

Alpha T-catenin (CTNNA3): a gene in the hand is worth two in the nest

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Abstract Alpha-T-Catenin (CTNNA3) is a key protein of the adherens junctional complex in epithelial cells playing a crucial role in cellular adherence. What makes this gene particularly interesting is that it is located within a common fragile site, is epigenetically regulated, is transcribed through multiple promoters, and generates a variety of alternate transcripts. Finally, CTNNA3 has a nested gene (LRTMM3) embedded within its genomic context transcribed in the opposite direction. Apart from the complexity of its regulation, alterations in both CTNNA3 and LRTMM3 are implicated in human disease.

Keywords CTNNA3 · LRTMM3 · Alpha-T-catenin · Adherens junction · Nested gene

The cell adhesion molecule alpha-T-catenin (CTNNA3)

The adherens junctional (AJ) complex is one of the critical mediators of cell–cell adhesion giving structural integrity to epithelial cells, a fundamental feature in maintaining the epithelial phenotype and a functional epithelial monolayer.

Alpha catenins are integral members of the AJ, functioning not only as static structural proteins but also as regulators of actin dynamics [1, 2]. The alpha catenin family of proteins consists of three members, alpha-E-catenin (CTNNA1, 5q31.2), alpha-N-catenin (CTNNA2, 2p12), and alpha-T-catenin (CTNNA3, 10q22.2).

Alpha-T-catenin (CTNNA3) is the most recently characterized member of the alpha catenin family. CTNNA3 encodes a protein of 895 amino acids with a predicted molecular weight of 100 kDa. At the amino acid level, the exon–exon boundaries of CTNNA3 are identical to CTNNA2, with most also identical in CTNNA1 [3]. This suggests that CTNNA2 and CTNNA3 are closely related, and that all members may have diverged from a common ancestor. The central role of CTNNA3 is to provide strong cell–cell adhesion. Janssens and colleagues [4] demonstrated that the overexpression of CTNNA3 in a CTNNA1 negative colon carcinoma cell line resulted in the reassembly of the adherens and tight junctions through the recruitment of CTNNA3 interacting partners such as E-cadherin, β -catenin, plakoglobin, and ZO-14.

CTNNA3 resides in a genomic locus that displays a high degree of organizational complexity, regulated by a number of intricate methods. Specifically, CTNNA3 is located within a common fragile site (CFS) and is epigenetically regulated. Moreover, a number of transcription factors control the functional expression of CTNNA3 and is predicted to be transcribed by multiple alternative promoters. Finally, LRTMM3, a leucine-rich repeat transmembrane neuronal gene, is nested within CTNNA3.

CTNNA3 is epigenetically regulated

Based on linkage studies in pre-eclampsia patients, it has been demonstrated that the 10q22.2 locus where CTNNA3

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resides displays a parent-of-origin phenotype, as affected sisters shared alleles that were maternal in origin. This hypothesis is supported by the observation that CTNNA3 expression is down-regulated in placentas of complete androgenetic (male) origin [5]. SNP analysis of CTNNA3 in placental samples has also shown polymorphic imprinted expression of CTNNA3, with monoallelic expression of CTNNA3 in one out of three informative samples. Subsequent staining of the placental tissue confirmed that the preferential expression status was cell-type-specific, with monoallelic expression and down-regulation of CTNNA3 in the villus trophoblast, compared to biallelic expression of CTNNA3 and normal staining in the extravillous trophoblast [6].

Transcription factor transactivation of CTNNA3

Conserved transcription factor binding sites between human, mouse, and rat for GATA family factors, MEF2C and E47/HAND have been identified in CTNNA3. GATA-4 and MEF2C are known to bind to the GATA box 2 in the major promoter of CTNNA3 and this element is essential in directly regulating expression of CTNNA3 in cardiac muscle cells. The co-transfection of GATA-4 with MEF2C leads to a synergistic activation of the CTNNA3 promoter [7].

STOX1 localizes to 10q21.3 adjacent to CTNNA3, and is a member of the winged-helix gene family related to the forkhead (FOX) multigene family. A common mutation of STOX1, a tyrosine to histidine amino acid substitution (Y153H), has been identified as a possible susceptibility variant in pre-eclampsia [8]. Transfection with the STOX1 Y153H variant led to a sixfold increase in CTNNA3 expression, negatively regulating trophoblast invasion [9].

