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Differential gene expression profile in the small intestines of mice lacking pacemaker interstitial cells of Cajal

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Abstract

Background: We previously identified eight known and novel genes differentially expressed in the small intestines of wild type and W/W^V mice, which have greatly reduced populations of the interstitial cells of Cajal, that are responsible for the generation of electrical slow waves, by using a differential gene display method.

Methods: By using the same method we isolated additional candidate genes that were specifically down- or up-regulated in W/W^V mice. Novel transcripts were designated as *DDWMEST*.

Results: We isolated seven candidates that were specifically down- or up-regulated in W/W^V mice. Two novel transcripts, *DDWMEST 1* and *-91* were increased in both *fed* and *fasted* W/W^V mice. Expression of another five genes was suppressed in W/W^V mice: *ARG2* (Arginase II), *ONZIN* (encoding leukemia inhibitory factor regulated protein), and three novel transcripts: *DDWMEST62*, *-84*, and *-100*. Together with the previous report, we identified fifteen differentially expressed genes in total in the small intestines of W/W^V mice. Eight of these genes were reduced in the jejunums of W/W^V mice compared to age matched wild type mice, whereas the other seven genes showed an increase in expression. Differential expression was the same in *fasted* and *fed* animals, suggesting that the differences were independent of the dietetic state of the animal.

Conclusions: Several known and novel genes are differentially expressed in the small intestines of W/W^V mice. Differential gene comparison might contribute to our understanding of motility disorders associated with the loss of the interstitial cells of Cajal.

Background

Interstitial cells of Cajal (ICC) are pacemakers [1–3] and mediators of enteric motor neuro-transmission [4,5]. KIT, a receptor tyrosine kinase, is essential for the development and maintenance of the ICC phenotype [6,7]. Animals with reduced KIT expression (e.g. the W/W^V mutant

mouse) develop few ICC at the level of the myenteric plexus (IC-MY) in the small intestine [2,3], and lose intramuscular ICC (IC-IM) in the stomach [4]. The loss of IC-MY is associated with a loss of electrical slow waves in the small intestine. Loss of IC-IM is associated with the disruption of enteric neuro-transmission in the gut [4].

Table 1: Genes differentially expressed in the small intestine of *W/W^v* mice

Fragment Code	Accession No.	Protein (UniGene No.)	<i>W/W^v</i> (a)	Status (b)	Subcellular location (c)	Chromosome (d)
DDWMEST117	U82256	ARG2	decreased	this report	mitochondria ^(s)	14q24.1-q24.3
DDWMEST6	AI263458	ONZIN	decreased	this report	cytoplasm ^(p)	4q21.22
DDWMEST100	W12272	(Mm28954)	decreased	this report	cytoplasm ^(p)	1q42.11-q42.3
DDWMEST1	AB052398	unknown	increased	this report	ND	ND
DDWMEST62	AB052399	unknown	decreased	this report	ND	ND
DDWMEST84	AB052400	(Mm5296)	decreased	this report	nuclear ^(p)	6
DDWMEST91	AB052402	unknown	increased	this report	ND	ND
DDWMEST54	AB030038	ACPL1	decreased	reported previously	cytoplasm ^(s)	1q21
DDWMEST101	AI173010	COX7B	decreased	reported previously	mitochondria ^(s)	Xq13.2
DDWMEST4	W33314	SORCIN	decreased	reported previously	cytoplasm ^(s)	7q21.1
DDWMEST43	U67771	RPL8	increased	reported previously	cytoplasm ^(s)	8q24.3
DDWMEST116	M10319	ADA	increased	reported previously	cytoplasm ^(p)	20q12-q13.11
DDWMEST90	M16229	MDH1	increased	reported previously	cytoplasm ^(s)	2p13.3
DDWMEST110	M74773	SPTB2	increased	reported previously	cytoplasm ^(s)	2p21
DDWMEST99	M27347	P6-5	increased	reported previously	cytoplasm ^(p)	12q13

(a) Gene expression pattern of each gene in the small intestine of *W/W^v* mice. (b) "this report" means the gene newly isolated by us as a differentially expressed gene in the small intestine of *W/W^v* mice. "reported previously" indicates that the differential expression of the gene was reported by us previously (ref. 15, 16). (c) Subcellular location determined by the SOURCE program <http://source.stanford.edu> or predicted by the PSORTII program <http://psort.ims.u-tokyo.ac.jp/form2.html> were shown as "(s)" or "(p)", respectively. ND: not determined. Human chromosomal localization of the gene.

