PLoS one

Functional Gene-Expression Analysis Shows Involvement of Schizophrenia-Relevant Pathways in Patients with 22q11 Deletion Syndrome

Nico J. M. van Beveren[1,2,6]*, Lianne C. Krab[2], Sigrid Swagemakers[3], Gabriella Buitendijk[2], Erik Boot[8], Peter van der Spek[3], Ype Elgersma[2], Therese A. M. J. van Amelsvoort[4,5,7]

1 Department of Psychiatry, Erasmus University Medical Centre, Rotterdam, The Netherlands, 2 Department of Neuroscience, Erasmus University Medical Centre, Rotterdam, The Netherlands, 3 Department of Bioinformatics, Erasmus University Medical Centre, Rotterdam, The Netherlands, 4 Mondriaan Mental Health Care, Heerlen, The Netherlands, 5 Department of Psychiatry and Psychology, Maastricht University, Maastricht, The Netherlands, 6 Delta Center for Mental Health Care, Rotterdam, The Netherlands, 7 Virenze Mental Health Care, Gronsveld, The Netherlands, 8 Department of Psychiatry, Academic Medical Centre, Amsterdam, The Netherlands

Abstract

22q11 Deletion Syndrome (22q11DS) is associated with dysmorphology and a high prevalence of schizophrenia-like symptoms. Several genes located on chromosome 22q11 have been linked to schizophrenia. The deletion is thought to disrupt the expression of multiple genes involved in maturation and development of neurons and neuronal circuits, and neurotransmission. We investigated whole-genome gene expression of Peripheral Blood Mononuclear Cells (PBMC's) of 8 22q11DS patients and 8 age- and gender-matched controls, to (1) investigate the expression levels of 22q11 genes and (2) to investigate whether 22q11 genes participate in functional genetic networks relevant to schizophrenia. Functional relationships between genes differentially expressed in patients (as identified by Locally Adaptive Statistical procedure (LAP) or satisfying $p < 0.05$ and fold-change > 1.5) were investigated with the Ingenuity Pathways Analysis (IPA). 14 samples (7 patients, 7 controls) passed quality controls. LAP identified 29 deregulated genes. Pathway analysis showed 262 transcripts differentially expressed between patients and controls. Functional pathways most disturbed were cell death, cell morphology, cellular assembly and organization, and cell-to-cell signaling. In addition, 10 canonical pathways were identified, among which the signal pathways for Natural Killer-cells, neurotrophin/Trk, neuregulin, axonal guidance, and Huntington's disease. Our findings support the use of 22q11DS as a research model for schizophrenia. We identified decreased expression of several genes (among which COMT, Ufd1L, PCQAP, and GNB1L) previously linked to schizophrenia as well as involvement of signaling pathways relevant to schizophrenia, of which Neurotrophin/Trk and neuregulin signaling seems to be especially notable.

Introduction

Deletions of chromosome 22q11 are associated with a high prevalence of dysmorphology (velo-cardio-facial syndrome; 22q11DS), cognitive and behavioural disorders and schizophrenia-like psychosis [1,2]. It is thought that a hemizygous 22q11 deletion disrupts the expression of multiple genes involved in maturation, development of neurons and neuronal circuits, and neurotransmission [3]. Several genes located in the 22q11 region have been linked to schizophrenia, including COMT, ProDH, Ufd1L, PCQAP [4], and, recently, GNB1L [5]. It is likely that genes present in the 22q11 deleted region form functional networks (i.e. metabolic-and signaling pathways) with other genes outside this region involved in development and neuronal functioning, and that the decreased expression of 22q11 genes alters the functional activity of these pathways. It is however unclear which pathways these are, and what their relationship is with pathways involved in idiopathic schizophrenia. More insight

into the nature of these functional networks may increase our understanding in the genetic networks involved in the developmental, psychiatric, cognitive and behavioural disturbances seen in 22q11DS including schizophrenia. Moreover, it will provide more understanding of the relationship between 22q11DS and schizophrenia and the value of 22q11DS for investigating schizophrenia pathology.

The combination of gene expression profiling and biomics is proven to be a powerful technology to identify functional changes in genetic networks [6]. However, for neuropsychiatric disorders a major limitation is the inaccessibility of the living brain and the heterogeneity of many psychiatric disorders. This is especially relevant for disorders such as schizophrenia, which show a prolonged life-long course during which the effects of normal and abnormal brain development, drug abuse and medication influence the results of post-mortem gene-expression studies [6,7]. Peripheral Blood Mononuclear Cells (PBMC's) express many brain relevant genes [8] and have been suggested as

'window-on-the-brain' surrogate tissue to investigate neuropsychiatric disorders. In schizophrenia, a number of recent studies have explored the use of various types of PBMC's [9–13]. The key assumption of these studies is that although peripheral blood cells in schizophrenia show no clear aberrations in function or morphology, subtle molecular-biological aberrations are present and can be informative about the molecular-biological underpinnings of schizophrenia. However, in non-22q11DS schizophrenia a multivariate genetic and environmental cause is suspected. Therefore PBMC gene expression of patients with idiopathic schizophrenia may be largely variable. We reasoned that in 22q11DS the deletion will most likely result in decreased PBMC expression of 22q11 genes which may in turn influence the expression of other genes outside the deleted region more robustly and persistently than in idiopathic schizophrenia. Furthermore, using functional pathway analysis, 22q11DS might offer a view of the concerted altered expression of genes both within and without the 22q11 deleted region that may alter the activity of functional genetic networks.

PBMC gene expression in 22q11DS patients has not yet been investigated, neither the individual expression levels of genes in the 22q11 deleted region, nor the influence of the deletion on functional genetic pathways. Thus, we investigated gene expression in PBMC's of 22q11DS patients, with the objective to (1) investigate the expression levels of 22q11 genes and to (2) investigate whether 22q11 genes, together with genes outside this region, participate in functional networks relevant for (neuro)developmental abnormalities in 22q11DS.

Materials and Methods

Subjects

8 22q11DS patients and 8 age-and gender matched controls were included in this study. Deletions at chromosome 22q11 were previously identified in all subjects by Fluorescence In-Situ Hybridization (FISH). This study was approved by the institutional review boards of the two participating centres (Erasmus University Medical Centre, Rotterdam, and Academic Medical Centre, Amsterdam) and was performed in accordance with the declaration of Helsinki. All subjects provided written informed consent.

