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Data in Brief

Transcriptional profiling of the postnatal brain of the Ts1Cje mouse model of Down syndrome

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ABSTRACT

The Ts1Cje mouse model of Down syndrome (DS) has partial trisomy of mouse chromosome 16 (MMU16), which is syntenic to human chromosome 21 (HSA21). It develops various neuropathological features demonstrated by DS patients such as reduced cerebellar volume [1] and altered hippocampus-dependent learning and memory [2,3]. To understand the global gene expression effect of the partially triplicated MMU16 segment on mouse brain development, we performed the spatiotemporal transcriptome analysis of Ts1Cje and disomic control cerebral cortex, cerebellum and hippocampus harvested at four developmental time-points: postnatal day (P)1, P15, P30 and P84. Here, we provide a detailed description of the experimental and analysis procedures of the microarray dataset, which has been deposited in the Gene Expression Omnibus (GSE49050) database.

Specifications	
Organism/cell line/tissue	*Mus musculus*/cerebral cortex, cerebellum and hippocampus
Strain(s)	Ts1Cje (on a C56BL/6 background) & disomic littermates (assumed to be C56BL/6)
Sex	Female
Sequencer or array type	Affymetrix Gene-Chip® Mouse Genome 430 2.0 arrays
Data format	Normalised data
Experimental factors	Ts1Cje vs disomic littermates; age (P1, P15, P30 and P84); brain regions (cerebral cortex, cerebellum and hippocampus)
Experimental features	72 microarray datasets encompass Ts1Cje vs disomic littermates, four postnatal time-points and three brain regions
Consent	n/a
Sample source location	n/a

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49050.

Experimental design, materials and methods

Experimental approach

Three main brain regions including the cerebral cortex, cerebellum and hippocampus were targeted in the study. Transcriptomes of these brain regions from 3 Ts1Cje and 3 disomic littermate control were compared at each of the following time-points: P1, P15, P30 and P84. The tissue samples were randomised prior to RNA extraction, quantitation of total RNA and quality/integrity, cRNA preparation and microarray hybridisation steps (Table 1). Fig. 1(A) is a simplified diagram of the experimental design and data processing flow/criteria used for the study.

Ts1Cje mouse breeding, ethics statement and genotyping

Ts1Cje and disomic mice were generated by mating Ts1Cje males (originally obtained from The Jackson Laboratory, Bar Harbour, USA)

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Table 1
Sample randomisation prior to RNA extraction, determination of total RNA sample quality/integrity, cRNA preparation and microarray hybridization steps.

