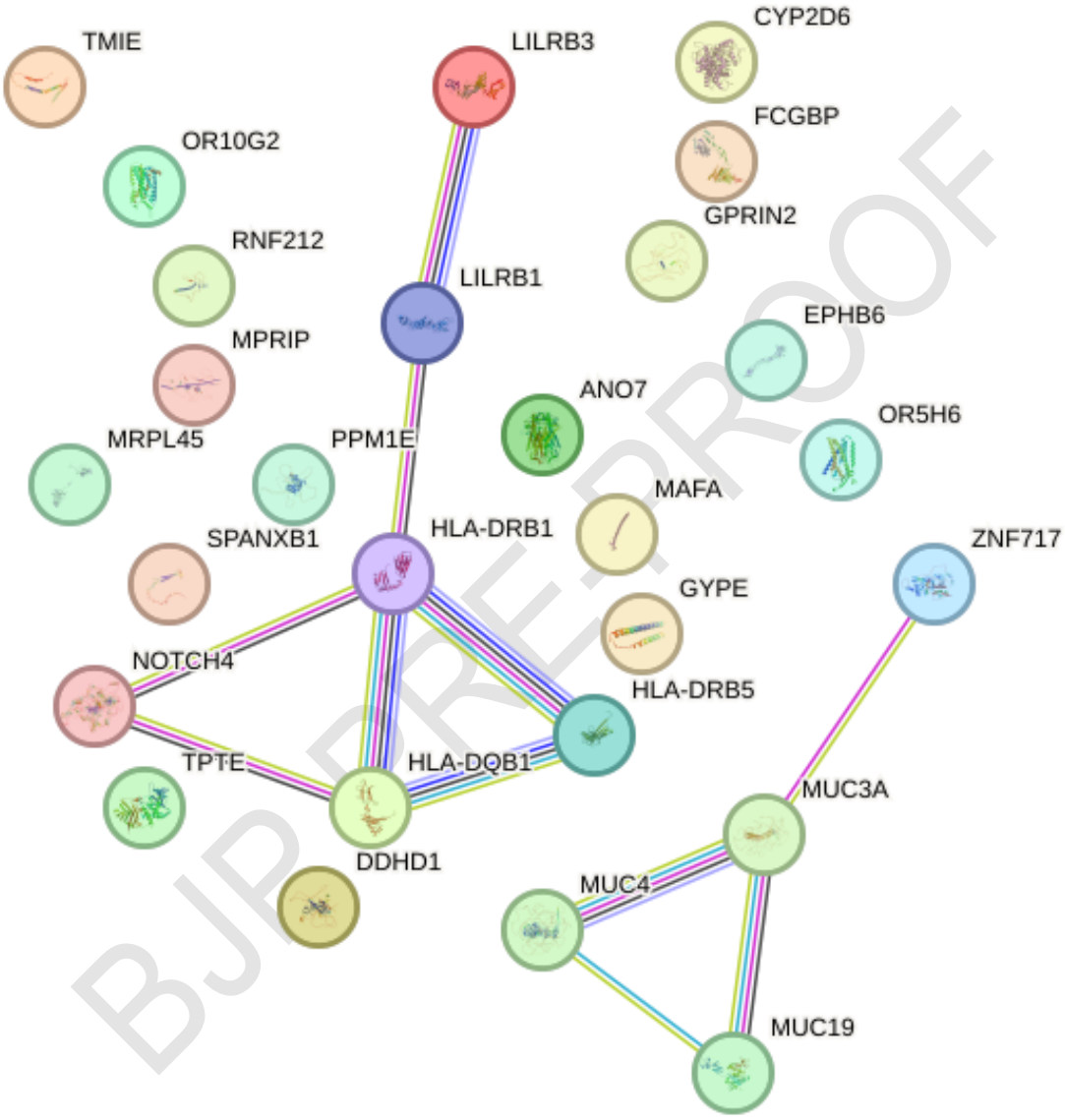

Figure 1: STRING network using genes presenting mutations in at least one-third of mediums subjects.

The nodes are genes, and the edges represent the predicted functional associations evidence (Red: Indicates the presence of fusion, Green: Neighborhood, Blue: Cooccurrence, Purple: Experimental, Yellow: Text mining, Light blue: Database, Black: Coexpression).