Absolute or relative loss of ICC has been reported in many gastrointestinal (GI) motility disorders [8–12]. The association between diseases of GI motility and loss of ICC suggests that a more comprehensive understanding of the molecular biology of ICC may contribute to our understanding of the etiology of GI motility disorders [13–16]. The loss of IC-MY and slow wave activity in the small intestines of *W/W^v* mice provides a unique opportunity to examine molecular changes that are associated with the loss of ICC in the gut. We hypothesized that there may be differential expression of genes in the small intestines of age-matched wild type and *W/W^v* mice [15]. By the differential display method, we were able to identify a novel gene, *ACPL1* and seven known genes that have been previously reported [15,16]. In both *fasted* and *fed* states, expression of the *ACPL1*, *COX7B* and *SORCIN* were considerably reduced in the jejunums of *W/W^v* mice compared to age matched wild type mice. Whereas, five other genes (*ADA*, *MDH1*, *RPL8*, *SPTB2*, and *p6-5*) showed an increase in expression in *fed* and *fasted W/W^v* mice Table 1.

In the present study we have combined molecular profiles from an additional seven genes: We have identified fifteen candidate genes that are either up- or down-regulated in jejunal tissues of *W/W^v* mice.

Methods

The use and treatment of experimental animals was approved by the guideline governing Animal Experiment Committee at the University of Yamanashi School of Medicine, Yamanashi, Japan and at the University of Nevada School of Medicine, Reno, USA. Six adult male *WBB6F1-+/+* (wild type) and *WBB6F1-W/W^v* mice, weighing 20 to 30 g, were purchased from Japan SLC Inc (Shizuoka, Japan). Animals were anesthetized with ether inhalation and sacrificed by cervical dislocation followed by exsanguination. Jejunal segments were resected and subsequently frozen in liquid nitrogen (-196°C).

For differential gene display, poly (A)⁺ RNA was isolated from each tissue sample by TRIZOL (Life Technologies, Inc., Gaithersburg, MD, USA). The differential display procedure was performed as previously described [15–18]. The blots were autoradiographed and analyzed with a fluorescent image analyzer (FMBIO, Takara Co., Kyoto, Japan). For reverse transcription-polymerase chain reaction (RT-PCR), total RNA was isolated from resected specimens by TRIZOL. cDNA were prepared from 2 µg of total RNA by (dT)₁₅ priming and synthesized in the same manner as in the differential gene display experiments. The levels of gene expression were analyzed by semi-quantitative RT-PCR using mRNAs from proximal jejunums of *fed*

and *fasted* wild type and W/W^V mice as templates. The PCR reactions were optimized for number of cycles to ensure product intensity within the linear phase of amplification.

Results

To identify genes that may be selectively associated with IC-MY, we compared gene expression patterns in jejunal tissues derived from age-matched wild type and W/W^V mice utilizing a differential gene display method. Using various primer sets, we identified a total of twelve cDNA bands that were likely to be expressed at the biggest different levels in these tissues. They showed either intense or weak labeling in the jejunums of wild type animals but very weak or intense labeling in W/W^V jejunums. To confirm the differential display profiles, we further examined the expression levels in murine jejunal mRNA derived from wild type and W/W^V mice maintained under *fed* or *fasted* conditions as templates, using semi-quantitative RT-PCR analysis. In this way, we identified a total of eight differentially expressed genes in the small intestines of W/W^V mice Table 1.

