Lack of reproducibility in re-evaluating associations between *GCH1* polymorphisms and Parkinson's disease and isolated dystonia in an Australian case-control group

Jeremy R. B. Newman¹, Michael Todorovic¹, Peter A. Silburn^{3,4}, Greg T. Sutherland⁵, George D. Mellick^{1,2,3}

1. Eskitis Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia

2. Department of Neurology, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia

3. Department of Neurology, Royal Brisbane and Women's Hospital, Herston, Queensland, Australia

4. University of Queensland Centre for Clinical Research, School of Medicine, Queensland, Royal Brisbane and Women's Hospital, Herston, Australia

5. Discipline of Pathology, Sydney Medical School, University of Sydney, Camperdown, New South Wales, Australia

Corresponding author: Associate Professor George D. Mellick, Deputy Director, Eskitis Institute for Drug Discovery, Griffith University, Don Young Road, Nathan QLD 4111, Australia, phone: +61 7 373 55019, fax: + 61 7 373 54255, E-mail: G.Mellick@griffith.edu.au

Short title: GCH1 in Parkinson's disease and dystonia

Abstract: 50 words **Text word count:** 1041 words

Number of: **text pages:** 4 **figures:** 0 **tables:** 2 **Supplementary files:** 1

Dystonia is a group of debilitating movement disorders characterised by the sustained or intermittent involuntary co-contraction of agonist and antagonist muscles. While the cause of the major of cases is not known (termed ‘idiopathic isolated dystonia’), mutations in at least 12 genes (‘DYT genes’) are known to cause hereditary dystonia syndromes [1]. We have previously investigated common single nucleotide polymorphisms (SNPs) in eight DYT genes as susceptibility factors for idiopathic isolated dystonia and reported modest associations between SNPs in the *GCH1* gene and idiopathic isolated dystonia [2]. An additional supplementary analysis using a Parkinson’s disease (PD) case group also demonstrated associations with the same *GCH1* SNPs [2]. Two of these SNPs are in high linkage disequilibrium with SNPs that constitute a so-called “pain-protective” haplotype [3]. Because this haplotype is associated with reduced *GCH1* expression and function [3], our initial findings suggested that there may be a shared functional genetic contribution to the pathogenesis of both dystonia and PD in the form of common *GCH1* SNPs [2]. However, the similar odds ratios observed in both PD and dystonia association analyses for each SNPs also suggested an unintentional bias in the selection of control subjects. We therefore decided to genotype these SNPs in a larger sample to reinvestigate our initial reported findings.

Dystonia cases were recruited from two private and two public movement disorders clinics as previously described [2]. PD cases and controls were recruited as part of the Queensland Parkinson’s Project, which is a collaborative research study cohort of over 3000 community-dwelling Queenslanders, recruited since 2005, who have agreed to participate in research into PD and which has contributed to several large-scale genetic studies [4]. Informed consent was obtained from all participating subjects and ethical clearance was obtained from all participating institutions. In total,

there were 233 dystonia cases (mean age = 61 ± 13 years, 82 males/151 females), 1337 PD cases (mean age = 71 ± 10 years, 836 males/501 females) and 1090 controls (mean age = 70 ± 10 years, 530 males/560 females). This included 226 PD cases and 228 controls from our initial study [2]. Supplementary Table 1 summarises the demographics of these groups (Supplementary File). Blood was collected from all subjects and DNA extracted by standard methods.

Assays for the three *GCHI* SNPs from our previous study – rs12147422, rs3759664 and rs10483639 – were designed and performed using the MassARRAY genotyping platform (Sequenom). We also designed and carried out genotyping assays for rs8007267 and rs3783641 to complete the pain-protective *GCHI* haplotype [3]. Genotyping was carried out on all subjects in this study. Reliability of genotyping was verified by re-genotyping a random selection of 16% of all subjects and also by comparing with our previously obtained genotypes [2]. No conflicts between our previous and current studies were discovered, and in total 98.6% of genotypes were successfully called. Statistical analyses were carried out in PLINK version 1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>). The odds ratios (ORs) for genotype associations (additive model) were calculated using logistic regression with adjustment for age and gender.