Table 4: Overrepresented pathways given by STRING tool.

Pathway	Description	Genes found[a]	Genes count[b]	Strength[c]
HSA-202430	Translocation of ZAP-70 to Immunological synapse	3	16	2.14
HSA-5083625	Defective GALNT3 causes HFTC	3	18	2.08
HSA-5083636	Defective GALNT12 causes CRCS1	3	18	2.08
HSA-202427	Phosphorylation of CD3 and TCR zeta chains	3	19	2.06
HSA-5083632	Defective C1GALT1C1 causes TNPS	3	19	2.06
HSA-389948	PD-1 signaling	3	20	2.04
HSA-977068	Termination of O-glycan biosynthesis	3	25	1.94
HSA-5621480	Dectin-2 family	3	28	1.89
HSA-202433	Generation of second messenger molecules	3	31	1.85
HSA-3906995	Diseases associated with O-glycosylation of proteins	4	69	1.63
HSA-877300	Interferon gamma signaling	3	90	1.39
HSA-202424	Downstream TCR signaling	3	92	1.38

Footnote:
[a]Genes within the metabolic pathway were identified as mutated in the sample.
[b]Total of genes within the pathway.
[c]Strength: Quantified by the log10 of the ratio between the number of genes in our network and the number of genes expected to be annotated with the respective term in a randomly generated network of equivalent size.

Table 5: Significant pathways according to enrichment analysis (Gene Ontology) using WebGestalt tool.

GO ID	GO Name	Gene Found[a]	Gene Count[b]	P-Value	Adjusted P-value[c]
GO:0002768	immune response-regulating cell surface receptor signaling pathway	6	304	6.0341e-7	0.0063
GO:0090345	cellular organohalogen metabolic process	2	2	0.0000011841	0.0063
GO:0090346	cellular organofluorine metabolic process	2	2	0.0000011841	0.0063
GO:0050778	positive regulation of immune response	7	648	0.0000031279	0.0124
GO:0002764	immune response-regulating signaling pathway	6	446	0.0000056032	0.0178
GO:0002429	immune response-activating cell surface receptor signaling pathway	5	277	0.0000098535	0.026
GO:0048584	positive regulation of response to stimulus	10	2027	0	0.0267
GO:0050776	regulation of immune response	7	837	0	0.0312
GO:0016098	monoterpenoid metabolic process	2	6	0	0.0312
GO:0002684	positive regulation of immune system process	7	894	0	0.0413

GO:0002223	stimulatory C-type lectin receptor signaling pathway	3	56	0	0.0449
GO:0002220	innate immune response activating cell surface receptor signaling pathway	3	58	0	0.0457

Footnote: GO: Gene Ontology.
[a]Genes within the metabolic pathway were identified as mutated in the sample.
[b]Total of genes within the pathway.
[c]The adjusted p-value was determined by applying the Benjamini & Hochberg procedure.

DISCUSSION

This study marks the inaugural endeavor to genomically assess individuals with mediumistic spiritual experiences. Notably, we meticulously chose mediums all over Brazil who were recognized by their peers for manifesting outstanding levels of mediumistic phenomena. The control group was composed of mediums' first-degree relatives without mediumship, with a shared context, ancestry, and religious background with the mediums, enabling us to control the findings for sociocultural and biological confounders. In a previous paper we have failed to find any relationship between this sample of mediums and psychoses or any other mental disorder, as mediums presented social adjustment and quality of life similar to their control subjects.[12]. In the present study, we investigated whether mediumship could be related to inherent genetic alterations so mediums could process information differently from controls.

After incorporating stringent criteria to consider valid genomic variants, our analyses allowed the identification of 7,269 genes, exclusively found to be altered in mediums but not in their controls. These gene variations potentially exert a moderate or high impact on the functionality of their respective encoded proteins. Within this set, 33 genes exhibited modifications in at least one-third of the mediums, while their corresponding relatives did not show such alterations. Notably, genes associated with the mucous protection of epithelial cells and antigen presentation topped this list.