Day	Week	Tube	Mouse ID	Strain	Age	Tissue	Accession ID
1	1	1	10.1.1	Disomic	P1	Hippocampus	GSM1193014
1	1	2	A40	Ts1Cje	P84	Cerebral cortex	GSM1193015
1	1	3	A104	Ts1Cje	P30	Cerebral cortex	GSM1193016
1	1	4	10.1.3	Ts1Cje	P1	Cerebellum	GSM1193017
1	1	5	A104	Ts1Cje	P30	Hippocampus	GSM1193018
1	1	6	A63	Disomic	P15	Cerebral cortex	GSM1193019
2	1	7	A40	Ts1Cje	P84	Cerebellum	GSM1193020
2	1	8	10.1.1	Disomic	P1	Cerebellum	GSM1193021
2	1	9	A41	Disomic	P84	Hippocampus	GSM1193022
2	1	10	A60	Ts1Cje	P15	Cerebral cortex	GSM1193023
2	1	11	A40	Ts1Cje	P84	Hippocampus	GSM1193024
2	1	12	10.1.3	Ts1Cje	P1	Hippocampus	GSM1193025
3	1	13	A103	Disomic	P30	Hippocampus	GSM1193026
3	1	14	A41	Disomic	P84	Cerebellum	GSM1193027
3	1	15	A63	Disomic	P15	Hippocampus	GSM1193028
3	1	16	A41	Disomic	P84	Cerebral cortex	GSM1193029
3	1	17	A60	Ts1Cje	P15	Hippocampus	GSM1193030
3	1	18	A104	Ts1Cje	P30	Cerebellum	GSM1193031
4	1	19	A103	Disomic	P30	Cerebellum	GSM1193032
4	1	20	10.1.3	Ts1Cje	P1	Cerebral cortex	GSM1193033
4	1	21	A60	Ts1Cje	P15	Cerebellum	GSM1193034
4	1	22	A103	Disomic	P30	Cerebral cortex	GSM1193035
4	1	23	A63	Disomic	P15	Cerebellum	GSM1193036
4	1	24	10.1.1	Disomic	P1	Cerebral cortex	GSM1193037
5	2	25	A44	Ts1Cje	P84	Hippocampus	GSM1193038
5	2	26	10.1.5	Ts1Cje	P1	Cerebellum	GSM1193039
5	2	27	A91	Disomic	P15	Hippocampus	GSM1193041
5	2	28	105	Disomic	P30	Cerebral cortex	GSM1193044
5	2	29	105	Disomic	P30	Hippocampus	GSM1193048
5	2	30	10.1.5	Ts1Cje	P1	Hippocampus	GSM1193049
6	2	31	A91	Disomic	P15	Cerebellum	GSM1193050
6	2	32	A33 3.2.4	Ts1Cje	P30	Cerebellum	GSM1193051
6	2	33	10.1.2	Disomic	P1	Cerebral cortex	GSM1193052
6	2	34	10.1.5	Ts1Cje	P1	Cerebral cortex	GSM1193053
6	2	35	10.1.2	Disomic	P1	Cerebellum	GSM1193054
6	2	36	A92	Ts1Cje	P15	Cerebral cortex	GSM1193055
7	2	37	10.1.2	Disomic	P1	Hippocampus	GSM1193056
7	2	38	A33 3.2.4	Ts1Cje	P30	Hippocampus	GSM1193057
7	2	39	A42/43	Disomic	P84	Cerebellum	GSM1193058
7	2	40	A91	Disomic	P15	Cerebral cortex	GSM1193059
7	2	41	A42/43	Disomic	P84	Cerebral cortex	GSM1193060
7	2	42	A92	Ts1Cje	P15	Cerebellum	GSM1193061
8	2	43	105	Disomic	P30	Cerebellum	GSM1193062
8	2	44	A42/43	Disomic	P84	Hippocampus	GSM1193063
8	2	45	A92	Ts1Cje	P15	Hippocampus	GSM1193064
8	2	46	A44	Ts1Cje	P84	Cerebral cortex	GSM1193065
8	2	47	A33 3.2.4	Ts1Cje	P30	Cerebral cortex	GSM1193066
8	2	48	A44	Ts1Cje	P84	Cerebellum	GSM1193067
9	3	49	A76	Ts1Cje	P15	Cerebellum	GSM1193068
9	3	50	A34 3.2.5	Ts1Cje	P30	Hippocampus	GSM1193069
9	3	51	10.1.9	Ts1Cje	P1	Cerebellum	GSM1193070
9	3	52	106	Disomic	P30	Cerebellum	GSM1193071
9	3	53	A49	Ts1Cje	P84	Hippocampus	GSM1193072
9	3	54	A76	Ts1Cje	P15	Hippocampus	GSM1193073
10	3	55	A76	Ts1Cje	P15	Cerebral cortex	GSM1193074
10	3	56	10.1.7	Disomic	P1	Hippocampus	GSM1193075
10	3	57	106	Disomic	P30	Cerebral cortex	GSM1193076
10	3	58	A49	Ts1Cje	P84	Cerebellum	GSM1193077
10	3	59	A50	Disomic	P84	Hippocampus	GSM1193078
10	3	60	A49	Ts1Cje	P84	Cerebral cortex	GSM1193079
11	3	61	A50	Disomic	P84	Cerebellum	GSM1193080
11	3	62	10.1.9	Ts1Cje	P1	Cerebral cortex	GSM1193081
11	3	63	A34 3.2.5	Ts1Cje	P30	Cerebral cortex	GSM1193082
11	3	64	10.1.7	Disomic	P1	Cerebral cortex	GSM1193083
11	3	65	A34 3.2.5	Ts1Cje	P30	Cerebellum	GSM1193084
11	3	66	A75	Disomic	P15	Cerebral cortex	GSM1193085
12	3	67	A75	Disomic	P15	Hippocampus	GSM1193086
12	3	68	10.1.7	Disomic	P1	Cerebellum	GSM1193087
12	3	69	A75	Disomic	P15	Cerebellum	GSM1193088
12	3	70	10.1.9	Ts1Cje	P1	Hippocampus	GSM1193089
12	3	71	106	Disomic	P30	Hippocampus	GSM1193090
12	3	72	A50	Disomic	P84	Cerebral cortex	GSM1193091

