Research article

A necdin/MAGE-like gene in the chromosome 15 autism susceptibility region: expression, imprinting, and mapping of the human and mouse orthologues

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Abstract

Background: Proximal chromosome 15q is implicated in neurodevelopmental disorders including Prader-Willi and Angelman syndromes, autistic disorder and developmental abnormalities resulting from chromosomal deletions or duplications. A subset of genes in this region are subject to genomic imprinting, the expression of the gene from only one parental allele.

Results: We have now identified the NDNL2 (also known as MAGE-G) gene within the 15q autistic disorder susceptibility region and have mapped its murine homolog to the region of conserved synteny near necdin (Ndn) on mouse Chr 7. NDNL2/MAGE-G is a member of a large gene family that includes the X-linked MAGE cluster, MAGED1 (NRAGE), MAGEL2 and NDN, where the latter two genes are implicated in Prader-Willi syndrome. We have now determined that NDNL2/Ndnl2 is widely expressed in mouse and human fetal and adult tissues, and that it is apparently not subject to genomic imprinting by the PWS/AS Imprinting Center.

Conclusion: Although NDNL2/MAGE-G in the broadly defined chromosome 15 autistic disorder susceptibility region, it is not likely to be pathogenic based on its wide expression pattern and lack of imprinted expression.

Background

Human chromosome 15q is prone to cytogenetic rearrangements, in part due to repetitive elements located therein [1,2]. Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are two neurodevelopmental disorders caused by deletions of 15q11-q13, and neurodevelopmental abnormalities are associated with supernumerary chromosomes derived from inverted duplications of 15q [3]. In addition, an autistic disorder susceptibility locus has been localized to proximal 15q by linkage and association studies [4,5]. AS is associated with UBE3A mutations [6,7]. Strong candidates for PWS have recently emerged and are likely to have an additive effect in causing this disorder [8–12]. In particular, two members of the NDN/MAGE gene family, NDN and MAGEL2, are located in the PWS deletion region and are inactivated in individuals with PWS [8,11–14]. Respiratory and behavioral abnormalities in a mouse deleted for Ndn, the murine orthologue of NDN, may suggest that NDN is implicated in the PWS phenotype [15,16]. We have therefore investigated the possibility that other NDN/MAGE genes may also be present on proximal 15q.
and may be involved in neurodevelopmental disorders. Indeed, we identified a novel necdin-related gene, \textit{NDNL2}, on proximal 15q within the critical region for autistic disorder susceptibility. We have examined the expression of \textit{NDNL2} and its murine orthologue, and placed the murine gene on Chr. 7. The proximal region of chromosome 15 is subject to genomic imprinting, the expression of a gene from only one allele depending on parent-of-origin. Furthermore, the maternal derivation of chromosome abnormalities seen in individuals with autistic disorder suggests a parent-of-origin effect \[4,5\]. We have therefore analyzed the imprinting of human and mouse \textit{NDNL2/Ndnl2}.

\textbf{Results and discussion} Using a BLAST search for genes with sequence similarity to \textit{NDN}, \textit{NDNL2} was identified as a MAGE-like protein on the BAC clone RP11-18H24 (GenBank accession AC016484). This clone also contains the \textit{APBA2} gene which has been physically mapped to within the critical region for a 15q autistic disorder susceptibility locus, and outside the PWS/AS deletion region \[4,5\] (Fig. 1). An open reading frame of 472 amino acids was predicted from the genomic sequence of \textit{NDNL2} and is located within a single exon of the predicted gene. A series of expressed sequence tags (ESTs) were identified through a BLAST search of the EST database and were found to be part of the Unigene cluster Hs.94011. Assembly of the ESTs into a consensus sequence was performed and this sequence was compared to our sequence of IMAGE clones representing \textit{NDNL2} to arrive at a final sequence for the predicted protein (Fig. 2) (also see partial sequence in GenBank AF320911, MAGE-G).