We identified a total of nine cDNA bands expressed at the biggest different levels in the small intestines of W/W^V mice by using primer sets, which were not tested in the previous study. By means of semi-quantitative RT-PCR analysis with subsequent cDNA screening, we were able to isolate seven additional novel candidate transcripts among the nine bands that were specifically down- or up-regulated in the small intestines of W/W^V mice; two of them (*ARG2* and *ONZIN*) were found to be known genes (Table 1, Fig 1). Semi-quantitative RT-PCR revealed that the other five candidates (*DDWMEST-1*, *-62*, *-84*, *-91*, and *-100*; tentative names) were differentially expressed in W/W^V mice (Table 1, Fig. 1), although in some of them full-length cDNA have not been obtained so far. A search with the FASTA program <http://www.ebi.ac.uk/fasta33/> revealed no significant homology between these 5 novel transcripts and any archived molecule in the public databases, however two of them, *DDWMEST84* and *-100* were found in the UniGene Cluster database that contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and genetic map location <http://www.ncbi.nlm.nih.gov/UniGene/>[19]. In both *fasted* and *fed* states, expression of the *DDWMEST 1* and *-91* were significantly increased in the jejunums of W/W^V mice compared to age matched wild type mice. Whereas, the other five transcripts (*ARG2*, *ONZIN*, *DDWMEST-62*, *-84*, and *-100*) showed a decrease in expression in *fed* and *fasted* W/W^V mice.

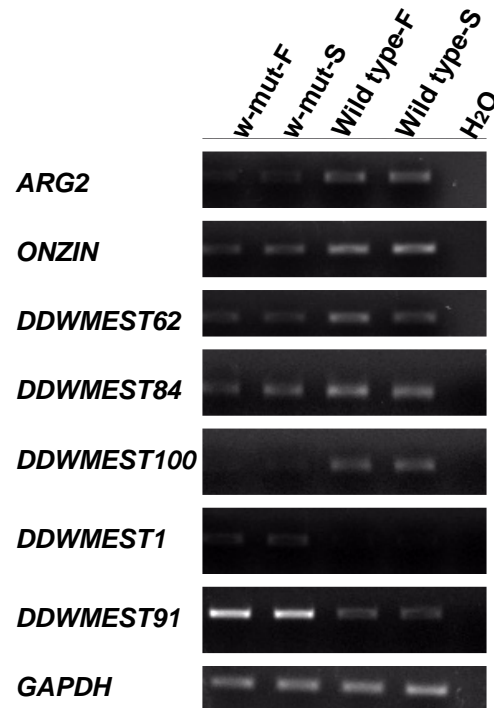


Figure 1

Expression of seven genes, using semi-quantitative RT-PCR analysis, in jejunum tissues from wildtype and W/W^V mutant mice maintained under *fed* and *fasted* conditions. F and S indicate samples of fed and starved mice respectively. *GAPDH* levels were measured as a control. Five genes showed a decrease in expression in both *fed* and *fasted* W/W^V mice. These transcripts included: the gene for *ARG2*, *ONZIN*, *DDWMEST62*, *-84*, and *-100*. Two transcripts, *DDWMEST1* and *-91* were increased in both *fed* and *fasted* W/W^V mice.

Discussion

In the present study we have identified seven additional differentially expressed genes in the small intestines of W/W^V mice. This animal model have the loss of IC-MY in the gut that are responsible for the generation of electrical slow wave activity. In combination with our previous reports [15,16], now we have completed the differential display analysis and all the twenty-one candidate cDNA bands that were likely to be expressed at the reliable different levels in those tissues confirmed with semi-quantitative RT-PCR, the differential expression of a total of fifteen genes, and obtained a molecular profile of the

small intestine of W/W^V mice. A comparison of differentially expressed genes from the jejunums of W/W^V mice using a differential display method has revealed that the expression of eight genes were significantly suppressed in W/W^V mice, whereas seven genes were dramatically up-regulated in these animals Table 1. Data obtained from these experiments suggest that expression of the genes were specifically regulated in W/W^V mice and were independent of the dietary conditions (i.e. *fed* or *fasted*) imposed.