Modest associations were seen in our original case-control series of 226 PD cases and 228 age- and gender-matched controls as previously reported [2] (Table 1). These associations were not observed in second series of 1105 PD cases and 862 controls, nor were they seen when both studies were combined into a single dataset ($P > 0.05$; Table 1).

Our initial study also comprised 230 isolated dystonia cases [2]. As only an additional three isolated dystonia cases were recruited into this study, we decided to analyse all

233 cases against each control group (Table 2). Similarly to our previous study, associations between *GCHI* SNPs and isolated dystonia were found when comparing to the original control group ($P < 0.05$), but were not replicated when comparing this case group against either the second control series or all controls combined (Table 2). We also looked at the “pain protective” haplotype comprised of rs10483639, rs3783641 and rs8007267, denoted in this study as the “H2” haplotype. No significant differences in haplotype frequencies were observed when comparing all PD cases or all dystonia cases to all controls individuals (Supplementary Table 2, Supplementary File).

We previously determined that an additional 770 cases and controls would be needed to conclusively determine if our initial results were real and not spurious findings [2]. The study we present here exceeds this requirement and has 90% power to detect ORs of at least 1.390 for the PD analysis and ORs of at least 1.700 for the isolated dystonia analysis, taking multiple testings into consideration. However, we were unable to replicate our original findings in this larger sample. Additionally, when we measured *GCHI* expression in patient-derived cell lines cultured in identical conditions, no differences were observed between PD cases and controls (Supplementary File). This suggests that *GCHI* SNPs do not alter susceptibility to PD. This is consistent with previously published literature regarding *GCHI* variants in PD [5].

Excluding our original analysis, there have been no other studies examining *GCHI* genetic variants in idiopathic dystonia. While our analysis would have benefitted from the recruitment of additional dystonia cases, this was not possible as the low prevalence of the disorder suggests that we have likely included most of the available cases in Queensland. As we have previously stated, the rarity of the dystonia phenotype requires collaborative efforts for sufficiently large genetic association

studies [2]. In this study, we attempted to address this limitation by the addition of larger control group. However, the lack of reproducibility in our analysis here suggests that the associations reported in our original study were spurious results. This lends supports to the conclusions of our previous study that susceptibility to idiopathic dystonia is not greatly affected by common genetic variants around dystonia-linked genes. Taken with our original analyses, this highlights the need to expand the search for candidate genes for idiopathic dystonia.

ACKNOWLEDGEMENTS

A special thanks to all participants in this study. We would also like to thank Nick Matigian, Ratneswary Sutharsan, Jessica Clark and Priscilla Hunt for valued assistance; Richard S. Boyle, John O'Sullivan, Nicole Limberg, Stephan Blum, Gerhard Siebert, Sarah Day, Daniel Buchanan, Nadeeka Dissanayaka, Coral Gartner, Madeline Minslow and Karen O'Maley for their expert assistance with patient recruitment and DNA extraction; and Sullivan and Nicolaides Pathology for assistance with blood collection.

REFERENCES

- [1] Charlesworth G, Bhatia KP, Wood NW. The genetics of dystonia: new twists in an old tale. *Brain*. 2013;136:2017-37.
- [2] Newman JR, Sutherland GT, Boyle RS, Limberg N, Blum S, O'Sullivan JD, et al. Common polymorphisms in dystonia-linked genes and susceptibility to the sporadic primary dystonias. *Parkinsonism & related disorders*. 2012;18:351-7.
- [3] Antoniades C, Shirodaria C, Van Assche T, Cunningham C, Tegeder I, Lotsch J, et al. GCH1 haplotype determines vascular and plasma biopterin availability in coronary

artery disease effects on vascular superoxide production and endothelial function.

Journal of the American College of Cardiology. 2008;52:158-65.

[4] Sharma M, Ioannidis JP, Aasly JO, Annesi G, Brice A, Van Broeckhoven C, et al.

Large-scale replication and heterogeneity in Parkinson disease genetic loci.

Neurology. 2012;79:659-67.

[5] Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, et al.

Comprehensive research synopsis and systematic meta-analyses in Parkinson's

disease genetics: The PDGene database. PLoS genetics. 2012;8:e1002548.