\begin{figure}
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\caption{Map of the human 15q11-q14 region. Location of \textit{NDNL2} was derived from the Ensembl Human Genome server [http://www.ensembl.org/] and the physical location of \textit{APBA2}, which is located on the same genomic clone as \textit{NDNL2}. The large double-headed arrow indicates the common PWS/AS deletion, and BP2 and BP3 indicate the most common proximal and distal breakpoint regions. The breakpoint region for the largest inv dup (15) chromosome \[27\] and the autistic disorder susceptibility region \[5\] are also indicated. The physical distance between BP3 and \textit{APBA2/NDNL2} is as yet unknown due to the presence of repetitive elements in the intervening region.}
\end{figure}

The sequence for murine \textit{Ndnl2} was identified using sequence data obtained from homology searches of mouse ESTs (UniGene cluster Mm.19944). Comparison of the sequence from multiple cDNAs revealed that the predicted mouse protein has 279 amino acids and has 83\% sequence identity with the human protein (Fig. 2). As with other NDN/MAGE family members, the maximum sequence conservation is in the C-terminus of the protein, suggesting a common function for this domain among family members. The murine protein is truncated at the N terminus compared to the human protein at the position equivalent to the methionine at position 168 in the human predicted protein, predicted based on the presence of a strong Kozak consensus surrounding the murine AUG, including a G at the minus three position. In at least two EST clones, including RIKEN clone AK010294 from a library enriched for full-length cDNAs, the predicted amino acids upstream of the putative murine start methionine are not conserved with the human sequence, in sharp contrast with those C-terminal to this methionine (Fig. 2). In addition, the lack of conservation does not appear to be due to the presence of an intron or a sequencing error. Closer inspection of the sequence revealed that \textit{NDNL2} contains only partial similarity to the antigenic nonapeptide characteristic of other MAGE family proteins (Fig. 2). \textit{Ndnl2} is identical in sequence to a predicted protein named "mage-g1" (GenBank accession AF319979).

We identified a DNA polymorphism within \textit{Ndnl2} by direct sequencing of a 469 bp PCR product amplified from \textit{M. musculus} strain C57BL/6J\textit{Ei} and \textit{M. spretus} SPRET/\textit{Ei} DNA. The presence of a T to C transition at position 42 of AV005659 abolishes an \textit{Msp}I site in C57BL/6J\textit{Ei} that is present in SPRET/\textit{Ei}. By genotyping the Jackson laboratory BSS backcross derived from the cross (C57BL/6J\textit{Ei} × SPRET/\textit{Ei})F\textit{i} (Fig. 1), we demonstrated linkage of \textit{Ndnl2} to chromosome 7, at a position genetically inseparable from that of \textit{Ndn} (see Mouse Genome Informatics, [http://www.informatics.jax.org/] for details). As predicted from the location of human \textit{APBA2}
near NDNL2, the murine Apba2 gene is also located in the central region of Chr 7, but the use of different mapping panels for the two genes does not permit their relative order to be established [17]. Based on the high sequence similarity between human and mouse sequences and on the location in a region of conserved synteny we conclude that the NDNL2 and Ndnl2 genes are orthologous in these two species.

The expression of NDNL2 and Ndnl2 was analyzed by northern blot studies (Fig. 3). The observed 1.9 kb RNA transcript roughly corresponds in size to the 1.5 kb cDNA present in IMAGE clone 1134795, which likely contains most of the transcript. The transcript was observed in all human tissues tested, and appeared to be most abundant in testis after adjustments were made for RNA loading. Expression in mouse is also widespread, with a moderately abundant 1.7 kb transcript observed in all tissues tested consistent with the 1.5 kb insert size for cDNA clone RIKEN ID 1010001J10. The origin of the smaller transcript in mouse testis is unknown but may represent an alternative polyadenylation site or alternative transcriptional start site. It is notable that expression of NDNL2/Ndnl2 is widespread although for other family members, including Ndn, Mage2 and Dlxin, the murine homologue of MAGED1, expression is more spatially and temporally limited [12,18,19].

