Effects of segmental trisomy on the expression of protein-coding and microRNA genes

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ABSTRACT:

Introduction: In our pilot study, we have used adult mice of the Ts43H mouse model of human aneuploidy syndromes (Fig. 3) carrying largest known segmental trisomy of an autosome with more than 300 genes [1]. First, we were interested in consequences of the segmental gene dosage imbalance on transcription of protein-coding genes located in the trisomic region in comparison with genes located in the disomic region of the same chromosome. Second, we have also measured expression levels of mature microRNA molecules located inside of the trisomic region of the chromosome 17 and one mature microRNA molecule located on another chromosome. MicroRNA genes are known to be example of non-coding RNA genes with strong regulatory potential and are therefore candidate genes in study of development of different pathological states including aneuploidy syndromes.

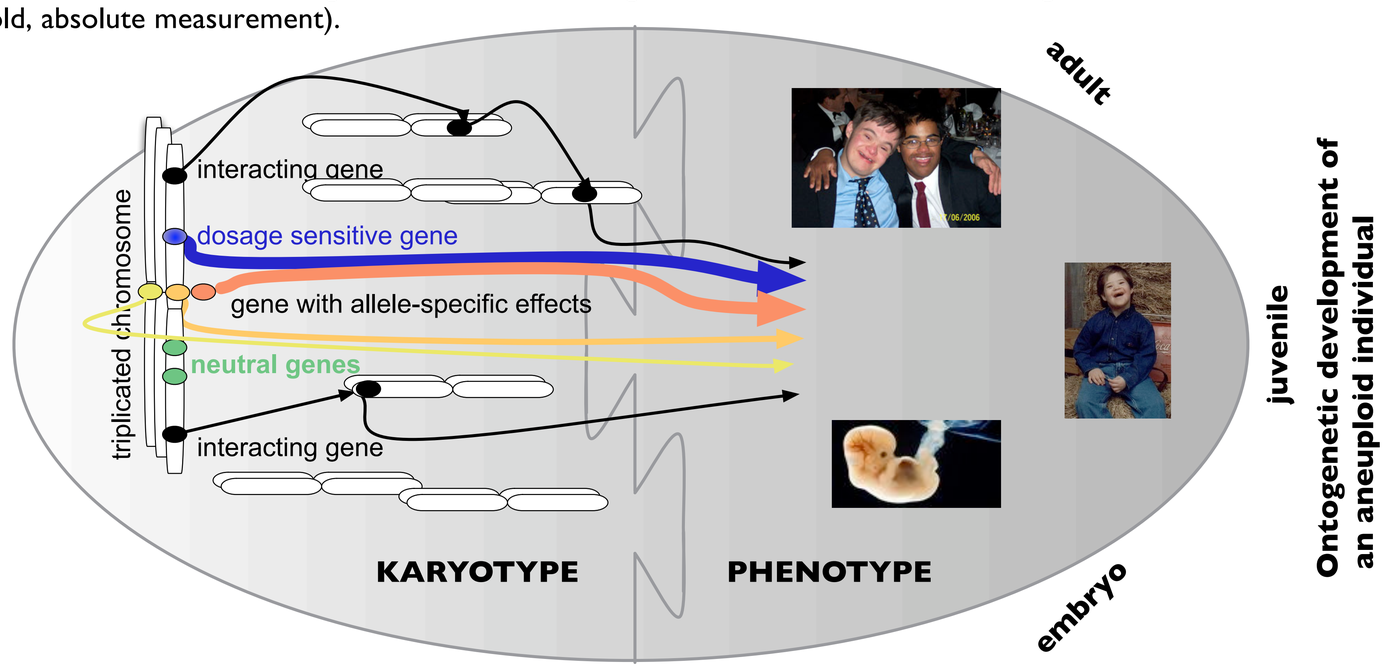
Results: We are reporting significant differences (overview of methods in Box 1) in both individual protein-coding genes and microRNA genes between control animals and trisomic animals, mainly for genes in the trisomic region. The average expression level of protein-coding genes in the trisomic region was ~1.6-fold in both liver and brain, which corresponds with the altered gene dosage and does not indicate any kind of dosage compensation (0.01% difference between the two tissues). In the disomic region, however, the average expression level was ~1.0-fold in liver and ~0.9-fold in brain (9% difference), which indicates slight downregulation of the disomic region in brain if we consider the liver tissue as a control (Tab. 1 and 2, Fig. 1 and 2). However, statistical significance of the difference remains unclear.