The Mucin 19 gene (*MUC19*) particularly stood out, being the most prevalent in both the mediums sample (87.04%) and the validation sample (91.67%). Furthermore, *MUC19* was identified in both twins and ranks among the genes with higher expression in the pineal gland which is intriguing considering the longstanding hypothesis that this gland serves as the epicenter for spiritual experiences.[35,36]

Our analysis of overrepresented pathways revealed that the translocation of ZAP-70 to the immunological synapse exhibited the highest strength. Zeta-Chain Associated Protein Kinase 70 kDa (ZAP-70) plays a crucial role in T-cell antigen receptor stimulation. Moreover, enrichment analysis further delineated 12 significantly distinct pathways, most of which are associated with immune system functions. In the first cluster identified in network analysis, both *LILRB1* and *LILRB3* are representatives of genes within the leukocyte immunoglobulin-like receptor (*LIR*) family. Predominantly expressed in immune cells, these receptors interact with major histocompatibility complex (MHC) class I molecules on antigen-presenting cells, thereby negatively regulating immune cell activation. Their putative role involves modulating inflammatory responses and cytotoxicity, contributing to the precise regulation of the immune response and the limitation of autoreactivity[37].

HLA-DRB1, *HLA-DRB5*, and *HLA-DQB1* belong to the MHC, Human Leukocyte Antigen (HLA), class II beta chain paralogs. MHC class II molecules play a crucial role in the immune system by presenting peptides derived from extracellular proteins[38]. Notably, *MHC* genes exhibit high polymorphism due to their functionality which determines peptide binding specificities. The *NOTCH4* gene encodes a member of the type I transmembrane protein family. Notch signaling represents an evolutionarily conserved intercellular pathway that regulates interactions between physically adjacent cells by binding Notch family receptors to their respective ligands.

Alterations in immune cells might play a crucial role in mediating information from external to internal environment. It is tempting to speculate that such signals might be transmitted through inflammatory responses and cytotoxicity like the immune response. In essence, immune cells may act as intermediaries, influencing how the body perceives and responds to stimuli from its surroundings and internal state. This complex interplay highlights the intricate relationship between the immune system and sensory perception, shedding light on the potential mechanisms underlying our perception of the world around us and our internal physiological conditions. Also, we may be on the brink of discovering novel functions for these genes, spurred by the recognition of our incomplete comprehension regarding the actions of each known gene.

In the second identified cluster, the *ZNF717* gene encodes a kruppel-associated box (KRAB) zinc-finger protein, which belongs to an extensive group of transcriptional

regulators in mammals. The *MUC4*, *MUC3A*, and *MUC19* genes encode mucins which serve as the primary components of mucus. This is pivotal in protecting epithelial cells and have been implicated in epithelial renewal and differentiation processes. Concerning these mutations, we are more likely on the verge of uncovering new functions for these genes. Interestingly, it is worth noting that the epithelium and the central nervous system share a common origin from the embryonic ectoderm.

The fundamental role of some of the genes found here is controversial. This is especially true for the FLAGS genes, including *HLA*, olfactory receptor genes, and mucins[39]. *HLA* and *MUC* genes are frequently identified in studies that investigate mutations related to diverse diseases such as autism[40], congenital heart disease[41] and pulmonary arterial hypertension[42]. These genes were excluded in all the studies mentioned above due to their hypervariability. However, the claim that genes like *MUC* and *HLA* genes are always found mutated in genomic analyses is not entirely accurate. Whereas these genes do appear relatively frequently in mutation lists, the reasons for their apparent prevalence depend on several factors, such as sequencing depth, filtering thresholds, and mutation hotspots. Finding them mutated is not a universal truth, and their significance depends on the study's specific context and the technical considerations involved. Other authors argue that, despite their frequent identification in many diseases, these genes, including *HLA* and mucins, may be involved in diseases such as passenger mutations that are relevant for some patient groups[43]. Finally, as the mutated genes in mediums were not associated with specific mediumistic abilities, it is conceivable that those genes could be related to the mediumistic skill, but not with any particular mediumship modality.

An important issue to highlight is that the genes with more frequent mutations in mediums are not specifically associated with mental or physical disorders. This non-specificity aligns with the understanding that spiritual well-being is strongly interconnected with overall health, encompassing physical, mental, and social dimensions [44,45]. Higher levels of self-transcendent spirituality appear to be robust indicators of an individual's enhanced ability to regulate health holistically, facilitated by well-integrated brain connectivity and dynamic gene expression that adapts to changing conditions. Consequently, gifted mediums, who exhibit better average health and unique genetic variants related to inflammation, immune response, and

adaptation, contrast with the pervasive markers of low self-awareness prevalent in poorly regulated populations under current global conditions.