with C57BL/6 female mice for over 10 generations. All mice were kept in a controlled environment of 12-h light/12-h dark cycle with unlimited access to a standard pellet diet and water. Breeding procedures, husbandry and all experiments were performed under the approval from the Walter and Eliza Hall Institute Animal Ethics Committee (Project numbers 2001.45, 2004.041 and 2007.007). Genomic DNA was extracted from mouse-tails and genotyping was performed using multiplex PCR with primers for neomycin (*neo*) and the glutamate receptor, ionotropic, kainite 1 (*Grik1*) as an internal control as described previously [4].

Tissue procurement

Three female Ts1Cje mice at four time-points (P1, P15, P30 and P84) with sex and age matched disomic littermates were used to avoid the effects of Y-linked genes such as *Sry* (sex-determining region of the Y chromosome), which contribute to neural sexual differentiation of the brain [5]. All mice were euthanized via cervical dislocation. Procurement of the cerebral cortex, cerebellum and hippocampus was conducted according to a method described previously [6].

RNA extraction and microarray hybridisation

The Qiagen RNeasy Micro kit (Qiagen) with a DNase I digestion step was used to extract total RNA from each tissue according to the manufacturer's instructions. All 72 tissues were randomised prior to RNA extraction to avoid biases (Table 1). The quality and quantity of each RNA sample were assessed using an Agilent 2100 Bioanalyzer (Agilent). The RNA Integrity Number (RIN) ranged from 7.0 to 10. Six micrograms of total RNA was used to prepare biotinylated cRNA according to the standard Affymetrix protocol (Expression Analysis Technical Manual, 2001, Affymetrix). Hybridisation of labelled RNA samples onto Affymetrix GeneChip Mouse Genome 430 2.0 Arrays was performed according to the Australian Genome Research Facility (AGRF) protocol. A probe cocktail (cRNA at 0.05 μg/μl), which included 1× Hybridisation Buffer (100 mM MES, 1 M NaCl, 20 mM EDTA, 0.01% Tween-20), 0.1 mg/ml Herring Sperm DNA, 0.5 mg/ml BSA, and 7% DMSO was prepared to a total of 300 μl for each sample and 200 μl was hybridised onto a single GeneChip. The chips were incubated at 45 °C for 16 h in an oven with a rotating wheel at 60 rpm, washed and stained with streptavidin-phycoerythrin (SAPE) using the appropriate fluidics script on the Affymetrix Fluidics Station 450 (Affymetrix). The GeneChips were scanned using a GeneChip Scanner 3000® (Affymetrix) with GeneChip® Operating Software (GCOS). Fig. 1(B) shows a simplified diagram of the sample preparation.