The two additional genes that were up-regulated in the small intestines of W/W^V mice were identified; *DDWMEST-1* and *-91*, both of which revealed no significant homology to any known genes. Two of five additional down-regulated genes in W/W^V mice, *ARG2* and *ONZIN* were identified as known genes by cDNA screening. *ARG2* has seven conserved residues known to be essential for activity of arginases [20], and has an important role in the regulation of nitric oxide synthesis and polyamine metabolism by modulating local arginine concentrations [21]. It also contains a mitochondrial import sequence, and is imported into the mitochondria with appropriate proteolysis [22]. It is reported that release of cytochrome c from the mitochondrial intermembrane space results in nuclear apoptosis [23]. An abundance of mitochondria is one of the criteria used to identify IC-MY by electron microscopy, and our data have possibly suggested the importance of mitochondria in generating pacemaker activity in GI muscles [24]. The loss of IC-MY in W/W^V mutant mice may be associated with the down-regulation of *COX7B* and *ARG2* in these animals, both of which were localized in the mitochondria. *ONZIN* was identified through a screening of LIF regulated genes. LIF is a multifunctional cytokine implicated in various functions and is supported to play a role in neuro-immune reactions [25] as well as an important role in aspects of neuronal development [26,27]. Expression of LIF protein is observed in the gut, especially in the myenteric and submucous ganglion cells at 13–31 weeks of gestation in childhood cases and adults [28]. The down-regulation of *ONZIN* in W/W^V mice therefore suggests the involvement of the LIF pathway in the GI motility disorders.

The other three transcripts whose expression were decreased in W/W^V mice showed no significant homology to any known genes, however two of them, *DDWMEST-84* and *-100*, matched to the assembled sequences in the UniGene Cluster database.¹⁹ *DDWMEST-84* (HomoloGene No.: Mm.5296) was reported to be expressed in brain, embryo, heart, lung, and mammary gland and to be mapped to mouse chromosome 10, and its human counterpart was localized at chromosome 6. *DDWMEST-100* (HomoloGene No.: Mm.28954) was suspected to be expressed ubiquitously in various tissues, such as embryo,

hypothalamus, liver, muscle, placenta, skin, lung, mammary gland, and uterus.

In the fifteen genes identified, we also determined the subcellular localization and human chromosomal mapping of the twelve known genes and two UniGene Cluster sequences using several web-available software (the SOURCE program: <http://source.stanford.edu> or the PSORTII program: <http://psort.ims.u-tokyo.ac.jp/form2.html>) Table 1. Interestingly, eleven genes were suggested to be localized in the cytoplasm or mitochondria. Another gene was supposed to encode a nuclear protein. Further analyses of these genes might enable us to elucidate not only their relationship with the KIT, a receptor tyrosine kinase, but also the molecular aspects of GI pacemaker system.

Today the Genome Project is considered to be one of the most important projects in biology and medicine. The discovery of the entire human and mouse genes through this project will revolutionize biological medicine including molecular diagnosis of various diseases and the development of novel treatments. The information will accelerate discovery of genes susceptible to or causing various diseases and contribute to screening of novel drugs that target these disease-gene products. In this regard, the effort of the molecular profiling project in which we attempt to discover genetic aberrations in animal disease models such as W/W^V mice will generate very valuable resources for further elucidation of GI motility disorders.

At the present time we do not have enough evidence whether these genes are important to the function of the pacemaker apparatus or were down-or up-regulated as a result of the loss of ICC and impairment of normal slow wave pacemaker activity. However, the molecular profile obtained can yield valuable insights into the molecular events underlying motility disorders in which ICC are lost. Application of the differential gene display method, when combined with the future progress of the human and mouse genome project, may reveal new genetic pathways that are common to those GI motility disorders characterized by a deficiency of ICC.