In sum, it is noteworthy that the mutated genes identified here are intricately associated with metabolic pathways relevant to interactions with the external environment, specifically involving the epithelial and immune systems. This observation prompts the intriguing hypothesis that individuals harboring these mutations may possess an inherent biological predisposition that influences the information processing of the external world in a manner distinct from those lacking such genetic variations. Analogous to certain animals that exhibit extraordinary senses beyond human capabilities[46], it is conceivable that these individuals may have evolved to perceive the environment uniquely.

This might be attributed to the hypothesis that their sensory system is a filter with more prominent pores or a less restrictive valve, enabling them to perceive aspects of reality that most individuals do not. Seminal authors such as William James (1898), C. S. Schiller (1891), and Aldous Huxley (1954)[47] have proposed that the brain would act as a filter or a "reducing valve" of the larger reality to select perceptions needed for survival[48,49]. Supporting this hypothesis, a recent experiment found that repetitive transcranial magnetic stimulation (rTMS) inhibiting the activity of the brain's left medial middle frontal lobe induced higher psi ("paranormal") skills[50]. Additionally, an alternative hypothesis is that the mutated genes may harbor functions beyond the scope recognized in previous studies.

To our knowledge, this is the first study that performed a large exome-wide investigation of genes potentially related to mediumistic experiences. We identified 33 genes significantly expressed in at least one-third of unrelated mediums from our sample but not in their first-degree relatives. These genes emerge as possible candidates for further investigations of the biological underpinnings that allow spiritual experiences such as mediumship. These findings warrant replication studies, which are necessary before any conclusion can be drawn.

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Data Availability Statement: The data that support the findings of this study, documentation, and code used in analysis are available from the corresponding author upon reasonable request. The data are not publicly available due to containing information that could compromise research participant privacy.

Author Contributions: WFG and AMA conceived and designed the study. AMA, MAC, and WFG conducted the study. AMA and MAC helped with data collection. WFG and LLT coordinated laboratory and exome analysis. ASO and DG conducted and help with the interpretation of statistical analysis. MAC, ASO, and DG wrote the manuscript. AMA and WFG supervised the manuscript writing. WFG acquired the funding. All authors reviewed and approved the final version of the manuscript.