Table 1. Genotyping associations of SNPs and Parkinson's disease

SNP	Alleles ^a	Control	PD	OR (95% CI)	P ^b
Original cohort					
rs10483639	G/C	127/75/21	142/75/6	0.681 (0.498-0.932)	0.016
rs12147422	T/C	183/37/3	171/48/5	1.400 (0.927-2.115)	0.110
rs3783641	T/A	125/78/14	140/76/4	0.711 (0.509-0.992)	0.045
rs3759664	C/T	135/76/12	146/74/3	0.734 (0.523-1.031)	0.074
rs8007267	C/T	134/74/11	150/70/4	0.730 (0.519-1.027)	0.071
Replication cohort					
rs10483639	G/C	548/269/36	693/338/45	1.006 (0.857-1.181)	0.941
rs12147422	T/C	646/196/16	840/217/25	0.895 (0.741-1.081)	0.250
rs3783641	T/A	547/262/36	678/339/47	1.051 (0.895-1.234)	0.547
rs3759664	C/T	593/236/26	731/299/42	1.080 (0.912-1.278)	0.372
rs8007267	C/T	598/236/25	738/300/39	1.072 (0.905-1.270)	0.423
Combined cohort					
rs10483639	G/C	666/341/57	835/413/51	0.929 (0.806-1.070)	0.305
rs12147422	T/C	819/231/19	1011/265/30	0.959 (0.809-1.138)	0.633
rs3783641	T/A	665/335/50	818/415/51	0.979 (0.848-1.131)	0.774
rs3759664	C/T	719/309/38	877/373/45	1.000 (0.861-1.161)	0.996
rs8007267	C/T	723/307/36	888/370/43	0.994 (0.855-1.156)	0.942

^a Major/minor allele^b adjusted for age and gender

OR, odds ratio; CI, confidence interval

Table 2. Genotyping associations of SNPs and idiopathic dystonia

SNP	Alleles ^a	Control	Dystonia	OR (95% CI)	P ^b
Original cohort					
rs10483639	G/C	127/75/21	151/67/11	0.704 (0.518-0.956)	0.025
rs12147422	T/C	183/37/3	169/57/6	1.534 (1.020-2.306)	0.040
rs3783641	T/A	125/78/14	148/66/7	0.687 (0.492-0.959)	0.027
rs3759664	C/T	135/76/12	166/60/6	0.626 (0.444-0.882)	0.007
rs8007267	C/T	134/74/11	157/58/6	0.670 (0.473-0.949)	0.024
Replication cohort					
rs10483639	G/C	548/269/36	151/67/11	0.974 (0.746-1.272)	0.848
rs12147422	T/C	646/196/16	169/57/6	1.194 (0.881-1.620)	0.254
rs3783641	T/A	547/262/36	148/66/7	0.909 (0.688-1.203)	0.505
rs3759664	C/T	593/236/26	166/60/6	0.909 (0.679-1.217)	0.521
rs8007267	C/T	598/236/25	157/58/6	0.948 (0.706-1.273)	0.722
Combined cohort					
rs10483639	G/C	666/341/57	151/67/11	0.885 (0.686-1.141)	0.346
rs12147422	T/C	819/231/19	169/57/6	1.260 (0.937-1.693)	0.126
rs3783641	T/A	665/335/50	148/66/7	0.856 (0.654-1.121)	0.260
rs3759664	C/T	719/309/38	166/60/6	0.828 (0.625-1.096)	0.187
rs8007267	C/T	723/307/36	157/58/6	0.873 (0.657-1.161)	0.350