Conclusion

Our data provide one of the first genetic evidences that several important proteins that have roles in DNA/RNA/protein synthesis and metabolism, oxygen metabolism, energy cycle, and development and differentiation of gut cells are significantly changed in the small intestines of W/W^V mice. Considering the number of novel genes identified by us in this study, we suggest that many unknown genes could be involved in the cellular changes that lead to motility disorders associated with ICC loss.

List of abbreviations

ACPL1: acid phosphatase-like protein 1,
 ADA: adenosine deaminase,
 ARG2: Arginase II,
 COX7B: cytochrome c oxidase subunit VIIb,
 DMP: deep muscular plexus,
 ESTs: expressed sequence tags,
 FISH: fluorescence *in situ* hybridization,
 GI: gastrointestinal,
 GAPDH: glyceraldehyde-3-phosphate dehydrogenase,
 ICC: interstitial cells of Cajal,
 IC-DMP: ICC at the level of the deep muscular plexus,
 IC-IM: intramuscular ICC,
 IC-MY: ICC at the level of the myenteric plexus,
 LIF: leukemia inhibitory factor,
 LOS: lower esophageal sphincter,
 MY: myenteric plexus,
 MDH1: malate dehydrogenase,
 SPTB2: spectrin, nonerythroid, beta subunit,
 RPL8: ribosomal protein L8,
 RT-PCR: reverse transcription-polymerase chain reaction.

Competing interests

NONE DECLARED.

Authors' contributions

YD and IT carried out the molecular studies and the design of study and coordination. BAJP and CC participated in the molecular studies. SW, KS and MF participated in the design of study and coordination. All authors read and approved the final manuscript.

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References

- Langton PD, Ward SM, Carl A, Norell MA and Sanders KM: **Spontaneous electrical activity of interstitial cells of Cajal isolated from canine proximal colon** *Proc Natl Acad Sci USA* 1989, **86**:7280-7284.
- Ward SM, Burns AJ, Torihashi S and Sanders KM: **Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine** *J Physiol (Lond)* 1994, **480**:91-97.
- Huizinga JD, Thuneberg L, Kluppel M, Malysz J, Mikkelesen HB and Bernstein A: **W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity** *Nature (Lond)* 1995, **373**:347-349.
- Burns AJ, Herbert TM, Ward SM and Sanders KM: **Interstitial cells of Cajal inhibit neurotransmission in the stomach** *Proc Natl Acad Sci USA* 1996, **93**:12008-12013.
- Ward SM, Beckett EAH, Wang XY, Baker F, Khoyi M and Sanders KM: **Interstitial cells of Cajal mediate enteric excitatory neurotransmission in the murine fundus** *J Neurosci* 2000, **20**:1393-1403.
- Nishi K, Tokutomi N, Sato D, Tokutomi Y, Sugita M, Lai ZF and Torihashi S: **Identification of gastrointestinal tract pacemaker cells and their functional significance** In: *Gastrointestinal Function, Regulation and Disturbance Volume 14*. Edited by: *Tsuchiya M, Matsuo Y, Kasuga Y, Muto T*. Tokyo: *Excepta Medica*; 1996:27-43.
- Takayama I, Takeda M, Ohno S and Fujino MA: **Experimental pacemaker disease model in mouse jejunum: Impairment of interstitial cells of Cajal by an antagonistic antibody for c-kit**. *Yamanashi Med J* 1998, **13**:53-63.
- Isozaki K, Hirota S, Miyagawa J, Taniguchi M, Shiomura Y and Matsuzawa Y: **Deficiency of c-kit + cells in patients with a myopathic form of chronic idiopathic intestinal pseudo-obstruction** *Am J Gastroenterol* 1997, **92**:332-334.
- Vanderwinden JM, Liu H, DeLaet NH and Vanderhagen JJ: **Interstitial cells of Cajal in human colon and in Hirschsprung's disease** *Gastroenterology* 1996, **111**:901-910.
- He CL, Burgart L, Wang L, Pemberton J, Young-Fadok L, Szurszewski J and Farrugia G: **Decreased interstitial cell of Cajal volume in patients with slow-transit constipation** *Gastroenterology* 2000, **118**:14-21.
- Ordog T, Takayama I, Cheung WKT, Ward SM and Sanders KM: **Remodeling of networks of interstitial cells of Cajal in a murine model of diabetic gastroparesis** *Diabetes* 2000, **49**:1731-1739.
- Takayama I, Seto E, Zai H, Ohno S, Tezuka H, Daigo Y and Fujino MA: **Changes of in vivo gastroenteric motor pattern in pacemaker-deficient (WsRC-Ws/Ws) rats** *Dig Dis Sci* 2000, **45**:1901-1906.
- Takayama I, Daigo Y, Kojima Y and Fujino MA: **Gastroenteric pacemaker system** *Nippon Shoukaku Gakkai Zasshi* 2001, **98**:922-934.
- Takayama I, Horiguchi K, Daigo Y, Mine T, Fujino MA and Ohno S: **The interstitial cells of Cajal and a gastrointestinal pacemaker system** *Arch Histol Cytol* 2002, **65**:1-26.
- Takayama I, Daigo Y, Ward SM, Sanders KM, Yamanaka T and Fujino MA: **Differential gene expression in the small intestines of wild type and W/W^v mice** *Neurogastroenterol Motil* 2001, **13**:163-168.
- Takayama I, Daigo Y, Ward SM, Sanders KM, Walker RL, Horowitz B and Fujino MA: **Novel human and mouse genes encoding an acid phosphatase family member and its down regulation in W/W^v mouse jejunum** *Gut* 2002, **50**:790-796.
- Liang P and Pardee AB: **Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction** *Science* 1992, **257**:967-971.
- Liang P and Pardee AB: **Differential display. A general protocol** *Mol Biotechnol* 1998, **10**:261-267.
- Schuler GD, Boguski MS, Stewart EA, Stein LD, Gyapay G and Rice K et al.: **A gene map of the human genome** *Science* 1996, **274**:540-546.
- Morris SM Jr, Bhamidipati D and Kepka-Lenhart D: **Human type II arginase: sequence analysis and tissue-specific expression** *Gene* 1997, **193**:157-161.
- Gotoh T, Sonoki T, Nagasaki A, Terada K, Takiguchi M and Mori M: **Molecular cloning of cDNA for no hepatic mitochondrial arginase (arginase II) and comparison of its induction with**