Samples

From each participant 30 ml of blood was drawn into heparinized tubes. PBMC's were isolated by Ficoll-gradient separation starting 90 minutes after the drawing of blood. Cells were subsequently disrupted (Qiashredder kit; Qiagen), and RNA was isolated (RNeasy minikit; Qiagen) with an additional DNAse digestion step (RNase-free DNase set; Qiagen), all according to the manufacturer's protocol, diluted in nuclease free water and frozen at −80 C before use. After thawing the isolated RNA was biotinylated into cRNA using the One-Cycle Target Labeling and Control Reagents Kit (Affymetric Co) according to the manufacturer's protocol. Before hybridization RNA quality and integrity was assessed using the Agilent 2100 BioAnalyzer (Agilent) and RNA purity and quantity with the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Biotinylated cRNA was hybridized to the Affymetrix Human Genome U133 plus 2.0 GeneChip© microarray containing 54,675 probe sets (Affymetrix Co). Each sample was individually biotinylated and hybridized to an individual microarray. Biotinylation was performed in two batches with randomization of samples across the batches. Hybridization was performed in one batch. The arrays were scanned and analyzed using Affymetrix Microarray Suite 4.2 software.

Statistical analysis

Probe sets that were absent in all samples (according to Affymetrix software) were omitted from further analysis. Raw intensities (33,196 probe sets) were normalized by quantile normalization. Data analysis was done using OmniViz version 5.0 (Biowisdom) and R program. Minimum thresholds were set at 30. To investigate the expression levels of genes in the 22q11 region between patients and controls we applied two separate methods, Significance Analysis of Microarrays (SAM) [14] (OmniViz) and Locally Adaptive Statistical procedure (LAP) [15] (R program). SAM uses permutations of the repeated measurements of the expression levels to estimate the false discovery rate (FDR). LAP combines a FDR approach with information about chromosomal location of the genes and is specifically suited to identify differentially expressed regions that are involved in known chromosomal aberrations [15]. It should be noted that LAP may identify significantly decreased expression of genes which are actually not expressed at all, due to way the LAP algorithm uses information about chromosomal location. These not expressed genes were removed from further analyses. To investigate the functional relationships between genes which are differentially expressed between patients and controls we used the Ingenuity Pathway Analysis (IPA) (Ingenuity Systems®; www.ingenuity.com; October 2007). The IPA is an internet-accessible database in which knowledge about the interaction between genes and gene products is stored based on known interactions in the literature [16]. Thus, the IPA defines genetic networks, functions, and metabolic-and signaling pathways which describe functional relationships between gene products and presents as output networks, functions and metabolic-and signaling pathways in which the genes in the dataset participate more than can be expected by change. It does so by calculating a significance score (using the right-tailed Fisher's exact test and expressed as a p-value) for each process by computing the number of deregulated genes that participate in a network or pathway relative to the total number of occurrences of these genes in all functional/pathway annotations stored in the IPA. The significance value associated with networks and pathways is a measure for how likely it is that genes from a dataset participate in that function or pathway. For genetic networks an IPA score >= 3 is considered significant, for canonical pathways a p-value<.05 is considered significant. We uploaded into the IPA a dataset which consisted of the genes identified by LAP and of genes with different expression levels between patients and controls according to the criteria: t-test between patients and controls significant (p<0.05) and a 1.5 or more fold-change (FC) in expression level in patients compared to controls.

Results

Subjects

8 22q11DS patients and 8 controls were included. Four patients had a previous history of psychotic symptoms, whereas four did not (further characteristics see **table 1**).

Routine quality controls of the gene expression results showed sub-optimal signal-to-noise ratios in two subjects (one patient with psychosis and one control). These two samples were excluded from further analyses.

Global significance analysis of gene expression

SAM showed 14 transcripts differentially expressed between patients and controls (False Discovery Rate (FDR) = 6.6%; falsely

Table 1. Subject characteristics.

Pair #	PATIENTS				CONTROLS	
	Psychosis	**Antipsychotic use**	**Age**	**gender**	**Age**	**gender**
1	No	none	18	F	19	F
2	No	none	39	F	39	F
3	No	none	25	M	25	M
4	No	none	17	F	18	F
5	Yes	zuclopentixole	27	M	27	M
6	yes*	risperidone	32	F	30*	F
7	Yes	risperidone	25	M	26	M
8	Yes	risperidone	21	M	21	M

*excluded cases (N = 2) because of bad signal-to-noise ratio of the expression data; coincidentally, the excluded cases form a matched pair.
doi:10.1371/journal.pone.0033473.t001

called<1) (see **table 2**). Ten of these transcripts are located in the 22q11 region.

LAP identified 44 genes (p value<0.05), all in the 22q11 region (see **figures 1 and 2**).

Of these 44 genes, 9 were not expressed in blood and 6 were not differentially expressed between patients and controls, resulting in 29 differentially expressed genes.

Pathway analysis

The criteria p<0.05 and FC>1.5 showed 262 transcripts differentially expressed between patients and controls. These 262 transcripts are described in **table S1**. Combining these with the genes identified by LAP (n = 29) and uploading these in the IPA generated 128 genes available for building networks and investigating relationships. Seven functional networks are considered to be significantly associated with the uploaded gene set according to IPA criteria (IPA score>3) These networks are involved in (among others) cardiovascular disease, cardiovascular system development and function, nervous system development and function, connective tissue development and function, cell

signaling, and cell-to-cell signaling and interaction. **Table 3** shows the seven highest ranking networks, and the biological processes associated with these networks.

Canonical pathways identified by the IPA in which the uploaded gene set participates more than can be expected by chance (p<.05) are Natural Killer (NK)-cell signaling (p = .0004), neurotrophin/Trk signaling (p = .003), Fibroblast Growth Factor (FGF) signaling (p = .006), leukocyte extravasation signaling (p = .007), neuregulin signaling (p = .002), complement and coagulation cascades (p = .002), Platelet Derived Growth Factor (PDGF) signaling (p = .003), ERK/MAPK signaling (p = .003), axonal guidance signaling (p = .047), and Huntington's disease signaling (p = .049).

Comparison of PBMC expression levels with brain expression levels in mouse models

The Df1/+ heterozygous mice (Df1/+), a model for 22q11DS, displays specific deficits in hippocampus-dependent functions. Sivagnanasundaram et al [17] analyzed the hippocampal gene expression of genes mapping to the deleted region as compared to wild type (WT) mice. Twelve genes were differentially expressed in the hippocampus. Of these twelve genes seven were also expressed in our PBMC samples (DGCR6, RANBP1, ZDHHC8, HTF9C, COMT, CLDN5, and UFD1L). The relative expression levels of these genes (hippocampal levels of Df1/+ vs WT mice (after Sivagnanasundaram et al [17]) and PBMC levels of 22q11DS patients vs controls) are shown in **table 4**.