^a Major/minor allele^b adjusted for age and genders

OR, odds ratio; CI, confidence interval

SUPPLEMENTARY METHODS

Dataset normalisation and analysis

Raw data (in the form of CEL files) were downloaded from the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) or ArrayExpress (<http://www.ebi.ac.uk/arrayexpress>). To exclude potential variability introduced from examining data generated by different platforms, only studies that utilised Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix) were considered. Data were normalised with Affymetrix Power Tools version 1.14.2 (Affymetrix), using the Robust Multichip Average (RMA) normalisation algorithm. Quantile normalisation was used and data was $\log_2$ -transformed. Because the existing Affymetrix probeset definitions were designed on older genome sequence annotations and may overlap polymorphic sequences, custom probeset definitions based on RefSeq transcript annotations, developed by Dai et al [1], were used and downloaded from <http://brainarray.mbni.med.umich.edu/brainarray/Database/CustomCDF/>. All individuals from each dataset were included in the normalisation, although ultimately only LPS-treated and untreated samples were included in subsequent analyses. Linearised fold change values were generated for each probeset using the “ComparativeMarkerSelection” module in GenePattern [2](<http://genepattern.broadinstitute.org/gp/>). Probesets were deemed “not expressed” if more than 50% of samples did not pass detection thresholds.

Cell culture

Cell lines derived from biopsies of the olfactory mucosa (termed human olfactory neurosphere-derived (hONS) cells) from donor subjects were established as previously described [3, 4]. Aliquots of hONS cell lines from PD patients (N = 6) and neurologically-normal controls (N = 10) were grown in Dulbecco’s Modified Eagles Medium/Ham’s F-12 (Gibco) containing 10% foetal calf serum (Gibco) and 1.5% penicillin-streptomycin (Gibco),

and incubated at 37°C with 5% CO₂. Cell lines were grown in these conditions until at least 80% confluent and harvested by enzymatic detachment.

Cell lines were seeded into 6-well plates at a density of 40000 cells/well in 2 mL of medium and allowed to attach overnight. The media was replaced the following day with 2 mL of fresh media containing 100 ng/mL γ -irradiated lipopolysaccharides (LPS) from *Salmonella enterica* serotype typhimurium (Sigma Aldrich; Catalogue# L6143) and incubated for 6 hours.

mRNA expression

Whole cell RNA was extracted using the TRIzol method (Invitrogen). TRIzol-extracted RNA quality was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and a Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA was reverse transcribed using the SuperScript III first-strand synthesis kit with oligo(dT)₁₈ primers (Invitrogen). Relative levels of mRNAs were calculated and normalised against *RPL32* using the $\Delta\Delta$ CT relative quantification method with correction for amplification efficiency [5]. PCRs were carried out using the Corbett Rotor-Gene 6000 Real-Time PCR system (Qiagen) and the Sensimix HRM Master Mix (Bioline). PCR conditions were 95°C for 10 minutes followed by 50 cycles at 95°C for 10 seconds, 60°C for 15 seconds and 72°C for 20 seconds. Melting curves were generated following amplification to confirm a single PCR product. Primer sequences were *GCHI* (NM_000161): F-agcaaacttgcgaggattgt, R-ttcaaccactaccccgactc; *RPL32* (NM_000994): F-gaagccaagatcgtaaaa, R-accctgtgtcaatgcctct; *SOD2* (NM_001024465): F-tgcatctgttggtgtccaag, R-gtagtaagcgtgctccaca; *PTGS2* (NM_000963): F-gcagggttgctggtgtag, R-tgcagacatttcctttctcc; *IL8* (NM_000584): F-ctgcgccaacacagaaatta, R-ggcatcttcactgattcttg; *LAPTM4A* (NM_014713): F-cagtgaccggttctacagca, R-gtcagcaaaattgccatcaa.

SUPPLEMENTARY RESULTS SUMMARY

Control transcript selection

Transcripts that were consistently upregulated following treatment of cell lines with LPS were selected from microarray datasets and served as positive controls for LPS stimulation. These datasets encompassed several studies examining the transcriptional response to LPS treatment. In total, 8 microarray datasets were selected from public repositories and were used for determining appropriate control transcripts (Supplementary Table 1).

Probesets were considered for control gene selection if they met the following criteria: (1) linearised fold change greater than 3 in all datasets examined; (2) expressed in greater than 50% of all samples in all datasets examined; (3) expressed in the hONS cell lines at baseline as per ArrayExpress studies E-TABM-724 [4] and E-TABM-879 [3]. Overall, 25 probesets were consistently upregulated across all datasets examined and 7 of these were expressed in the hONS cell lines (Supplementary Table 2) and from this list assays were designed to amplify three RefSeq transcripts: NM_001024465 (*SOD2*), NM_000963 (*PTGS2*), NM_000584 (*IL8*).