Previous studies have suggested that the imprinted domain on chromosome 15q is limited to a region of about 1.5 Mb surrounding the imprinting center [20,21]. However, given the observed parent-of-origin effects in chromosomal disorders of chromosome 15 [4], we performed experiments to analyze the imprinting of NDNL2 and Ndnl2. To test imprinting of Ndnl2, we examined newborn brain, liver and lung, and embryonic day 12.5 whole embryo RNA samples from the offspring of an interspecific mouse cross for allelic expression using the same murine Msp1 polymorphism as used for genetic mapping. Both parental alleles were detected equally, indicating that expression of Ndnl2 is not imprinted (Fig. 4A). We were unable to identify an intragenic polymorphism in NDNL2 that would allow direct evaluation of the levels of expression of each allele. Instead, we evaluated the effect of deletion of the chromosome 15q11-q13 imprinting center (IC) on the expression of NDNL2. Sporadic PWS and AS patients with de novo deletions not only have a loss of the genes located inside the deletion interval, they also have a loss of the imprinting control element located therein. In a previous study, a somatic, de novo deletion of the IC impaired imprinting of target genes [22]. These target genes are not necessarily limited to the region typically deleted in PWS and AS, which is defined by unstable genomic elements located at the deletion boundaries [1], but could include other genes within the range of the imprinting center. However, our previous study had predicted that it is unlikely that the range of the IC extended beyond the deletion itself [20]. To test this hypothesis and to examine the possibility that NDNL2 is acted on by the IC, we tested the effect of the loss of the IC in sporadic PWS and AS patients on the expression of NDNL2. Using a previously defined assay [20], RNA from lymphoblast and fibroblast cell lines that carry either a paternal 15q11-q13 deletion (PWS) or the

The human and mouse predicted amino acid sequences are aligned with conserved amino acids shaded. Partial sequence of two cDNA clones has been included for comparison of the predicted translation with the longer human open reading frame. The predicted position of a MAGE-like nonapeptide present in other MAGE proteins is underlined.

Figure 2
Sequence comparison of human NDNL2 and mouse Ndnl2 sequence. The human and mouse predicted amino acid sequences are aligned with conserved amino acids shaded. Partial sequence of two cDNA clones has been included for comparison of the predicted translation with the longer human open reading frame.
equivalent maternal deletion (AS) were tested by RT-PCR for expression of *NDNL2*. On repeated trials, both cell lines expressed *NDNL2*, indicating that *NDNL2* is not imprinted by the imprinting center located in the deletion region (Fig. 4B).

The names *NDNL2/Ndnl2* have been approved by the human and mouse nomenclature committees, based on sequence similarity to *NDN* and chromosomal localization. In a recent review of the NDN/MAGE gene family, a multiple sequence alignment clustered the chromosome 15 family members (*NDN*, *MAGEL2/NDNL1*, and *NDNL2/MAGE-G*) together with the X-linked *MAGE-D*, *MAGE-E* and chromosome 3-linked *MAGE-F* genes [23]. *MAGE-D*, known more commonly as *NRAGE* (human) or *dlxin* (mouse) may be ancestral to other members of the NDN/MAGE gene family. We recommend that the human and mouse nomenclature committees evaluate the arguments for the names of genes in this gene family to establish a unified nomenclature that clarifies the relationships among the family members.

Necdin was originally identified as a gene induced on neuronal differentiation of embryonic carcinoma cells, but has more recently been implicated in PWS. Members of the MAGE gene family are recognized for their expression in tumor cells, and a new family member *MAGED1* (*NRAGE/dlxin*) is postulated to have roles in chondrogenesis, neurotrophin signaling and apoptosis [19,24,25]. Although the widespread expression of *NDNL2* does not immediately suggest a role in the neurodevelopmental disorders associated with abnormalities of chromosome 15, *NDNL2* is located within the broad region associated with developmental disorders.