The relative expression levels of mice and humans correlated strongly and significantly (r: 0.677, p = 0.05, one sided). In another study, Meechan et al [18] investigated the expression levels of nine 22q11 orthologues in a 22q11DS mouse model (IDD, PRODH2, ZDHHC8, RANBP1, T10, COMT-MB, TBX1, UFD1L, and HIRA) in the developing and adult mouse brain. They also found diminished expression for the entire set of orthologues. Their findings indicated a fairly consistent decrease in expression levels with a magnitude between 40% and 60%, quite similar to our PBMC findings. Taken together, these results in mice cautiously suggest that diminished expression levels of 22q11 genes in PBMC's in humans might reflect decreased expression levels in the mouse brain for those genes which are also expressed in neuronal tissue.

Table 2. Significance Analysis of Microarrays (SAM).

Probe Set ID	Gene Title	Gene Symbol	Entrez Gene	Chromosomal Location
229906_at	Armadillo repeat containing 7	ARMC7	79637	chr17q25.1
217427_s_at	HIR histone cell cycle regulation defective homolog A (S. cerevisae)	HIRA	7290	chr22q11.2| chr22q11.21
1553974_at	Hypothetical protein 1	LOC128977	128977	chr22q11.2
202483_s_at	RAN binding protein 1	RANBP1	5902	chr22q11.2
203152_at	Mitochondrial ribosomal protein L40	MRPL40	64976	chr22q11.2
209103_s_at	Ubiquitin fusion degradation 1 like (yeast)	UFD1L	7353	chr22q11.2
210010_s_at	Solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1	SLC25A1	6576	chr22q11.2
32032_at	DiGeorge syndrome critical region gene 14	DGCR14	8220	chr22q11.2| chr22q11.2
208818_s_at	Catechol-O-methyltransferase	COMT	1312	chr22q11.2-q11.23
206184_at	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	CRKL	1399	chr22q11.2| chr22q11.21
212180_at	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	CRKL	1399	chr22q11.2| chr22q11.2
202206_at	ADP-ribosylation factor-like 4C	ARL4C	10123	chr2q37.1
235289_at	Eukaryotic translation initiation factor 5A2	EIF5A2	56648	chr3q26.2
230685_at	Hypothetical protein LOC644873	FLJ33630	644873	chr5q23.3

doi:10.1371/journal.pone.0033473.t002

gene.id	locuslink	symbol	score	smoothed.score	chromosome	gene.map	gene.position	q.value	median CO	median VCF	VCF/CO	log2 VCF/co
219180_s_at	55670	PEX26	0.31485	1.793781252	22	22q11.21	16940704	0	87.6	86.7	0.989726	-0.0149
220069_at	51807	TUBA8	0.981132	1.917861395	22	22q11.21	16973558	0	30	30	1	0
219211_at	11274	USP18	0.446857	2.066129479	22	22q11.21	17012757	0	76	65.1	0.856579	-0.22334
215603_x_at	2679	GGT2	-0.02505	2.523903834	22	c("22q11.2	17141174	0	62.9	66.1	1.050874	0.07159
208024_s_at	8214	DGCR6	4.862316	2.949790973	22	c("22q11.2	17273778	0	300.4	147.4	0.490679	-1.02715
227028_s_at	9993	DGCR2	3.990962	3.346799347	22	22q11.21	17403800	0	38.9	30	0.771208	-0.37481
239730_at	26221	DGCR13	0.928571	3.624496017	22	22q11	17497792	0	30	30	1	0
204383_at	8220	DGCR14	14.39046	3.634961642	22	c("22q11.2	17501451	0	134	83.1	0.620149	-0.68931
210010_s_at	6576	SLC25A1	6.782165	3.750364612	22	22q11.21	17543094	0	145.7	67.4	0.462594	-1.11218
240451_at	7290	HIRA	7.138001	4.105658037	22	c("22q11.2	17698223	0	41.8	30	0.717703	-0.47854
203152_at	64976	MRPL40	8.159832	4.260705025	22	22q11.21	17800035	0	400.8	194.9	0.486277	-1.04015
1553974_at	128977	LOC12897	12.51765	4.273502747	22	22q11.21	17810894	0	309.2	139.5	0.451164	-1.14828
209103_s_at	7353	UFD1L	7.190132	4.281149677	22	22q11.21	17817701	0	822.9	429.7	0.522178	-0.93739
204482_at	7122	CLDN5	0.416221	4.347187431	22	22q11.21	17890549	0	54	30.5	0.564815	-0.82415
206655_s_at	2812	GP1BB	1.82882	4.422411971	22	c("22q11.2	18091065	0	184.1	136.2	0.739815	-0.43476
207662_at	6899	TBX1	0	4.419058183	22	22q11.21	18124225	0	30	30	1	0
226204_at	54584	GNB1L	5.191292	4.40950642	22	22q11.2	18155939	0	103.3	69.7	0.674734	-0.56761
219560_at	79680	C22orf29	1.410881	4.373725262	22	22q11.21	18213670	0	37.1	30	0.808625	-0.30646
210803_at	10587	TXNRD2	4.941368	4.347271321	22	22q11.21	18243039	0	30	30.1	1.003333	0.004801
208818_s_at	1312	COMT	6.993038	4.275342606	22	c("22q11.2	18309308	0	849.2	461.3	0.543217	-0.8804
235396_at	128989	C22orf25	4.892061	4.161717579	22	22q11.21	18388630	0	244.9	181.4	0.74071	-0.43302
218650_at	54487	DGCR8	3.752243	4.0744775	22	22q11.2	18447833	0	120.9	102.7	0.849462	-0.23538
218475_at	27037	HTF9C	4.688983	4.030278781	22	c("22q11.1	18479397	0	63.4	30	0.473186	-1.07952
202483_s_at	5902	RANBP1	5.80296	4.022109915	22	22q11.21	18485023	0	715.2	287	0.401286	-1.3173
225744_at	29801	ZDHHC8	4.862845	4.000747456	22	22q11.21	18499364	0	112.9	71.3	0.631532	-0.66307
228550_at	65078	RTN4R	1.149472	3.832453675	22	22q11.21	18608939	0	30	30	1	0
205881_at	7625	ZNF74	2.551791	3.174154163	22	c("22q11.2	19078477	0	49.4	30	0.607287	-0.71955
221838_at	84861	KLHL22	4.842764	3.127260579	22	22q11.21	19125805	0	88.2	58.7	0.665533	-0.58742
221175_s_at	51586	PCQAP	3.315321	3.044264972	22	22q11.2	19191885	0	319.7	228.4	0.71442	-0.48516
223628_at	84222	DKFZp434	0.899982	2.722664167	22	22q11.21	19385401	0	56.6	53.7	0.948763	-0.07588
207081_s_at	5297	PIK4CA	4.708394	2.709945032	22	22q11.21	19391990	0	410.5	239	0.582217	-0.78037
218327_s_at	9342	SNAP29	3.712754	2.398500785	22	22q11.21	19543291	0	309.4	154.1	0.498061	-1.00561
212180_at	1399	CRKL	6.762857	2.285366627	22	c("22q11",	19601713	0	658.4	332.6	0.505164	-0.98518
244084_at	150209	AIFL	0.948051	2.19852904	22	22q11.21	19649433	0	60.9	48	0.788177	-0.34341
203412_at	8216	LZTR1	3.832558	2.169034501	22	c("22q11.2	19666655	0	137.6	98.6	0.71657	-0.48082
218492_s_at	80764	THAP7	5.002873	2.140414446	22	22q11.2	19684060	0	215.3	123.8	0.575012	-0.79834
206880_at	9127	P2RXL1	-0.19305	2.11589133	22	22q11.21	19699448	0	31.4	34	1.082803	0.11477
233374_at	23119	HIC2	-1.45296	1.454573723	22	22q11.21	20101692	0	30	30	1	0
213408_s_at	220686	LOC22068	2.219998	1.357961705	22	22q11.21	20157288	0	1127.7	926.3	0.821406	-0.28383
200682_s_at	7332	UBE2L3	1.152725	1.200855685	22	22q11.21	20251956	0.026938	2243.6	2215.5	0.987475	-0.01818
227042_at	150223	LOC15022	-0.14804	1.111671641	22	22q11.21	20312380	0.048977	144.3	143	0.990991	-0.01306
218681_s_at	23753	SDF2L1	1.915884	1.092181796	22	22q11.21	20326541	0.048977	214.4	183.6	0.856343	-0.22374
214986_x_at	23759	PPIL2	-1.20472	1.060760572	22	22q11.21	20350275	0.048977	123.6	111.4	0.901294	-0.14993
213996_at	29799	YPEL1	3.27043	1.021279608	22	22q11.2	20381825	0.048977	218.4	145.2	0.664835	-0.58893