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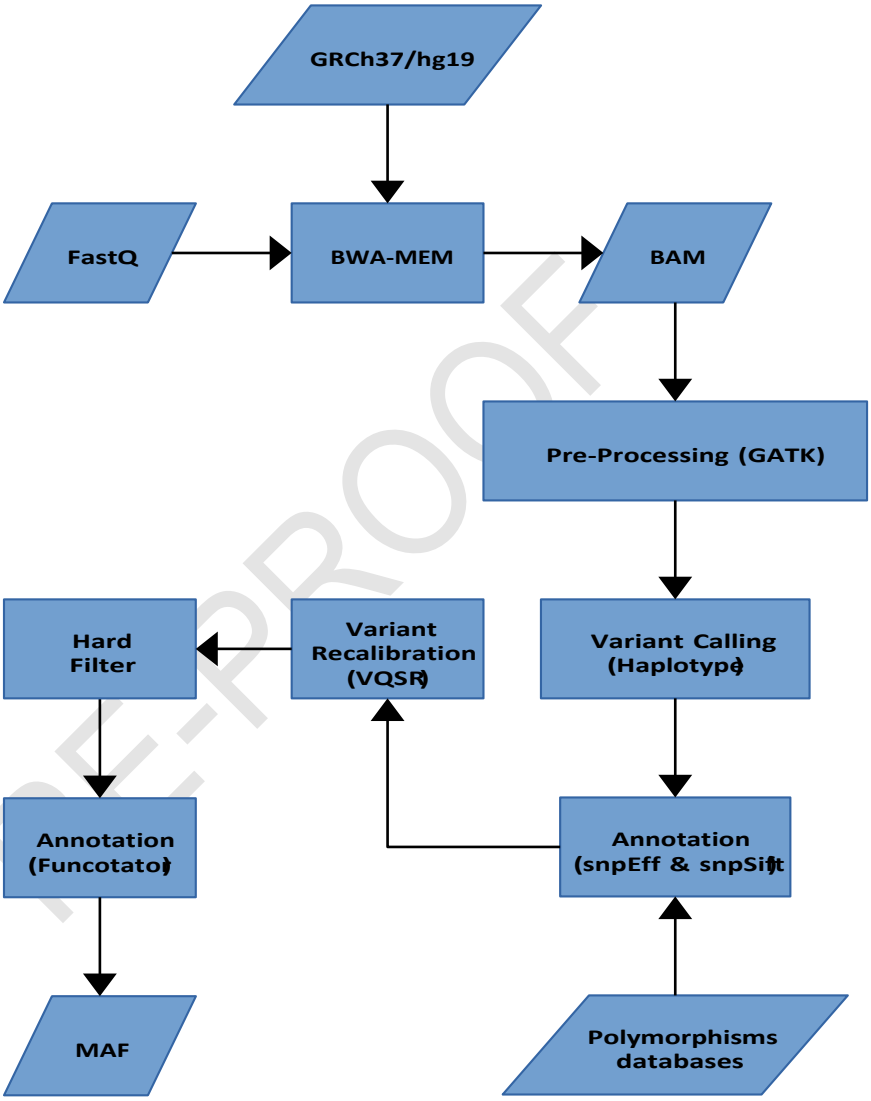
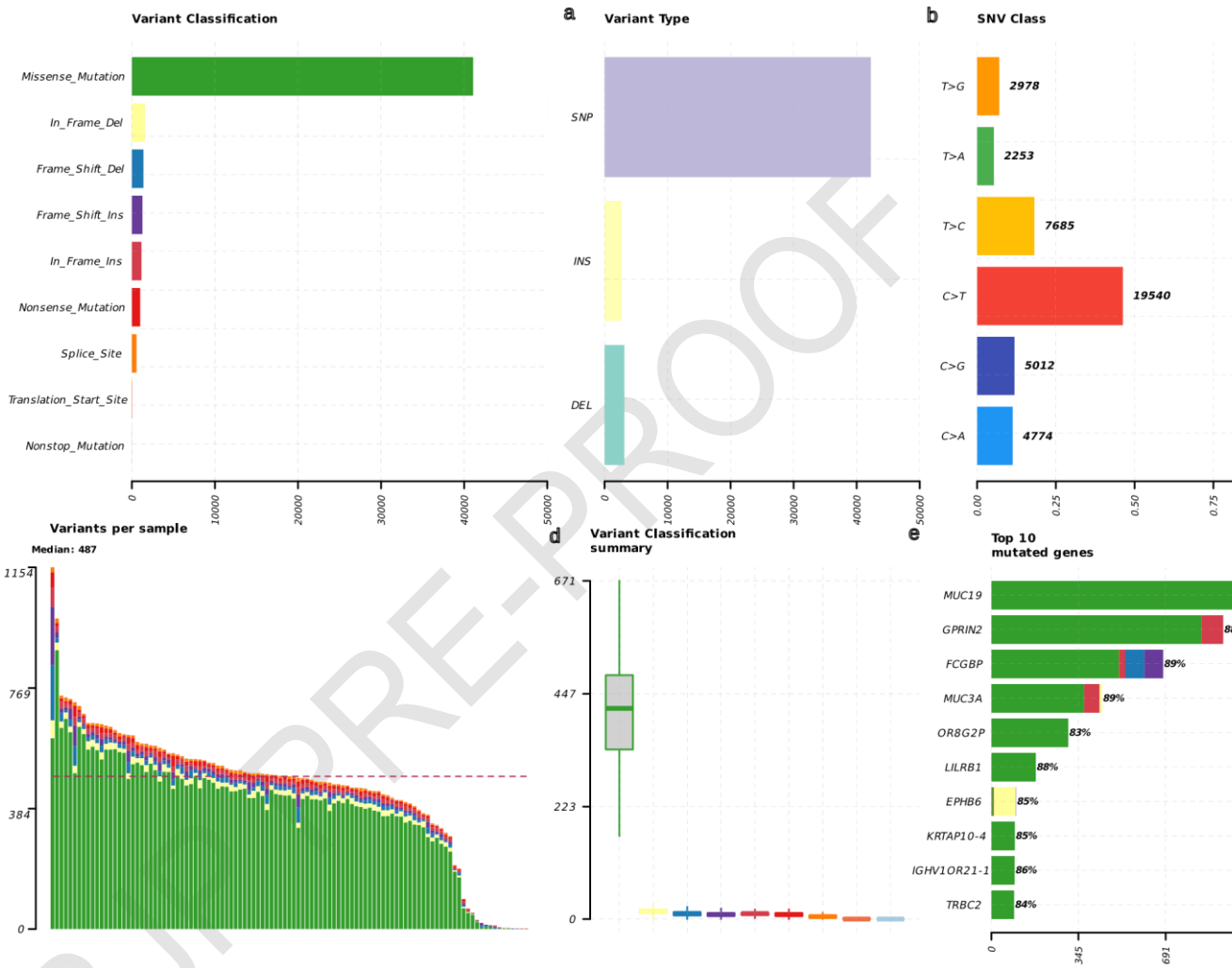
Supplementary material