Figure 3
Expression analysis necdin-like 2 in embryonic and adult human and mouse tissues. (A) Northern blots of adult (left and center) and fetal (right) human tissues. The 1.9 kb *NDNL2* transcript is indicated by the arrow. Size markers are as indicated. (B) Northern blot of adult (left) and embryonic (right) mouse tissues shows expression of a 1.7 kb transcript (arrow).
In particular, the location of NDNL2 near D15S1019, proximal to D15S165 implies that copies of NDNL2 are present on most phenotypically relevant inv dup(15) chromosomes [26,27] and may contribute to the findings in patients carrying these supernumerary chromosomes through a doubling of NDNL2 expression levels. However, NDNL2 is outside the more narrowed interstitial duplication interval associated with some autistic phenotypes [28], although long range effects on NDNL2 expression may still occur.

Conclusions
Given the wide range of processes that members of this intriguing gene family participate in and the possibility of redundant function within the gene family, we suggest that the 15q NDN/MAGE family members be considered as candidates for involvement in the neurodevelopmental disorders associated with chromosome 15q.

Materials and methods

**cDNA clones**

Human and mouse cDNAs were obtained from Research Genetics or the MRC Genome Resource Facility and were sequenced using the Thermo Sequenase fluorescent labeled primer cycle sequencing kit with 7-deaza dGTP (Amersham-Pharmacia Biotech, Buckinghamshire, England) and LiCor technology (LiCor 4200 Automatic Sequencer, Lincoln, Nebraska). The human cDNA was represented by IMAGE clone 1147395; mouse cDNAs were IMAGE clones 483464, 2101532 and RIKEN #101001J10.

**Northern blot expression studies**

A PCR product derived from the 3' end of human NDNL2 was generated with oligonucleotide primers NDNL2-3F (5'-GTCTACCCCAAGAAAGAC) and NDNL2-4R (5'-CCTTCCCCAATCTTTCGC), in a 20 µl PCR reaction containing 20 pmol of each oligonucleotide. The PCR was performed as follows: 94°C for 5 min. followed by 30 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 30 s, and final extension at 72°C for 10 min. The PCR product was random prime 32P-dCTP labeled with the Random Primers DNA Labeling System (Life Technologies, Rockville, MD). The labeled probe was hybridized to human adult and fetal Multiple Tissue Northern (MTN) Blots (Clontech Laboratories, Palo Alto, CA., Cat. #7760-1, 7759-1 and 7756-1) in ExpressHyb solution according to manufacturer's directions. The final wash was at 50°C in 0.1X SSC, 0.1%SDS twice for 20 min each time. Exposure to Hyperfilm (Amersham-Pharmacia Biotech) was for...
four days at -80°C. Oligonucleotide primers Ndl2-1F (5'-CTTGGAGTACCCGGGAGATAAC) and Ndl2-2R (5'-CAACACATCTCTAAGGCCTCA) were used for mouse northern blots. These primers amplified a 343 bp DNA fragment corresponding to the 3' end of the Ndl2 gene. The PCR was performed as above but with an annealing temperature of 58°C. Mouse adult and embryo MTN blots (Cat. #7762–1 and 7763–1, Clontech Laboratories) were similarly hybridized with the radioactively labeled Ndl2F/R PCR product. To control for the amount of loaded RNA the same blot was subsequently hybridized with a β-actin or ubiquitin probe demonstrating approximately equal loading in all lanes.