Figure 1. Results of the Locally Adaptive Statistical Procedure (LAP). Green rows show genes which are not expressed in PBMC's; red rows show genes not differentially expressed in PBMC's between patients and controls. Columms respectively show (from left to right): Probe Set ID; Entrez Gene ID; Gene Symbol; SAM score (d_i); smoothed score (S_i); chromosomal localization and position; qvalues; median intensity of the controls (CO); median intensity of the 22q11DS patients; ratio 22q11DS/CO and log2 ratio 22q11DS/CO.
doi:10.1371/journal.pone.0033473.g001

Discussion

To our best knowledge our study is the first to examine gene expression of 22q11DS patients. We show decreased expression of several genes present in the 22q11 deleted region. Among these are the genes which have been previously associated with schizophrenia, COMT, Ufd1L, PCQAP [4], and GNB1L [5]. Canonical pathway analyses show the significant involvement of the canonical pathways NK-cell signaling, neurotrophin/Trk signaling, FGF signaling, leukocyte extravasation signaling, neuregulin signaling, complement and coagulation cascades, PDGF signaling, ERK/MAPK signaling, axonal guidance signaling, and Huntington's disease signaling. The phenotypical expression of 22q11DS is highly variable. Among the major phenotypic features encountered are, apart from schizophrenia, cardiac anomalies, immunodeficiencies, craniofacial defects,

hypocalcaemia, as well as a broad range of other psychiatric, cognitive and behavioral problems [19]. The most significantly involved canonical pathway in our study is NK-cell signaling. Immunodeficiency is one of the key features of 22q1DS, occurring secondary to thymic aplasia or hypoplasia with subsequent impaired thymocyte development. The majority of patients present with low or absent T-lymphocytes, but with spared B-lymphocytes and NK cells. An increased proportion of NK-cells have been described in patients with 22q11DS [20–22].However, the spectrum of severity of the immune deficit varies widely and does not appear to be related to other phenotypic abnormalities [23].Altered NK-cell activity in idiopathic schizophrenia has been reported in some studies [24] and is hypothesized to be related to the altered immunity and the reduced occurrence of autoimmune diseases and malignancies that has been observed in schizophrenia [25]. However, generally there is no clear agreement among
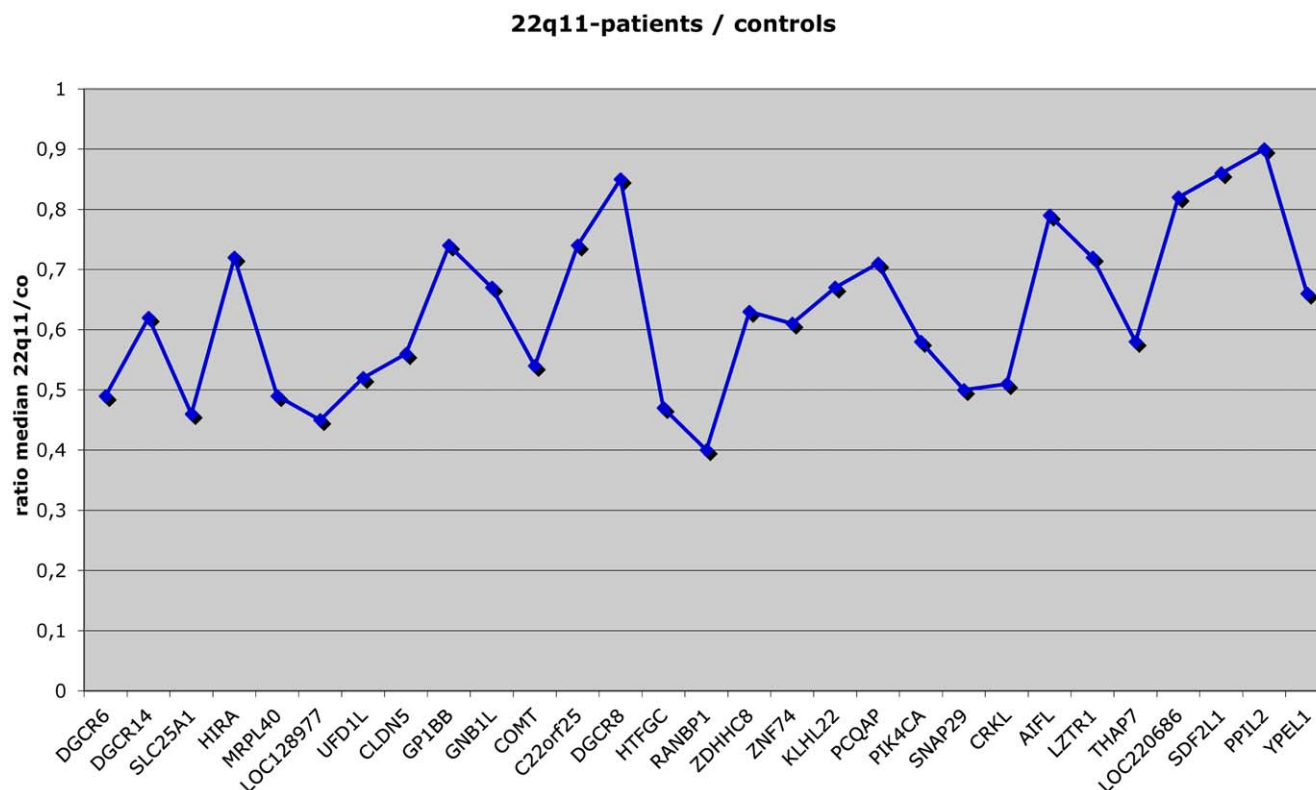
22q11-patients / controls