Microarray data normalisation and analysis

The microarray data was analysed using R (www.r-project.org) and Bioconductor (www.bioconductor.org) [7]. The probe-level intensities for the 72 arrays were background corrected, normalised and summarised using the GC Robust Multi-array Average (GC-RMA) algorithm [8] to obtain gene (probe-set) level summaries (see Supplementary File 1 for GC-RMA script used). Differential expression between Ts1Cje and their disomic littermates at different time-points and in different brain regions was assessed using the limma package [9]. A linear model was fitted for multiple contrasts (corresponding to the Ts1Cje vs disomic comparisons) for each gene using the lmFit procedure and differential expression was

Notes to Table 1:
Note: 'Day' and 'Week' refer to the different days in different weeks on which RNA was extracted from these samples. All samples were relabelled with a set of continuous numbers under the 'Tube' column. 'Mouse ID' refers to the internal identification number used in the animal facilities where these mice were bred and maintained. 'Accession ID' refers to GEO sample ID deposited under the GSE49050 data series. Within the 'Strain' column, Ts1Cje denotes mouse model of Down syndrome mice. Under the 'Age' column, P denotes 'Postnatal Development Day'.

assessed using empirical Bayes moderated *t*-statistics [10]. P-values corresponding to the moderated *t*-statistics were adjusted for multiple testing using the false discovery rate (FDR) procedure of Benjamini and Hochberg [11]. Fig. 1(C) shows a simplified diagram of the microarray analysis.

Stringent criteria were applied to identify differentially expressed genes (DEGs) from the datasets, which included *t*-statistic values of ≥4 or ≤ -4 and a FDR of ≤0.05. As reported in Ling et al. [12], a total number of 317 DEGs were identified from all spatiotemporal comparisons. A top-down screening approach was then used to analyse the 317 DEGs in order to identify any disrupted molecular pathways. Initially, a functional ontology clustering analysis based on all 317 DEGs collectively using the Database for Annotation, Visualisation and Integrated Discovery (DAVID) [13] was performed. The functional clustering analysis was performed under a stringent classification criteria (a kappa similarity threshold of 0.85, a minimum term overlap of three, two initial and final group membership with a 0.50 multiple linkage threshold and a modified Fisher-exact P-value or enrichment thresholds of 0.05) using the following databases: Biological Biochemical Image Database (BBID), BioCarta database, EC_number, Kyoto Encyclopedia of Genes and Genomes (KEGG) database, PANTHER pathway database and Reactome pathway database [13]. Subsequently, a more refined analysis was carried out involving the DEGs identified from the comparisons that were based on a specific time-point or brain region. Finally, the significant ontologies identified through all analyses were manually curated based on common genes that were found involved in the