Alternative transcripts of CTNNA3

CTNNA3 was originally identified in testis, and recent studies in mice have identified two alternative transcripts of CTNNA3 with expression restricted to testis [10]. The first transcript, alpha-T-catenin B (AT-B), is transcribed from a putative upstream promoter in CTNNA3. This transcript lacks the first non-coding exon of full-length CTNNA3, which is replaced by an alternative exon. However, the open reading frame of CTNNA3 is preserved in this transcript, encoding a protein of 100 kDa. The second alternative transcript identified in this study was alpha-T-catenin X (AT-X). This transcript lacks the first six exons of full-length CTNNA3 and is transcribed from an alternative promoter. The transcript encodes a protein, Isoform-X, of 628 amino acids with a predicted molecular weight of

70 kDa. The first 14 amino acids of this protein is Isoform-X specific, the remaining sequence encodes for the central and carboxy regions of the full-length CTNNA3 protein. The AT-X transcript lacks the vinculin homology (VH1) domain of full-length CTNNA3, rendering it unable to bind β -catenin. Moreover, overexpression of Isoform-X is unable to restore cell adhesion in an alpha catenin-negative colon carcinoma cell line. Expression of this truncated isoform has been shown to be both tissue- and stage-specific, as Isoform-X expression is restricted to testis, with upregulation of expression from the onset of puberty. This indicates that the Isoform-X transcript may play a role in spermatogenesis [10]. Alternative transcripts of CTNNA3 have also been identified in human brain tissue [11]. Specifically, a second transcript has been identified lacking the first non-coding exon of CTNNA3 and substituted by two other exons transcribed from a putative alternative promoter, containing a possible binding site for the nervous-system-specific transcription factor N-Oct 3 [11].

CTNNA3 is located in a common fragile site

CTNNA3 is the fifth largest gene in the human genome, spanning 1.78 Mbs in chromosome 10 and is located in the common fragile site FRA10D. Over half of the 20 largest human genes emanate from CFS regions [12]. Common fragile sites are regions of profound genomic instability, and are biologically significant due to their role in a number of genomic alterations that are frequently found in many different types of cancer [13]. Interestingly, many of these large common fragile genes such as the tumor suppressor genes FHIT located in FRA3B, WWOX/FOR, located in FRA16D and Parkin located in FRA6E are down-regulated in cancer, and express distinct alternative transcripts during tumorigenesis [13–15]. FHIT has been implicated as a downstream target of NF kappa B and the AKT-survivin pathway and along with WWOX/FOR is thought to be involved in the cellular response to stress [16]. The other alpha catenins, E (located at 5q31) and N (located at 2p12), are also situated on or near common fragile sites (5q31.1 and 2p13, respectively), however, the genomic instability observed at these sites is less than at FRA10D [17].

CTNNA3 in Alzheimer's disease

The gene encoding alpha-T-catenin, CTNNA3, is positioned within a region on chromosome 10 that shows strong evidence of linkage to Alzheimer's disease (AD), specifically late-onset Alzheimer's disease (LOAD). Therefore, CTNNA3 has been postulated as a good positional candidate gene for this disorder. For this reason,

numerous groups have investigated if there is a relationship between CTNNA3 and LOAD using SNP analysis with conflicting results (Table 1).

The first study by Ertekin-Tanner et al. [18] looked at plasma amyloid beta protein (A β 42) levels; a protein implicated in the development of LOAD. They found that two single nucleotide polymorphisms (SNPs) (rs7070570 and rs12357560) within the CTNNA3 gene were associated with elevated plasma A β 42 levels in two separate cohorts of 10 and 12 LOAD families [18]. Subsequent to this, however, Blomqvist et al. [19] found no association between the same two SNPs and clinical markers of LOAD such as age-at-onset (AAO), mini-mental state examination (MMSE) scores, CSF A β 42, tau levels, and senile plaque and neurofibrillary tangle (SP-NFT) scores. This was a case-controlled study of 1,006 patients from Sweden and Scotland [19]. Data from a further study genotyping 30 SNPs in >700 case-matched patients, again found no significant association [11]. However, this latter study did demonstrate that alpha-T-catenin was expressed in brain

tissue and demonstrated that similar to other catenins alpha-T-catenin inhibits Wnt signaling in the brain [11].

In a study of 738 AD families, Martin et al. [20] demonstrated that two SNPs (rs7911820 and rs7074454) within CTNNA3 and a SNP located in its nested gene LRRTM3 (SNP1) demonstrated an association with AD (Table 1). However, when they looked at a case-control sample of 1,442 patients, no such association was found. Equally, a subsequent case-control study from Italy again demonstrated no association between AD and SNP genotyping in CTNNA3 [21].