Figure 2. Expression levels of genes in the 22q11 deleted region. The figure shows the ratio of the median expression levels of patients versus controls for each of the genes shown along the x-axis.
doi:10.1371/journal.pone.0033473.g002

studies on the involvement of NK cell activity in schizophrenia, with some studies showing lower activity, some higher activity, and the majority no change in activity between schizophrenia patients and controls [24]. Alterations in NK signaling as observed in our patient sample may be related to these previously reported immune deficits; however, it should be kept in mind that the finding of immune perturbations could also be a confounding epiphenomenon of the specific tissue under investigation.

There is far more support for the involvement of neurotrophin/ Trk signaling in schizophrenia. This pathway is activated via the Trk family of receptors by various neurotrophic factors. The neurotrophic factors best known for their association with schizophrenia are Brain-derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), and neurotrophin-3 (NT-3). BDNF, NGF, and NT-3 are involved in a range of neuroplastic processes. Disturbed neurotrophin signaling is thought to underlie the neurodevelopmental disturbances seen in schizophrenia. Altered brain-(reviewed in Shoval and Weizmann [26]) and serum levels (reviewed in van Beveren et al ([27]) of neurotrophins have repeatedly been reported in schizophrenia.

There is also considerable support for an association of neuregulin with schizophrenia. Neuregulin is considered one of the genes most strongly associated with schizophrenia [28–30]. Neuregulin has been implicated in neuronal differentiation and migration. However, no consistent changes in neuregulin expression have been detected in schizophrenia. A recent report has shown that genetic variation in neuregulin (NRG1) is associated with variance in white matter brain content in normal individuals [31], as investigated by magnetic resonance imaging. Barnes et al [31] showed that genetic variation in the neuregulin variant SNP8NRG243177 is associated with variation in frontal

brain structure in both grey and white matter. Reduced global white matter, most prominently in posterior and temporal regions, has been reported in 22q11DS [32–34]. So, altered neuregulin signaling as observed in our 22q11DS sample may be related to dysmaturation of white matter. Disturbances in axonal guidance signaling and Huntington's disease signaling are at present not specifically associated with schizophrenia but may underlie the general cognitive and behavioural disturbances found in schizophrenia. Moreover, in 22q11DS frequently motor disturbances, obsessive-compulsive symptoms, tics, and neurological aberrations are being observed [25], which are also found in Huntington's disease, and recent studies suggest that cognitive deterioration in a 22q11DS may already start in childhood [35], with a dramatic cognitive decline in adulthood in some psychotic 22q11DS patients.

There is an interesting possible relationship between 22q11DS and FGF signaling. Aggarwal et al [36] and Guo et al [37] described epistatic relationships between the FGF system and Tbx1. It is firmly established that Tbx1 is responsible for most of the congenital defects seen in 22q11DS patients and mouse models [38–40]. Tbx1 point mutations were found in patients who displayed the key physical symptoms of the classic 22q11DS phenotype, but did not have a 22q11 deletion [41]. There is incidental evidence for an association of FGF signaling with idiopathic schizophrenia. One publication reports decreased serum levels of FGF in schizophrenia [42,43]. We could not identify reports describing involvement of PDGF signaling in schizophrenia. However, both FGF and PDGF are important growth factors of which involvement in schizophrenia has been suggested on theoretical grounds [30]. Disturbances in leucocyte extravasation signaling, and complement and coagulation

Table 3. Significantly deregulated genetic networks as identified by the Ingenuity Pathways Analysis (IPA).