Genetic mapping and imprinting studies
A 469 bp product corresponding to the 3' end of Ndl2 was generated from mouse genomic DNA (C57BL/J or SPRET/Ei) using oligonucleotide primers Ndl2-7F (5'-TGGAAACCCAGCAAGTAAAA) and Ndl2-8R (5'-AGCTACCTGGTTTCTTACGT). DNA samples were sequenced on both strands using the LiCor automated sequencer. DNA amplification products were digested with MspI to produce a 469 bp undigested product in C57BL/6J and 180 bp and 289 bp digested products in DNA from M. spretus. Genetic mapping was performed on the Jackson Laboratories BSS backcross. Cell lines used for human imprinting analysis were PWS lymphoblast GM11515 (both from the NIGMS Human Genetic Mutant Cell Repository) and AS lymphoblast GM11515 (both used for human imprinting analysis were PWS lymphoblast on the Jackson Laboratories BSS backcross. Cell lines DNA from C57BL/6J and 180 bp and 289 bp digested products in approximately equal loading in all lanes.

List of abbreviations
AS (Angelman syndrome), ESTs, (expressed sequence tags), IC (imprinting center), PWS (Prader-Willi syndrome).

References
1. Amos-Landgraf JM, Ji Y, Gottlieb W, Depinet T, Wandstrat AE, Cassidy SB, Driscoll DJ, Rogan PK, Schwartz S, Nichols RD: Chromosome breakage in the Prader-Willi and Angelman syndromes involves recombination between large, transcribed repeats at proximal and distal breakpoints. Am. J. Hum. Genet 1999, 65:370-386
2. Christian SL, Fantes JA, Mewborn SK, Huang B, Ledbetter DH: Large genomic duplicons map to sites of instability in the Prader-Willi/Angelman syndrome chromosome region (15q11-13). Hum. Mol. Genet 1999, 8:1025-1037
3. Nicholls RD: The impact of genomic imprinting for neurobehavioral and developmental disorders. J. Clin. Invest 2000, 105:413-418
4. Maddox LO, Menold MM, Bass MP, Rogala AR, Pericak-Vance MA, Vance JM, Gilbert JR: Autistic disorder and chromosome 15q11-q13: construction and analysis of a BAC/PAC contig. Genomics 1999, 62:325-331
5. Bass MP, Menold MM, Wolfert CM, Donnelly SL, Ravan SA, Hauser ER, Maddox LO, Vance JM, Abramson RK, Wright HH, Gilbert JR, Cuccaro ML, DeLong GR, Pericak-Vance MA: Genetic studies in autistic disorder and chromosome 15. Neurogenetics 2000, 2:219-226
6. Matsuura T, Sutcliffe JS, Fang P, Galjaard R-J, Jiang Y-h, Benton CS, Roevers JM, Beauvieux M: The novel truncated E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nature Genet 1997, 15:74-77
7. Kishino T, Lalande M, Waggstaff J: UBE3A/E6-AP mutations cause Angelman syndrome. Nature Genet 1997, 15:70-72
8. MacDonald HR, Wier B, Kieferman M, Lalande M, Brannan CI, Horstemke B, Bachelier JP, Broius J, Huttonhofer A: From the cover: identification of brain-specific and imprinted small nuclear RNA genes exhibiting an unusual genomic organization. Proc. Natl. Acad. Sci U S A 2000, 97:14311-14316
9. de los Santos T, Schweizer J, Rees CA, Francke U: Small evolutionarily conserved RNA, resembling C/D box small nuclear RNA, is transcribed from PWCR1, a novel imprinted gene in the Prader-Willi deletion region, which is highly expressed in brain. Am. J. Hum. Genet 2000, 67:1067-1082
10. Boccaccio I, Glatt-Deely H, Watrin F, Roeckel N, Lalande M, Muscatelli F: The human MAGEL2 gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. Hum. Mol. Genet 1999, 8:2497-2505
11. Lee S, Kozlov S, Hernandez L, Chamberlain SJ, Brannan CI, Stewart CL, Wier B: Expression and imprinting of MAGEL2 suggest a role in Prader-Willi syndrome and the homologous murine imprinting phenotype. Hum. Mol. Genet 2000, 9:1813-1819
12. Jay P, Rougeulle C, Massacrier A, Moncla A, Mattei MG, Malzac P, Roeckel N, Taviaux S, Lefranc JL, Cau P, Berta P, Lalande M, Muscatelli F: The human necdin gene, NDN, is maternally imprinted and its mouse homologue is similarly expressed and located in the Prader-Willi syndrome chromosomal region. Nature Genet 1997, 17:357-361
13. Sutcliffe JS, Han M, Christian SL, Ledbetter DH: Neuronally expressed necdin gene: an imprinted candidate gene in Prader-Willi syndrome. Lancet 1997, 350:1520-1521
14. Gerard M, Hernandez L, Wier B: Disruption of the mouse necdin gene results in early postnatal lethality: a model for neonatal distress in Prader-Willi syndrome. Nature Genet 1999, 23:199-202
15. Muscatelli F, Abrous DN, Massacrier A, Boccaccio I, Moal ML, Cau P, Cremer H: Disruption of the mouse necdin gene results in hypothalamic and behavioral alterations reminiscent of the human Prader-Willi syndrome. Hum. Mol. Genet 2000, 9:3101-3110
16. Blanco G, Irving NG, Brown SD, Miller CC, McLoughlin DM: Mapping of the human and murine X1-like genes (APBA2 and apb2), the murine Fe65-like gene (Fe65b), and the human Fe65-like gene (APBB2): genes encoding phosphotyrosine-binding domain proteins that interact with the Alzheimer's disease amyloid precursor protein. Mamm. Genome 1998, 9:473-475
17. Yoshikawa K: Cell cycle regulators in neural stem cells and postmitotic neurons. Neurosci. Res 2000, 37:1-14
18. Masuda Y, Sasaki A, Shibuya H, Ueno N, Ikeda K, Watanabe K: Dixin-1, a novel protein that binds Dlx5 and regulates its transcriptional function. J. Biol. Chem 2000, 17:5331-5339
19. Lee S, Wier B: Identification of novel imprinted transcripts in the Prader-Willi/Angelman syndrome deletion region: further evidence for regional imprinting control. Am. J. Hum. Genet 2000, 66:848-858
21. Meguro M, Mitsuwa K, Nomura N, Kohda M, Kashiwagi A, Nishigaki R, Yoshioka H, Nakao M, Oishi M, Oshimura M: Large-scale evaluation of imprinting status in the Prader-Willi syndrome region: an imprinted direct repeat cluster resembling small nucleolar RNA genes. *Hum. Mol. Genet* 2001, 10:383-394