Supplementary Figure 1: The pipeline utilized for processing exome data to produce a MAF file involves several key steps. Initially, sequencing reads (FastQ files) are aligned to the human reference genome (GRCh37/hg19) using BWA-MEM (v0.7.17-r1188), adjusting the thread count to 8. Subsequently, aligned files (BAMs) undergo preprocessing with GATK version 4.2, including steps like MarkDuplicates, BaseRecalibrator, and PrintReads with default parameters and 8 threads. Variant calling is conducted using HaplotypeCaller with specific parameters for each sample, followed by CombineGVCs and GenotypeGVCFs to identify variants in a 'joint analysis' format. Identified variants are annotated using snpEff and SnpSift software, leveraging various DNA polymorphism databases. Filtering steps include GATK's Variant Recalibration (VQSR) method with specific parameters and a Hard Filter based on GATK Best Practices. Common variants (>1% VAF - Variant Allele Frequency) found in non-M subjects, potentially less correlated with observed

phenotypes, are removed after comparison with public databases like dbSNP, 1000 Genomes, gnomAD, and the ABRaOM database. Finally, Funcotator annotates the remaining variants, ultimately generating the MAF file.



Supplementary Figure 2: The summary of the distribution of non-synonymous variants with depth>8 and vaf>30%. (a) The classification of variants according to the type of alteration with their count displayed on the x-axis. (b) The types of the variants (SNP: Single Nucleotide Polymorphism; INS: insertion and DEL: deletion). (c) Single Nucleotide Variant (SNV) class that shows the most frequent nucleotide substitutions. (d) The distributions of the variants per sample, with the dashed line being the median (487 variants per sample). (e) The box plot of the variant classification. (f) Top 10 genes with the highest counts of the variants on the x-axis. The color code of d, e and f corresponds to the variant classification. The percentages of participants that have at least one variant of these genes are displayed on the right side of the bars. The color code corresponds to the variant classification.

Supplementary Table 1: Genes presenting mutations in at least one-third of mediums subjects of this study