ID	Molecules in Network	Score	# of Focus Molecules	Top Functions
1	ARNT2, B2M, ↑BASP1, ↓CDC42, CYBA, H2-Q1, HLA-E, JAK2, ↑KCNJ15, KLRC1, ↓KLRC2, ↓KLRC3, ↓KLRD1, ↑MEF2C, ↑MXD1, MYCN, PAK1, ↓PARD6A, PARD6G, ↓PIK3R3, ↑PPM1F, ↓PTPN4, QDM, RAC2, ↓RANBP1, Rb, RB1, RBL2, ↓SEPT5, ↓SNAP29, ↑SNX5, STX1A, ↑STXBP2, ↑TLR4, VAMP2	26	18	Cell Cycle, Connective Tissue Development and Function, Nervous System Development and Function
2	↓ACVR2B, ↓CD2, ↓COMT, CRK, ↓CRKL, ↓CXCL10, ↑DDEF1, ↑DOCK4, E2F4, EPHB6, ESR1, ↑ETV6, ↓EXOSC2, EXOSC7, EXOSC8, EXOSC9, EXOSC10, ↑HSF1, Hsp70, HSPA7, ↑MAFG, MAP4K5, MYC, NFE2L3, ↓NQO1, ↑P2RX1, ↑PCID1, PIK3R1, PLAG1, ↓PPP2R2B, RICS, ↑SENP6, ↑SLC11A1, SUMO1, XRN2	24	17	Cancer, Hematological Disease, Cell-To-Cell Signaling and Interaction
3	APEX1, ATF3, CARM1, ↓CCL5, CCR4, ↑CD24, CD63, CKM, ↓CPT1A, ↓DGCR6, EP300, ↑GK, ↑HK2, MDM2 (includes EG:4193), ↓MYO6, NEUROG3, ↑OLIG1, ↓PIK4CA, PPARGC1A, ↓PRF1, PTGDS, ↓PYHIN1, RELA, SELP, SLC2A4, ↑SLC2A5, ↓TBX21, TFDP1, ↑TNFRSF10C, TP53, TRIM28, TSC22D3, ZBTB17, ↓ZDHHC8, ↓ZNF74	22	16	Gene Expression, Cancer, Respiratory Disease
4	↑ABCA1, ↓API5, CDKN2A, CKB, ↓CR1, CR2, ↓4S234E, ↓DGCR14, DLG4, ↓F2R, FGF2, FLT1, ↓GZMA, HMGB2, ↑HPSE, Hsp27, KCNJ4, KCNJ12, KPNB1, LAMA1, ↑LIN7A, ↑NAB2, ↓PCQAP, RNA polymerase II, ↓RNGTT, RNMT, SMAD3, Smad2/3, SP1, STX12, SUPT5H, ↑TFE3, ↓UFD1L, ↓ZNF83, ↑ZNF451	22	16	RNA Post-Transcriptional Modification, Cell Signaling, Cardiovascular System Development and Function
5	↑ADAM9 (includes EG:8754), APOB, ↓APOBEC3G, ↓ARL4C, CEBPA, CIAA1, CIAA2, COL10A1, COL11A2, COL18A1, COL1A1, COL2A1, COL3A1, ↓COL5A3, ↑CSF2RA, EGR2, GRM5, ↑HES7, HMGB2, Homer, HOMER1, HOMER3, ↓HP, ITGA2, ITGA9 (includes EG:3680), ITGB1, ITPR, NFYB, NFYC, ↑PTPRE, SHANK3, ↑THBD, ↑TNFRSF8, ↓TXNRD2, ↑ZBTB7B	17	13	Dermatological Diseases and Conditions, Cardiovascular Disease, Cell Cycle
6	↑AQP9, ARF1, CARM1, CBX1, CBX5, ↑CD53, ↓CENTG2, ↓CHAF1A, Creb, ↑CREB5, CREM, ↑ERAF, GRIA2, GRM7, H3F3B, ↓HIRA, HIRIP3, Histone h3, KIR3DL1, MBD1, NCAPD2, NCOA1, NCOA2, PICK1, Pkc(s), PLD1, ↑PRKCA, ↓RORA, ↓RORC (includes EG:6097), SETDB1, SFTPA1, ↓SMC2, SMC4, ↓THAP7, TRIM24	15	12	Cell-To-Cell Signaling and Interaction, Nervous System Development and Function, Cell Morphology
7	↑ACSL4, Actin, Arp2/3, ↑BACH2 (includes EG:60468), ↓C19ORF12, CAM, CD44, ↓CLDN5, ↓CLDN19, ↓CLIC5, DNMBP, HDAC4, MARK4 (includes EG:57787), NCOR2, PARD3, ↓PARD6A, PARD6B, PARD6G, ↑PDK1, PFN1, ↓PHLDB2, PKC (λ,ζ), PRKCI, ↑RAPGEF2, SMARCA4, STK11, TJP1, TJP2, TJP3, VIM, WAS, ↑WASF1, WASF2, YWHAQ, YWHAZ	13	11	Cell Morphology, Cellular Development, Cell-To-Cell Signaling and Interaction

The table displays the seven functional networks identified by the IPA as significantly (score>3) associated with the geneset defined by the two critera (1) deregulated between patients and controls according to LAP or (2) t-test<0.05 and Fold Change>1.5. The table displays the genes associated with the functional networks (genes which are present in the uploaded gene set are in bold). The arrows behind a gene indicate the direction of change (arrow pointing upwards: increased expression in patients, arrow pointing downwards: decreased expression in patients).The column 'score' gives the significance score for the network. The column '# of genes' gives the number of genes in the network. Each network consists –by definition of the IPA- of 35 genes. The last column shows the functions in which each network is predominantly involved.
doi:10.1371/journal.pone.0033473.t003

cascades cannot be clearly related with 22q11DS and schizophrenia pathology. It has been postulated that subtle aberrations in cellular machinery may be present in all organs and cell types

Table 4. Relative hippocampal gene expression levels.

Gene	Relative hippocampal expression Df1/+ vs WT	Relative PBMC expression 22q11DS patients vs controls
DGCR6	0.45	0.49
RANBP1	0.53	0.40
ZDHHC8	0.86	0.63
HTF9C	0.63	0.47
COMT	0.54	0.54
CLDN5	0.68	0.56
UFD1L	0.78	0.52

The table dispays the relative hippocampal expression levels of Df1/+ vs WT mice (after Sivagnanasundaram et al, 2007 [17]) and PBMC gene expression levels of 22q11DS patients vs controls (this study) for seven genes expressed in both samples (mice and humans).
doi:10.1371/journal.pone.0033473.t004

in schizophrenia [44], but only give rise to overt pathology in the nervous system. Such subtle aberrations may however become present in in-vitro situations, explaining differences in coagulation and leucocyte functions between patients and controls.