For the first time, we are presenting measurements of gene-dosage effects on microRNA genes in a mammalian genome (Tab. 3). The three microRNA genes located in the trisomic region were upregulated ~1.5-fold when compared to a reference microRNA gene in the disomic region. However, the microRNA gene located on another chromosome (mmu-let-7a) was significantly downregulated (0.8-fold, absolute measurement).

MOTIVATION: A central challenge of genetic research is to precisely define relationship between genotype and phenotype. This is especially critical in aneuploid syndromes like Down syndrome in humans, which is a product of genetic effects on different cells, structures, and functions throughout development, many of which may have cascading effects.

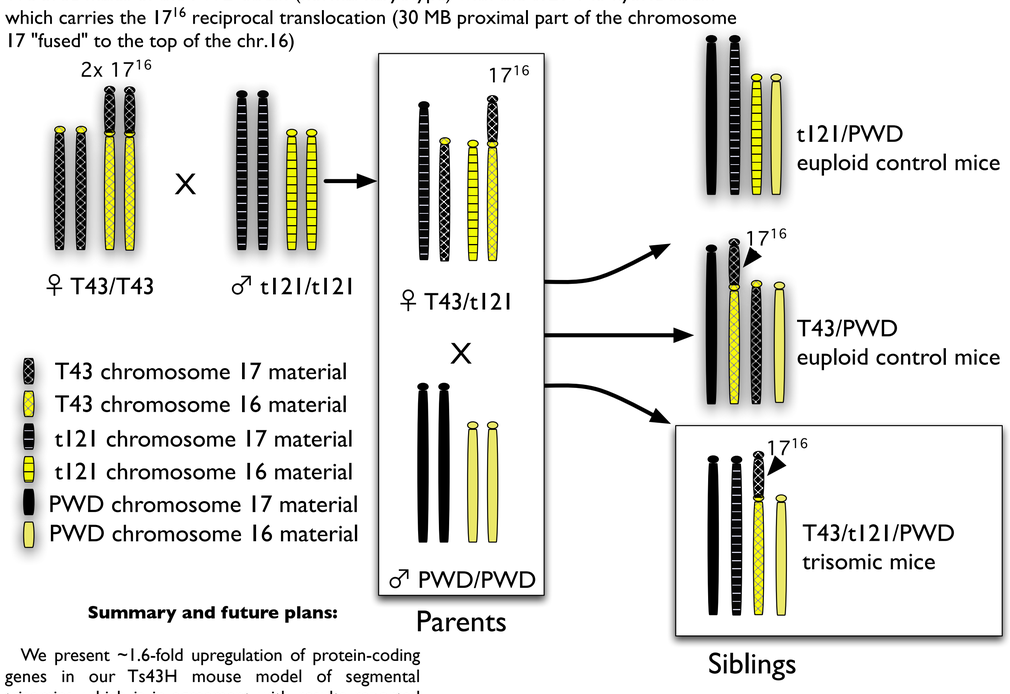
We want to separate those effects of trisomy that disturb development from those that alter function of cells that have reached an end point of differentiation. These are obviously not independent concepts; any "developmental" perturbation derives from alteration of some function in a developing cell.

Altered functions of a mature cell may have little or nothing to do with up-regulation of trisomic genes in that cell, but rather could reflect a developmental error caused by trisomy that has downstream consequences that affect function. That is, a specific phenotype may be a consequence of but not a direct product of trisomic gene expression (developmental versus functional effects).



Genetic puzzle: from the trisomic genotype to the complex phenotype

Fig. 3: Generation of trisomic mice:
We bred males of the PWD strain (normal karyotype) with the t121/T43 hybrid strain which carries the 17^{16} reciprocal translocation (30 MB proximal part of the chromosome 17 "fused" to the top of the chr.16)



Summary and future plans:

We present ~1.6-fold upregulation of protein-coding genes in our Ts43H mouse model of segmental trisomies, which is in agreement with results reported in other mouse models of aneuploidy syndromes and in human samples affected by the Down syndrome [4, 5, 6, 7].

We publish the first report on the expression levels of triplicated microRNA genes. The observed ~1.5-fold upregulation does not indicate any special mode of regulation of microRNA gene expression in comparison to the protein-coding genes.