Bertram et al.'s study [22] identified a new SNP in CTNNA3 (rs4548513) and genotyped it in a cohort of 437 mainly LOAD families finding an association with AD ($p = 0.02$). However, the same group in a case-controlled sample of 489 discordant siblings showed no association [22]. Finally, Miyashita et al.'s [23] 2,762 patients (1,313 LOADs and 1,449 control) found an association with LOAD and one out of seven SNPs (rs713250) in females (but not males) independent of APOE alterations.

Table 1 Studies analyzing the association between SNP genotyping in CTNNA3 and AD

Ref.	Compared to	Study type	SNPs	Cohort size	Significant result
Ertekin-Tanner et al. [18]	Serum A β 42	Cohort	rs7070570 rs12357560	10 families (292 patients)	$p = 0.0001$ $p = 0.0006$
			rs7070570 rs12357560	12 families (88 patients)	$p = 0.08$ $p = 0.004$
Blomqvist et al. [19]	CSF A β 42 Tau levels age of onset (AAO) MMSE scores senile plaque and neurofibrillary tangle (SP-NFT)	Case-control	rs7070570 rs12357560	1,006 patients	N/S N/S
Busby et al. [11]	Alzheimer's disease	Case-control	31 SNPs	792 patients	N/S
Martin et al. [20]	Alzheimer's disease	Cohort	rs7074454 rs7911820 SNP1(LRRTM3)	738 families	$p = 0.01$ $p = 0.03$ $p = 0.007$
		Case-control	rs7074454 rs7911820 SNP1(LRRTM3)	1,442 patients	N/S N/S N/S
Cellini et al. [21]	Alzheimer's disease	Case-control	rs7070570 rs12357560	704 patients	N/S N/S
Bertram et al. [22]	Alzheimer's disease	Cohort	rs4548513	437 families	$p = 0.02$
		Case-control	rs4548513	489 patients	N/S
Miyashita et al. [23]	Alzheimer's disease	Case-control	rs7909676 rs2394287 rs4459178 rs10997307 rs12258078 rs10822890 7132507 ^a	2,762 patients	N/S

^a Significant in females only

Overall, these studies demonstrate conflicting results as to whether CTNNA3 plays a role in AD specifically LOAD. If a link exists, it appears to be in familial cohorts of LOAD and not in sporadic cases and possibly associated with a gender bias to female patients.

CTNNA3 and cardiomyopathy

Similar to AD, CTNNA3's location on chromosome 10 has suggested a possible linkage to autosomal-dominant dilated cardiomyopathy (DCM). Specifically, as alpha-T-catenin is expressed in heart tissue, it was postulated as a candidate disease gene in a family showing DCM linkage to the 10q21-q23 locus. To examine this, Janssens et al. [3] screened all 18 exons of CTNNA3 for mutations but failed to detect any association with DCM [3]. The same group, however, later demonstrated that alpha-T-catenin co-localizes with plakophilin 2; mutations of which lead to cardiac muscle malfunction [24]. Finally, Christensen et al. [25] identified mutations in CTNNA3 using direct sequencing of DNA from 65 patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) in an attempt to find a link with alpha-T-catenin. No association between CTNNA3 mutations and ARVC was demonstrated [25].

The nested gene LRRTM3

Nested genes are a form of overlapping gene and are often referred to as a "gene within a gene." Nested genes are totally embedded within their host gene and may be transcribed in the same or the opposite direction to their host [26]. It is proposed that they have emerged by the insertion of a DNA sequence, which arose by gene duplication or retrotransposition, into an intron of a pre-existing gene. A genome-wide study of nested genes has identified that "host" genes are generally larger than the genome average, and nested genes are much smaller than average, with almost half of nested genes having only one exon. Interestingly, nested genes display high levels of tissue-specific expression [27].