- nitric oxide synthase in a murine macrophage-like cell line** *FEBS Lett* 1996, **395**:119-122.
22. Vockley JG, Jenkinson CP, Shukla H, Kern RM, Grody WW and Cedarbaum SD: **Cloning and characterization of the human type II arginase gene** *Genomics* 1996, **38**:118-123.
 23. Liu X, Kim CN, Yang J, Jemmerson R and Wang X: **Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c** *Cell* 1996, **86**:147-157.
 24. Ward SM, Ordog T, Koh SD, Abu Baker S, Jun JY, Amberg G and Sanders KM: **Pacemaking in interstitial cells of Cajal depends upon calcium handling by endoplasmic reticulum and mitochondria** *J Physiol (Lond)* 2000, **525**:355-361.
 25. Rissoan MC, Duhon T, Bridon JM, Bendriss-Vermare N, Peronne C, de Saint VB, Briere F and Bates EE: **Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells** *Blood* 2002, **100**:3295-3303.
 26. Escary JL, Perreau J, Dumenil D, Ezine S and Brulet P: **Leukaemia inhibitory factor is necessary for maintenance of haematopoietic stem cells and thymocyte stimulation** *Nature* 1993, **363**:361-364.
 27. Koblar SA, Turnley AM, Classon BJ, Reid KL, Ware CB, Cheema SS, Murphy M and Bartlett PF: **Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor** *Proc Natl Acad Sci USA* 1998, **95**:3178-3181.
 28. Wester T and Olsen L: **Expression of leukaemia inhibitory factor during the development of the human enteric nervous system** *Histochem J* 2000, **32**:345-334.

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