GCHI mRNA expression in hONS

We then examined if *GCHI* was differentially expressed between PDs and controls using hONS cell lines. These are human-derived cell lines cultured from biopsies of the olfactory neuroepithelium [6], and provide a useful cell model system in which to study disease-specific transcriptional differences in human neurological disorders, including PD [3, 4]. *GCHI* mRNA is typically expressed at low levels in the hONS cell lines (see ArrayExpress accession# E-TABM-724 and E-TABM-879), and cannot be reliably distinguished from background noise. Several studies have demonstrated that *GCHI* expression can be

stimulated by the addition of LPS into culture medium, and transcriptional differences between carriers and non-carriers of the functional “pain protective” haplotype are observable following treatment [7]. As these studies have largely utilised immune and other blood-derived cell lines, we reviewed microarray data to select transcripts that could be used as positive controls for LPS treatment (see Supplementary File for dataset selection, normalisation and analysis). From these datasets we selected transcripts of three genes (*PTGS2*, *IL8* and *SOD2*) that were consistently upregulated following LPS stimulation in several independent studies. We also selected the *LAPTM4A* transcript from these same datasets as a negative treatment control as its expression was relatively stable across these studies.

To demonstrate the responsiveness of the hONS cell lines to LPS treatment, we measured the gene expression after 6 hours of treatment with 100ng/mL LPS. *GCHI*, *IL8*, *PTGS2* and *SOD2* were all significantly upregulated, whereas *LAPTM4A* expression was unchanged, consistent with a response to LPS (Figure 2). We then compared the expression of *GCHI* after LPS treatment between PD and control hONS cell lines and found no significant differences in *GCHI* expression (Figure 2). When cell lines were regrouped according to whether or not they carried the “pain protective” haplotype, the mean *GCHI* expression was slightly lower in the haplotype carriers but this difference did not reach statistical significance (Figure 2).

Supplementary Figure 1. Genomic features of the *GCH1* SNPs genotyped in this study. LD map (r^2) was generated from all control individuals in this study and visualised in Haploview [8].

Supplementary Figure 2. Expression of *GCHI* in hONS cells. (A) Relative expression of *GCHI*, positive “LPS treatment” control genes (*IL8*, *PTGS2*, *SOD2*) and negative control gene (*LAPT4A*) in hONS cells after 6 hours treatment with 100ng/mL LPS. (B) Expression of *GCHI* in control and PD patient-derived hONS cells after LPS treatment. (C) Expression of *GCHI* in hONS cells that carry or do not carry “pain-protective” haplotype after LPS treatment. Values normalised to the average of vehicle-treated, control hONS or haplotype non-carriers. Error bars indicate standard deviation. *** $P < 0.001$.

Supplementary Table 1. Study demographics

Group	Total	Female/Male	Mean age $\pm$ SD	Mean age at onset $\pm$ SD
Controls (all)	1090	560/530	70 $\pm$ 10	n/a
Controls (original)	228	144/84	66 $\pm$ 11	n/a
Controls (replication only)	874	422/452	71 $\pm$ 10	n/a
PD (all)	1338	501/837	71 $\pm$ 11	59 $\pm$ 11
PD (original)	232	144/88	65 $\pm$ 12	53 $\pm$ 12
PD (replication only)	1107	358/749	73 $\pm$ 10	61 $\pm$ 11
Dystonia	233	151/82	61 $\pm$ 14	46 $\pm$ 15

Supplementary Table 2. Associations between PD and isolated dystonia and the functionally-significant “pain protective” haplotype