Since microRNA genes are considered potent regulators of gene expression themselves, targeting tens to hundreds of other genes, we propose that the effects of their dysregulation might play prominent role in the in the development of pathological states occurring in aneuploidy syndromes. More investigation will be needed to better understand this matter.

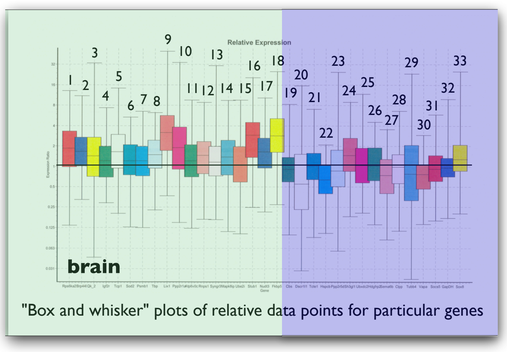
By conducting the above-mentioned experiments on samples from adult mice, we have started research into relationship between genes dysregulated in aneuploid genomes and their complex effects on different stages of ontogenetic development. We would like to continue by analyzing the embryonic stage of development.

Significant downregulation of the mmu-let-7a molecule located on a different chromosome than the segmentally triplicated chromosome 17 might raise several speculations. Since let-7a molecule belongs to the same family of microRNA molecules as the upregulated mmu-let-7e, the downregulation of its expression might suggest kind of compensation mechanism.

Box 1:
Overview of samples and methods used:
- 5 trisomic mice vs.10 disomic (euploid) control mice
- adult animals (age 129-133 d)
- total RNA from brain and liver (Trizol® isolated)
- Sybr Green I qRT PCR, Applied Biosystems TaqMan® MicroRNA Assay, Invader® MicroRNA Assay
- reference genes: mir-7b, U6 snRNA/total RNA, GapDH, actin beta, Hprt
- statistical analysis: REST 2005 [2], R statistical environment [3]

Tab. 3:
Expression of microRNA genes in the trisomics - microRNA cluster in the trisomic region of Ts43H is upregulated ~1.5-fold:
Four microRNA genes were measured - three of them located inside the trisomic region of the mouse chromosome 17 and and one located at the on another chromosome(s).

microRNA	chromosome	expression in trisomics (%)	p-value (REST 2005, t-test)	standardized to	reference gene	assay
let-7e	MMU 17 (trisomic region)	140	0.006	total RNA/ U6 sn RNA	mir-7b	AB TaqMan
mir-99b	MMU 17 (trisomic region)	149	0.04	total RNA / U6 sn RNA	mir-7b	AB TaqMan
mir-125a	MMU 17 (trisomic region)	153	0.02	total RNA / U6 sn RNA	mir-7b	AB TaqMan
let-7a	MMU 9+13	80	0.03	total RNA / U6 sn RNA	absolute measurement	Invader

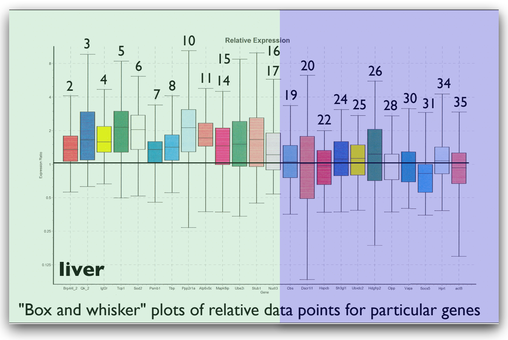


"Box and whisker" plots of relative data points for particular genes

Fig. 1: Chromosome 17 gene expression in brain:
- 8 genes in the trisomic region significantly up
- 1 gene in the disomic region down
- avg. expression in the trisomic region: 166 %
- avg. expression in the disomic region: 93 %
- reference gene: GapDH
- SybrGreen I (Roche)

Tab. 1:
Brain: relative expression values, charts and statistics produced using REST 2005 [2].