22. Bielinska B, Blaydes SM, Buiting K, Yang T, Krajewksa-Walasek M, Horsthemke B, Brannan CI: De novo deletions of SNRPN exon 1 in early human and mouse embryos result in a paternal to maternal imprint switch. *Nat Genet* 2000, 25:74-78

23. Chomez P, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S: An overview of the MAGE gene family with the identification of all human members of the family. *Cancer Res* 2001, 61:5544-5551

24. Jordan BW, Dinev D, LeMellay V, Troppmair J, Gotz R, Wixler L, Sendner M, Ludwig S, Rapp UR: NRAGE is an inducible IAP-interacting protein that augments cell death. *J. Biol. Chem* 2001, 6:1-6

25. Salehi AH, Roux PP, Kubu CJ, Zeindler C, Bhakar A, Tannis LL, Verdi JM, Barker PA: NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron* 2000, 27:279-288

26. Wandstrat AE, Leana-Cox J, Jenkins L, Schwartz S: Molecular cytogenetic evidence for a common breakpoint in the largest inverted duplications of chromosome 15. *Am. J. Hum. Genet* 1998, 62:925-936

27. Vandstrat AE, Schwartz S: Isolation and molecular analysis of inv dup(15) and construction of a physical map of a common breakpoint in the largest inverted duplications of chromosome 15. Am. J. Hum. Genet 1998, 62:925-936

28. Repetto GM, White LM, Bader PJ, Johnson D, Knoll JH: Interstitial duplications of chromosome region 15q11q13: clinical and molecular characterization. *Am. J. Med. Genet* 1998, 79:82-89