Haplotype	Alleles ^a	Controls	Cases	OR ^b (95% CI)	P ^b
PD vs Control					
H1	GTC	2033	1996	1.066 (0.936-1.210)	0.334
H2	CAT	452	399	0.930 (0.799-1.080)	0.350
H3	CTC	74	51	0.765 (0.531-1.100)	0.149
H4	CAC	59	56	0.920 (0.625-1.350)	0.670
H5	GAT	31	39	1.227 (0.754-2.000)	0.409
Others		37	43	1.245 (0.794-1.950)	0.339
Total		2686	2584		
Dystonia vs. Control					
H1	GTC	2033	348	1.264 (0.981-1.630)	0.066
H2	CAT	452	62	0.753 (0.559-1.010)	0.054
H3	CTC	74	12	1.076 (0.574-2.020)	0.821
H4	CAC	59	9	0.960 (0.452-2.040)	0.915
H5	GAT	31	6	1.273 (0.512-3.170)	0.612
Others		37	3	0.464 (0.139-1.540)	0.164
Total		2686	440		

^a rs10483639, rs3783641 and rs8007267^b Compared against all others^c adjusted for age and gender

OR, odds ratio; CI, confidence intervals

Supplementary Table 3. Summary of microarray studies used for control gene selection

Study	Cell type	[LPS]	Time (hours)	N treated	N untreated
E-MEXP-1958	MDDC ^a	1 µg/mL	48	3	3
GSE4984	MDDC	100 ng/mL	48	3	3
GSE9916	THP-1 cells	1 µg/mL	4	3	3
GSE10316	MDDC	1 µg/mL	4, 8, 16 ^b	1	1
GSE10739	MM6 cells	1 ng/mL	24	2	2
GSE11327	MDDC	30 ng/mL ^c	6, 12, 24, 48 ^b	1	1
GSE19627	THP-1 cells	1 µg/mL	4	3	3
GSE40885	Alveolar macrophages	4ng/kg	6	7	7

^a Monocyte-derived dendritic cells^b each time point analysed individually^c Plus 1U/mL IFN-γ

Supplementary Table 4. Linearised fold changes for transcripts most consistently upregulated following LPS treatment