Taken together, our pathway analyses show a number of pathways previously associated with idiopathic schizophrenia among the pathways significantly associated with the deregulated gene set in our 22q11DS sample. Most notably for their involvement in schizophrenia are neurotrophin/Trk signaling and neuregulin signaling. Our findings cautiously suggest that the molecular-biological underpinnings of the psychotic phenomena observed in 22q11DS are at least partly related with those seen in 'regular' schizophrenia. Moreover, our findings are present even though only three patients show psychotic phenomena, and four do not. This suggests that molecular-biological pathways involved schizophrenia are deregulated in 22q11DS patients both with and without psychosis, and that, like in idiopathic schizophrenia, environmental factors modulate the expression of the psychosis phenotype in 22q11DS. Furthermore, the psychiatric phenotype in 22q11DS is extremely variable, and besides schizophrenia a range of other (neuro)psychiatric disorders, including autism spectrum disorders, mood disorder, attention deficit hyperactivity

disorder, developmental delay, and learning disabilities. The co-occurrence of various psychiatric phenomena in 22q11DS may be an example where a general genetic vulnerability for mental impediments leads to variable phenotypic expression, probably dependent on (time-critical) environmental and stochastic influences. It is tempting to ascribe the other mental disorders present in 22q11DS to alterations in signaling pathways related to neuronal functioning, and/or pathways known to be disturbed in neuropsychiatric syndromes, such as axonal guidance signalling and Huntington's disease signalling, neuregulin signalling, and neurotrophin signalling. One may speculate that in the 22q11DS alteration in several distinct functional signaling cascades are present early on in life, with alterations in these cascades becoming more or less prominent, depending on environmental influences, and epistatic interaction with present or absent variants of risk genes outside the 22q11 deleted region.

Our study has a number of limitations; first, the number of subjects is small and heterogeneous (i.e. with-and without psychosis). Second: to identify functional networks we used a liberal statistical approach by not correcting for repeated measurements to obtain the gene set with criteria $p<0.05$ and FC>1.5, with a risk of identifying false-positives. However, we assume that combining this set with the genes present in the 22q11 deleted region (the set identified by LAP), which are certainly no false-positives, and investigating the functional relationships of combined gene set will 'pull out' the true positive genes as those will be the ones that predominantly have functional relationships with the 22q11 genes. Not so much a limitation as well as a caveat is that the approach presented here (investigating PBMC gene expression to gain insight in neuropsychiatric phenomena) relies on the assumption that molecular-biological aberrations observed in peripheral tissue can be informative about brain molecular-biological processes. Though may signaling pathways are similar in peripheral tissue and brain, and there are some reports that this approach is feasible [8,45], the precise validity of this assumption remains to be further elucidated. In summary, this study (1) shows decreased expression of genes in the 22q11 deleted region in PBMC's of 22q11DS patients and (2) suggests the presence of deregulated signaling pathways relevant for schizophrenia pathol-ogy in PBMC's of 22q11DS patients. Generally, our findings support the use of the 22q11DS as a suitable, more homogeneous research model for schizophrenia. A prominent feature of both 22q11DS and schizophrenia is that they have a clear developmental aspect. Clinical features, including cognitive deficits and psychotic phenomena develop over the life span. Symptoms are thought to arise in genetically vulnerable individuals in interaction with environmental, epistatic, and stochastic processes. This aspect of 22q11DS may be highly relevant for the idiopathic schizophrenia syndrome. Approximately 30% of children with a microdeletion of 22q11 will develop a form of schizophrenia that clinically and neurocognitively cannot be distinguished from the idiopathic disorder. Important insights into the trajectory from risk to disorder in idiopathic schizophrenia may be gained from ongoing longitudinal studies of these children comparing cognitive, affective and neural development in those who do and do not develop psychosis among this cohort with a similar genomic deletion [46]. Thus, future research in this field should longitudinally investigate PBMC gene expression in relationship with the phenotypical expression of symptoms in a larger sample of young 22q11DS patients.

Supporting Information

Table S1 Full list of transcripts (N = 262) differentially expressed between patients and controls ($p<0.05$ and Fold Change>1.5). Columns show (from left to right): Affymetrix Probe Set ID; Gene Abbreviation; Chromosomal location; log-transformed ratio of gene expression patients/controls; significance (not corrected for multiple comparisons: see text methods).
(XLS)

Author Contributions

Conceived and designed the experiments: NJMvB. Performed the experiments: LCK SS YE. Analyzed the data: PvdS GB. Contributed reagents/materials/analysis tools: LCK SS YE TvA EB. Wrote the paper: NJMvB TvA EB.

References

1. Murphy KC, Jones LA, Owen MJ (1999) High rates of schizophrenia in adults with velo-cardio-facial syndrome. Arch Gen Psychiatry 56(10): 940–5.
2. van Amelsvoort T, Daly E, Henry J, Robertson D, Ng V, et al. (2004) Brain anatomy in adults with velocardiofacial syndrome with and without schizophrenia: preliminary results of a structural magnetic resonance imaging study. Arch Gen Psychiatry 61: 1085–1096.
3. Meechan DW, Maynard TM, Gopalakrishna D, Wu Y, LaMantia AS (2007) When half is not enough: gene expression and dosage in the 22q11 deletion syndrome. Gene Expr 13: 299–310.
4. Maynard TM, Haskell GT, Peters AZ, Sikich L, Lieberman JA, et al. (2003) A comprehensive analysis of 22q11 gene expression in the developing and adult brain. Proc Natl Acad Sci U S A 100: 14433–14438.
5. Williams NM, Glaser B, Norton N, Williams H, Pierce T, et al. (2008) Strong evidence that GNB1L is associated with schizophrenia. Hum Mol Genet 17: 555–566.
6. Konradi C (2005) Gene expression microarray studies in polygenic psychiatric disorders: applications and data analysis. Brain Res Brain Res Rev 50: 142–155.
7. Mirnics K, Levitt P, Lewis DA (2006) Critical appraisal of DNA microarrays in psychiatric genomics. Biol Psychiatry 60: 163–176.
8. Sullivan PF, Fan C, Perou CM (2006) Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet 141: 261–268.
9. Vawter MP, Ferran E, Galke B, Cooper K, Bunney WE, et al. (2004) Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree. Schizophr Res 67: 4–52.
10. Bowden NA, Weidenhofer J, Scott RJ, Schall U, Todd J, et al. (2006) Preliminary investigation of gene expression profiles in peripheral blood lymphocytes in schizophrenia. Schizophr Res 82: 175–183.
11. Middleton FA, Pato CN, Gentile KL, McGann L, Brown AM, et al. (2005) Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches. Am J Med Genet B Neuropsychiatr Genet 136: 12–25.
12. Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sasik R, et al. (2005) Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc Natl Acad Sci U S A 102: 15533–15538.
13. Tsuang MT, Nossova N, Yager T, Tsuang MM, Guo SC, et al. (2005) Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report. Am J Med Genet B Neuropsychiatr Genet 133: 1–5.
14. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 98: 5116–5121.
15. Callegaro A, Basso D, Bicciato S (2006) A locally adaptive statistical procedure (LAP) to identify differentially expressed chromosomal regions. Bioinformatics 22: 2658–2666.
16. Kasamatsu A, Endo Y, Uzawa K, Nakashima D, Koike H, et al. (2005) Identification of candidate genes associated with salivary adenoid cystic carcinomas using combined comparative genomic hybridization and oligonucleotide microarray analyses Int J Biochem Cell Biol 37: 1869–1880.
17. Sivagnanasundaram S, Fletcher D, Hubank M, Illingworth E, Skuse D, et al. (2007) Differential gene expression in the hippocampus of the Df1/+ mice: a model for 22q11.2 deletion syndrome and schizophrenia. Brain Res 1139: 48–59.
18. Meechan DW, Maynard TM, Wu Y, Gopalakrishna D, Lieberman JA, et al. (2006) Gene dosage in the developing and adult brain in a mouse model of 22q11 deletion syndrome. Mol Cell Neurosci 33: 412–428.