	Gene	Expression	Std. Error	P-value	Result
1	Rps6ka2	1.76	0.691 - 4.608	0.030	UP
2	Brp44l	1.67	0.793 - 3.516	0.019	UP
3	Qk_2	1.41	0.484 - 4.614	0.306	
4	Igf2r	1.23	0.574 - 2.566	0.298	
5	Tcp1	1.69	0.734 - 4.085	0.032	UP
6	Sod2	1.18	0.524 - 2.660	0.418	
7	Psmb1	1.17	0.528 - 2.336	0.450	
8	Tbp	1.47	0.707 - 2.889	0.040	UP
9	Lix1	3.19	1.092 - 8.353	0.000	UP
10	Ppp2r1a	1.84	0.617 - 5.230	0.048	UP
11	Atp6v0c	1.20	0.469 - 2.822	0.449	
12	Rnps1	1.40	0.492 - 3.962	0.166	
13	Syngr3	1.25	0.562 - 2.706	0.435	
14	Mapk8ip	1.29	0.493 - 2.949	0.292	
15	Ube2i	1.07	0.411 - 2.880	0.801	
16	Stub1	2.52	1.156 - 6.430	0.001	UP
17	Nudt3	1.56	0.670 - 3.514	0.064	
18	Fkbp5	2.90	1.252 - 7.065	0.000	UP
19	Cbs	0.90	0.330 - 2.481	0.698	
20	Dscr1l1	0.55	0.107 - 2.433	0.150	
21	Tcte1	0.95	0.416 - 1.730	0.811	
22	Hspcb	0.63	0.343 - 1.297	0.020	DOWN
23	Ppp2r5d	0.95	0.293 - 3.206	0.882	
24	Sh3gl1	1.50	0.687 - 3.238	0.137	
25	Ubxdc2	1.08	0.458 - 2.608	0.786	
26	Hdgfrp2	1.02	0.458 - 2.320	0.925	
27	Sema6b	0.69	0.314 - 1.577	0.156	
28	Clpp	0.94	0.285 - 2.586	0.820	
29	Tubb4	0.80	0.193 - 3.106	0.591	
30	Vapa	0.72	0.404 - 1.253	0.108	
31	Socs5	0.97	0.453 - 2.032	0.891	
32	GapDH	1.00	0.614 - 1.621	1.000	
33	Sox8	1.41	0.678 - 2.641	0.152	



"Box and whisker" plots of relative data points for particular genes

Fig. 2: Chromosome 17 gene expression in liver:
- 8 genes significantly up, 0 down
- avg. expression in the trisomic region: 164 %
- avg. expression in the disomic region: 101 %
- reference genes: Hprt, actin beta
- SybrGreen I (Roche)

	Gene	Expression	Std. Error	P-value	Result
2	Brp44l_2	1.42	0.969 - 2.185	0.024	UP
3	Qk_2	1.86	0.956 - 3.426	0.022	UP
4	Igf2r	1.67	1.028 - 2.752	0.006	UP
5	Tcp1	2.02	1.080 - 3.536	0.012	UP
6	Sod2	2.01	1.157 - 3.608	0.005	UP
7	Psmb1	1.29	0.848 - 1.749	0.170	
8	Tbp	1.45	0.957 - 2.057	0.022	UP
10	Ppp2r1a	1.99	0.963 - 4.007	0.033	UP
11	Atp6v0c	1.70	1.193 - 2.739	0.022	UP
14	Mapk8ip	1.37	0.775 - 2.498	0.187	
15	Ube2i	1.55	0.719 - 3.329	0.126	
16	Stub1	1.71	0.838 - 3.356	0.058	
17	Nudt3	1.32	0.722 - 2.413	0.189	
19	Cbs	1.05	0.579 - 1.786	0.786	
20	Dscr1l1	0.91	0.391 - 2.047	0.783	
22	Hspcb	0.92	0.587 - 1.433	0.572	
24	Sh3gl1	1.13	0.662 - 1.995	0.507	
25	Ubxdc2	1.08	0.605 - 1.795	0.681	
26	Hdgfrp2	1.20	0.562 - 2.548	0.525	
28	Clpp	0.96	0.638 - 1.319	0.787	
30	Vapa	0.99	0.536 - 1.643	0.957	
31	Socs5	0.83	0.479 - 1.150	0.292	
34	Hprt	1.12	0.717 - 1.740	0.160	
35	actB	0.90	0.558 - 1.453	0.224	

Tab. 2:
Liver: relative expression values, charts and statistics produced using REST 2005 [2]

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Acknowledgments:
The project is supported by the Czech Science Foundation (project num.: 301/07/126) and the 6th Framework Programme of EU (project "AnEUploidy")

Thanks to:
Drs. Petr Jansa, Sona Gregorova, Zdenek Trachtulec, David Homolka and Petr Divina