Two hypotheses have been proposed for the expression of nested gene pairs. The functional co-regulation hypothesis predicts a positive correlation between levels of expression in different tissues and the transcriptional collision hypothesis predicts a negative correlation [28]. Co-regulation of nested gene pairs has often been observed when both genes produce products of related function. It is thought that the gain from this relationship is that the combined action of the two gene products may be more effective than each separately. For example, the oligodendrocyte-myelin glycoprotein gene (OMgp) is nested

within the first intron of the neurofibromin 1 gene (NF1). Both of these genes are expressed at similar levels within the central nervous system and both function to inhibit cell proliferation [29]. A negative correlation may arise when there is transcriptional interference between the partners. The interference might take place by direct competition for the transcription apparatus or by formation of double-stranded RNAs. This is anticipated to occur when the products of the gene partners have different functions [30]. Moreover, recent research has shown that overlapping genes are four times more likely to be co-expressed than expected by random probability, lending credence to the functional co-regulation hypothesis [26].

Interestingly, all three members of the alpha catenin family harbor leucine-rich repeat transmembrane neuronal (LRRTM) genes nested within the largest intron of each catenin family member. LRRTM1 is nested within alpha-N-catenin (CTNNA2) and LRRTM2 is nested within alpha-E-catenin (CTNNA1). Likewise, LRRTM3 is located within the largest intron (400 kb) of alpha-T-catenin (CTNNA3). LRRTM4 is closely related to LRRTM3 and may have arisen as a result of an ancestral duplication [31]. However, unlike the other three LRRTM family members, LRRTM4 is not located within an alpha catenin. All four LRRTM genes contain leucine-rich repeats (LRRs). These 20–29 residue sequence motifs are found in a large number of functionally unrelated proteins and are frequently involved in the formation of protein–protein interactions [32]. Amino acid analysis has shown that all LRRTM proteins possess a similar domain structure characterized by a signal sequence; ten extracellular LRRs flanked by cysteine-rich domains, and a transmembrane region followed by an intracellular tail of 71 or 72 amino acids.

Functionally, LRRTM3 is a transmembrane protein and similar to CTNNA3 is thought to mediate cell adhesion [31]. The LRRTM family members are also proposed to function as synaptic organizers during synapse development [33]. Interestingly, LRRTM3 displays the weakest activity of all LRRTMs, suggesting that its role is different in comparison to the other family members. Indeed, given LRRTM3's tissue-specific localization to the brain, notably in the hippocampus and its chromosomal localization to 10q22.2, has also resulted in its candidacy as a possible LOAD susceptibility gene [34].

Nested genes often show tissue-specific expression [27]. LRRTM3 displays mRNA expression predominantly in the brain [31]. Interestingly, LRRTM3 also is predicted to contain a number of alternative promoters that produce alternatively spliced transcripts. This allows for an effective regulation of expression of the gene in different cell populations and at different stages of development [31].

The expression of tissue-specific genes such as LRRTM3 may be controlled by DNA methylation. Unlike

the classical thinking that CpG Islands are normally unmethylated, tissue-specific differentially methylated regions (T-DMRs) silence the expression of genes in tissues where it should not be expressed. However, to date, few T-DMRs have been identified [35]. Interestingly, STOX1 (10q22.1), a transcriptional activator of CTNNA3 containing a winged-helix domain harboring a T-DMR within a CpG Island in its promoter, [36] resides in the same chromosomal locus as CTNNA3. The T-DMR in STOX1 occupies part of the CpG Island, and has been shown to be methylated in a number of somatic tissues such as kidney and brain, but unmethylated in sperm [36].

Conclusions

In summary, CTNNA3 is a key protein of the AJ complex in epithelial cells, playing a crucial role in cellular adherence. This extraordinary gene is epigenetically regulated, is transcribed through multiple promoters, and generates a variety of alternative transcripts. The location of this catenin at 10q22.2 has identified it as a candidate gene in the development of both AD and DCM. However, conflicting genotyping SNP analyses associating CTNNA3 with the development of AD has immersed. It does, however, appear to have some implication in the development of LOAD in familial cohorts. In relation to CTNNA3 gene expression, it appears to be regulated by a variety of different mechanisms involving alternative promoters, the generation of alternative transcripts and indeed subjected to epigenetic regulation. Analyzing both the epigenetic and transcriptional regulation in conjunction with STOX1 may prove beneficial in elucidating how and if CTNNA3 interacts in the development of LOAD. Similarly, with DCM, investigation to date has dealt with mutation analysis but transcription and epigenetic analysis may uncover a link with plakophilin 2 function and the development of DCM. In cancer, specifically urothelial carcinoma of the bladder (UCB), we have demonstrated a significantly lower level of CTNNA3 in the tumor samples compared to the paired normals, [37] suggesting that in this setting, removal of this protein may facilitate an increased capacity for invasion and metastases and deserves further investigation in this arena.

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