19. McDonald-McGinn DM, Sullivan KE (2011) Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). Medicine (Baltimore) 90(1): 1–18.
20. Jawad AF, Donald-McGinn DM, Zackai E, Sullivan KE (2001) Immunologic features of chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). J Pediatr 139: 715–723.
21. Kanaya Y, Ohga S, Ikeda K, Furuno K, Ohno T, et al. (2006) Maturational alterations of peripheral T cell subsets and cytokine gene expression in 22q11.2 deletion syndrome. Clin Exp Immunol 144: 85–93.
22. Kornfeld SJ, Zeffren B, Christodoulou CS, Day NK, Cawkwell G, et al. (2000) DiGeorge anomaly: a comparative study of the clinical and immunologic characteristics of patients positive and negative by fluorescence in situ hybridization. J Allergy Clin Immunol 105: 983–987.
23. Gennery AR (2012) Immunological aspects of 22q11.2 deletion syndrome. Cell Mol Life Sci 69(1): 17–27.
24. Yovel G, Sirota P, Mazeh D, Shakhar G, Rosenne E, et al. (2000) Higher natural killer cell activity in schizophrenic patients: the impact of serum factors medication, and smoking. Brain Behav Immun 14: 153–169.
25. Kobrynski IJ, Sullivan KE (2007) Velocardiofacial syndrome, DiGeorge syndrome: the chromosome 22q11.2 deletion syndromes. Lancet 370: 1443–1452.
26. Shoval G, Weizman A (2005) The possible role of neurotrophins in the pathogenesis and therapy of schizophrenia. Eur Neuropsychopharmacol 15: 319–329.
27. Van Beveren NJM, Van der Spelt JJ, De Haan L, Fekkes D (2006) Schizophrenia-associated neural growth factors in peripheral blood. A review. Eur Neuropsychopharmacol 16: 469–480.
28. Carter CJ (2006) Schizophrenia susceptibility genes converge on interlinked pathways related to glutamatergic transmission and long-term potentiation, oxidative stress and oligodendrocyte viability. Schizophr Res 86: 1–14.
29. Straub RE, Weinberger DR (2006) Schizophrenia genes -famine to feast. Biol Psychiatry 60: 81–83.
30. Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT (2006) Neurobiology of schizophrenia. Neuron 52: 139–153.
31. Barnes A, Isohanni M, Barnett JH, Pietiläinen O, Veijola J, et al. (2012) Neuregulin-1 genotype is associated with structural differences in the normal human brain. Neuroimage 1;59(3): 2057–61.
32. van Amelsvoort T, Daly E, Robertson D, Suckling J, Ng V, et al. (2001) Structural brain abnormalities associated with deletion at chromosome 22q11: quantitative neuroimaging study of adults with velo-cardio-facial syndrome. Br J Psychiatry 178: 412–9.
33. Baker K, Chaddock CA, Baldeweg T, Skuse D (2011) Neuroanatomy in adolescents and young adults with 22q11 deletion syndrome: comparison to an IQ-matched group. Neuroimage 15;55(2): 491–9.
34. da Silva Alves F, Schmitz N, Bloemen O, van der Meer J, Meijer J, et al. (2011) White matter abnormalities in adults with 22q11 deletion syndrome with and without schizophrenia. Schizophr Res 132(1): 75–83.
35. Duijff SN, Klaassen PWJ, Swanenburg de Veye HFN, Beemer FA, Sinnema G, et al. (2012) Cognitive development in children with 22q11.2 deletion syndrome. Br J Psychiatryin press.
36. Aggarwal VS, Liao J, Bondarev A, Schimmang T, Lewandoski M, et al. (2006) Dissection of Tbx1 and Fgf interactions in mouse models of 22q11DS suggests functional redundancy. Hum Mol Genet 1;15(21): 3219–28.
37. Guo T, McDonald-McGinn D, Blonska A, Shanske A, Bassett AS, et al. (2011) Genotype and cardiovascular phenotype correlations with TBX1 in 1,022 velo-cardio-facial/DiGeorge/22q11.2 deletion syndrome patients. Hum Mutat 32(11): 1278–89.
38. Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, et al. (2001) Tbx1haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. Nature 1;410(6824): 97–101.
39. Merscher S, Funke B, Epstein JA, Heyer J, Puech A, et al. (2001) TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. Cell 23;104(4): 619–29.
40. Jerome LA, Papaioannou VE (2001) DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. Nat Genet 27(3): 286–91.
41. Yagi H, Furutani Y, Hamada H, Sasaki T, Asakawa S, et al. (2003) Role of TBX1 in human del22q11.2 syndrome. Lancet 25;362(9393): 1366–73.
42. Klejbor I, Myers JM, Hausknecht K, Corso TD, Gambino AS, et al. (2006) Fibroblast growth factor receptor signaling affects development and function of dopamine neurons -inhibition results in a schizophrenia-like syndrome in transgenic mice. J Neurochem 97: 1243–1258.
43. Hashimoto K, Shimizu E, Komatsu N, Nakazato M, Okamura N, et al. (2003) Increased levels of serum basic fibroblast growth factor in schizophrenia. Psychiatry Res 120: 211–218.
44. Prabakaran S, Wengenroth M, Lockstone HE, Lilley K, Leweke FM, et al. (2007) 2-D DIGE analysis of liver and red blood cells provides further evidence for oxidative stress in schizophrenia. J Proteome Res 6: 141–149.
45. Gladkevich A, Kauffman HF, Korf J (2004) Lymphocytes as a neural probe: potential for studying psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 28: 559–576.
46. Insel TR. Rethinking schizophrenia (2010) Nature 11;468(7321): 187–93.