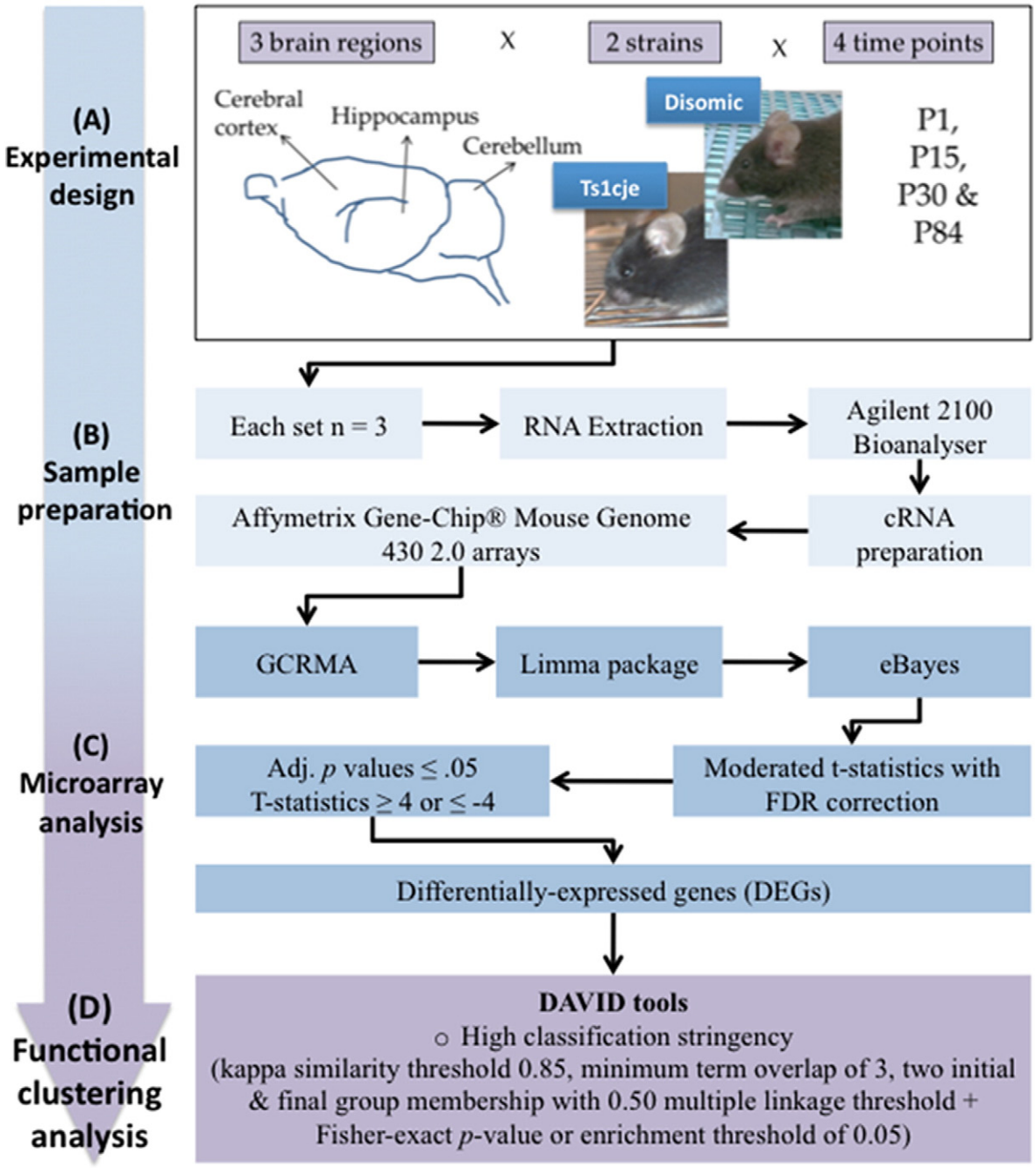

Fig. 1. Outline of experimental design (A), sample preparation (B), microarray data analysis (C), and functional clustering analysis using DAVID tools (D). Ts1Cje denotes Down syndrome mice, cRNA denotes complementary RNA, GCRMA denotes Guanine Cytosine Robust Multi-Array Analysis, FDR denotes false discovery rate, DAVID denotes Database for Annotation, Visualization and Integrated Discovery.

ontologies leading to the identification of 7 significant functional clusters. Fig. 1(D) shows a simplified diagram of the functional clustering analysis.

Discussion

Here we provide a detailed description of the generation of a 72 microarray dataset, which is comprised of transcriptome profiling data derived from three brain regions, at four postnatal time-points from the Ts1Cje mouse model of DS and disomic littermates. The strategy used to identify DEGs between the Ts1Cje and disomic littermate data and functional clustering analysis is also described. This comprehensive and well-controlled microarray dataset encompasses postnatal developmental stages from P1 to P84 in the cerebral cortex, cerebellum and hippocampus providing a platform to understand the differences between the Ts1Cje and disomic mouse brain in these regions at a transcriptome level. The analysis of the dataset was fully described and discussed in the study by Ling et al. [12], which demonstrated that the interferon-related pathways were significantly dysregulated in the Ts1Cje brain as compared to their disomic littermates.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2014.09.009.

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