RefSeq Probeset	Gene	E-MEXP-1958	GSE4984	GSE9916	GSE10316 (4 hours)	GSE10316 (8 hours)	GSE10316 (16 hours)	GSE10739	GSE11327 (6 hours)	GSE11327 (12 hours)	GSE11327 (24 hours)	GSE11327 (48 hours)	GSE19627	GSE40885	Expressed in hONS? ^a
NM_000675_at	<i>ADORA2A</i>	31.3	9.2	11.6	33.4	23.4	17.3	5.4	20.9	34.3	18.4	16.7	5.8	15.8	
NM_005623_at	<i>CCL8</i>	17.8	5.3	7.6	160.7	225.2	110.7	8.7	7.4	53.4	3.0	4.2	11.2	19.2	
NM_004591_at	<i>CCL20</i>	18.6	10.2	157	277.3	149.0	38.9	12.2	305.3	420.3	535.7	133.8	8.7	12.5	
NM_001130046_at	<i>CCL20</i>	18.6	10.2	157.0	277.3	149.0	38.9	12.2	305.3	420.3	535.7	133.8	8.7	12.5	
NM_005191_at	<i>CD80</i>	21.7	6.8	8.8	17.4	23.6	11.6	7.5	25.7	32.4	35.6	22.7	6.3	4.5	
NM_001511_at	<i>CXCL1</i>	3.3	15.2	26.0	71.9	88.1	10.4	23.9	149.5	158.4	117.0	29.2	4.1	13.9	
NM_001565_at	<i>CXCL10</i>	920.4	37.9	9.1	334.5	323.1	104.9	3.7	103.5	155.6	108.5	78.7	11.7	9.4	
NM_005755_at	<i>EBI3</i>	110.0	59.7	62.6	45.7	84.6	96.4	21.8	59.4	69.0	123.9	69.7	13.7	4.2	
NM_024930_at	<i>ELOVL7</i>	37.5	21.0	69.3	84.1	55.8	34.8	3.3	20.5	11.0	45.9	18.5	43.2	8.0	
NM_001104558_at	<i>ELOVL7</i>	37.5	21.0	69.3	84.1	55.8	34.8	3.3	20.5	11.0	45.9	18.5	43.2	8.0	
NM_001549_at	<i>IFIT3</i>	76.2	20.3	4.2	33.2	26.1	17.1	24.4	41.9	26.4	12.1	12.6	3.9	3.2	
NM_001031683_at	<i>IFIT3</i>	76.2	20.3	4.2	33.2	26.1	17.1	24.4	41.9	26.4	12.1	12.6	3.9	3.2	Yes
NM_000575_at	<i>IL1A</i>	14.6	6.9	5.0	288.0	164.0	95.1	9.1	531.9	588.6	336.7	181.9	4.5	4.1	
NM_000576_at	<i>IL1B</i>	12.8	26.8	60.3	72.6	57.3	46.2	42.2	113.9	98.7	98.7	91.0	9.3	6.9	
NM_000584_at	<i>IL8</i>	4.3	6.1	72.3	219.0	281.2	218.1	44.7	108.4	8.5	11.6	6.3	9.2	11.4	Yes
NM_000963_at	<i>PTGS2</i>	13.6	3.7	6.6	286.6	336.4	235.0	40.5	340.2	111.5	181.1	155.1	3.1	21.6	Yes
NM_017585_at	<i>SLC2A6</i>	5.6	3.7	9.7	5.6	7.3	6.2	3.4	8.1	19.6	29.5	21.2	4.4	3.4	Yes
NM_001145099_at	<i>SLC2A6</i>	5.6	3.7	9.7	5.6	7.3	6.2	3.4	8.1	19.6	29.5	21.2	4.4	3.4	
NM_000636_at	<i>SOD2</i>	25.3	4.6	53.1	14.4	18.2	16.3	16.5	45.8	9.2	8.1	17.1	12.4	3.8	
NM_001024465_at	<i>SOD2</i>	27.7	4.8	64.4	28.4	36.7	34.0	24.4	59.6	9.6	7.7	15.8	16.6	3.9	Yes
NM_001024466_at	<i>SOD2</i>	28.8	5.0	64.9	27.3	36.8	33.3	23.7	50.9	9.6	7.6	15.7	15.2	3.8	
NM_007115_at	<i>TNFAIP6</i>	514.0	105.9	264.4	395.0	386.7	435.6	12.9	116.8	87.3	128.4	104.6	86.5	9.8	Yes
NM_001128843_at	<i>TNIP3</i>	4.0	12.8	18.9	228.4	202.1	71.3	17.4	7.0	11.0	14.9	3.3	3.8	5.3	
NM_024873_at	<i>TNIP3</i>	4.0	12.8	18.9	228.4	202.1	71.3	17.4	7.0	11.0	14.9	3.3	3.8	5.3	
NM_025079_at	<i>ZC3H12A</i>	3.9	3.3	4.5	18.3	16.3	6.6	6.0	6.5	7.9	6.5	5.4	3.4	5.2	Yes

^a Based on data from E-TABM-724 and E-TABM-879

REFERENCES

- [1] Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, et al. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. *Nucleic acids research*. 2005;33:e175.
- [2] Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP. GenePattern 2.0. *Nat Genet*. 2006;38:500-1.
- [3] Cook AL, Vitale AM, Ravishankar S, Matigian N, Sutherland GT, Shan J, et al. NRF2 activation restores disease related metabolic deficiencies in olfactory neurosphere-derived cells from patients with sporadic Parkinson's disease. *PLoS One*. 2011;6:e21907.
- [4] Matigian N, Abrahamsen G, Sutharsan R, Cook AL, Vitale AM, Nouwens A, et al. Disease-specific, neurosphere-derived cells as models for brain disorders. *Disease models & mechanisms*. 2010;3:785-98.
- [5] Pfaffl MW. Quantification strategies in real-time PCR. In: Bustin SA, editor. *A-Z of quantitative PCR*. La Jolla, CA, USA: International University Line; 2004. p. 87-112.
- [6] Murrell W, Feron F, Wetzig A, Cameron N, Splatt K, Bellette B, et al. Multipotent stem cells from adult olfactory mucosa. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2005;233:496-515.
- [7] Tegeder I, Adolph J, Schmidt H, Woolf CJ, Geisslinger G, Lotsch J. Reduced hyperalgesia in homozygous carriers of a GTP cyclohydrolase 1 haplotype. *Eur J Pain*. 2008;12:1069-77.
- [8] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263-5.