Gene Name	Higher expression tissue[a]	Function	Frequency n (%)				
			Mediums Sample (n = 54)	Twins[b] (n = 2)	Validation Sample[c] (n = 12)	Sensory System	Pineal G
Mucin 19	Minor salivary gland	Mucous protection of epithelial tissues	47 (87.04%)	2 (100%)	11 (91.67%)	-	X
Mucin 3a	Small intestine	Mucous protection of epithelial tissues	36 (66.67%)	2 (100%)	11 (91.67%)	-	-
Mucin 4	Colon	Mucous protection of epithelial tissues	35 (64.81%)	2 (100%)	8 (66.67%)	-	-
Major histocompatibility complex class II DR Beta 5	Lung	Antigen presentation	34 (62.96%)	2 (100%)	9 (75%)	-	-
Zinc finger protein 717	Thyroid	Transcriptional regulation	31 (57.41%)	1 (50%)	8 (66.67%)	-	-
Major histocompatibility complex class II DQ Beta 1	Lung	Antigen presentation	31 (57.41%)	2 (100%)	8 (66.67%)	-	-
Major histocompatibility complex class II DR Beta 1	Lung	Antigen presentation	30 (55.56%)	2 (100%)	9 (75%)	-	-
Transmembrane phosphatase with Tensin homology	Testis	Signal transduction	28 (51,85%)	2 (100%)	11 (91.67%)	-	-
Notch receptor 4	Adipocytes	Regulates cell fate determination	27 (50.00%)	2 (100%)	5 (41.67%)	-	-
Ephrin type-B receptor 6	Brain cortex	Modulates cell adhesion and migration	27 (50.00%)	2 (100%)	10 (83.33%)	-	-
Fc gamma binding protein	Colon	Maintenance of mucosal structure	27 (50.00%)	2 (100%)	11 (91.67%)	-	-
Transmembrane Inner ear	Pituitary gland	Protein/vesicle trafficking	24 (44.44%)	-	7 (58.33%)	-	-
Anoctamin 7	Prostate gland	Cell-cell interaction	24 (44.44%)	2 (100%)	7 (58.33%)	-	-
MAF bZIP transcription factor A	Skeletal muscle	Transcription factor that regulates pancreatic beta cell-specific expression.	23 (42.59%)	-	8 (66.67%)	-	-
Leukocyte immunoglobulin like receptor B3	WBC	Receptor of class I MHC antigens	23 (42.59%)	-	7 (58.33%)	-	-
Nuclear pore assoc. protein 1	Testis	Unknown. nucleoporin-derived?	23 (42.59%)	-	7 (58.33%)	-	-
Sperm protein associated with the nucleus on the X chromosome	Testis	Regulates transcription and translation of several testis-specific genes	22 (40.74%)	2 (100%)	8 (66.67%)	-	-
Protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1E	Brain	Inactivates CaM kinases	22 (40.74%)	2 (100%)	9 (75%)	-	-

LOC101930307	Unknown	Unknown	22 (40.74%)	-	11 (91.67%)	-	-
Olfactory receptor Family 8 Subfamily G member 2	Testis	Olfactory receptor	21 (38.89%)	-	9 (75%)	-	-
Immunoglobulin heavy constant gamma P (non-functional)	Lymph node	Pseudogene	21 (38.89%)	2 (100%)	6 (50%)	-	-
Myosin phosphatase Rho interacting protein	Prostate gland	Regulates actin cytoskeleton	21 (37.04%)	-	6 (50%)	-	-
Mitochondrial ribosomal protein L45	Breast	Pseudogene	20 (37.04%)	-	8 (66.67%)	-	-
Putative POM121-like	Testis	Unknown	20 (37.04%)	-	6 (50.00%)	-	-
Immunoglobulin heavy variable 3-20	Lymph node	Antigen recognition	19 (35.19%)	-	11 (91.67%)	-	-
Leukocyte immunoglobulin like receptor B1	Spleen	Receptor of class I MHC antigens	19 (35.19%)	-	11 (91.67%)	-	-
DDHD domain containing 1	Testis	Phospholipase A1	19 (35.19%)	-	8 (66.67%)	-	-
Cytochrome P450 family 2 subfamily D member 6	Liver	Metabolism of fatty acids, steroids and retinoids	19 (35.19%)	-	9 (75%)	-	-
Ring finger protein 212	Testis	Regulator of crossing over during meiosis	19 (35.19%)	-	9 (75%)	-	-
Olfactory receptor family 5 subfamily H member 6	Testis	Olfactory receptor	19 (35.19%)	2 (100%)	10 (83.33%)	hsa04740	-
G-protein regulated inducer of neurite outgrowth 2	Skin	Neurite outgrowth	18 (33.33%)	-	11 (91.67%)	-	-
Glycophorin E	Spleen	Minor syaloglycoprotein of erythrocyte membranes	18 (33.33%)	-	5 (41.67%)	-	-
Olfactory receptor family 10 subfamily G member 2	Liver	Olfactory receptor	18 (33.33%)	-	9 (75%)	hsa04740	-

Gene name, tissue expression and function are given as provided by genecards.org.
[a]Tissue with higher expression according to RNA-Seq data, WBC: white blood cells, CNS: central nervous system, from Genecards; higher expression as determined by RNASeq experiments from (genecards.org).
[b]Twins: a pair of monozygotic twins presenting mediumship.
[c]Validation sample: 12 mediums without controls used for independent validation.
[d]In this column, "X" indicates that the gene is on the gene set related to pineal gland function.