# *RAB3GAP1*, *RAB3GAP2* and *RAB18*: disease genes in Micro and Martsolf syndromes

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## Abstract

**Micro syndrome (OMIM 60018) and Martsolf syndrome (OMIM 21270) are related rare autosomal recessive disorders characterized by ocular and neurological abnormalities and hypothalamic hypogonadism. Micro syndrome has been associated with causative mutations in three disease genes: *RAB3GAP1*, *RAB3GAP2* and *RAB18*. Martsolf syndrome has been associated with a mutation in *RAB3GAP2*. The present review summarizes the current literature on these genes and the proteins they encode.**

## Micro and Martsolf syndromes

Micro syndrome and Martsolf syndrome are autosomal recessive disorders first described in 1993 [1] and 1978 [2] respectively. Multiple cases of Micro syndrome have been reported in the literature, and a broad consensus has emerged with regard to its presentation [3–10]. One feature of the disease is significant visual impairment, with eye abnormalities including congenital bilateral cataracts, microphthalmia, microcornea (<10 mm in diameter), and small atonic pupils that do not react to light or mydriatic agents. Despite early cataract surgery, patients' vision remains poor (only light perception), and this is as a result of progressive optic atrophy and severe cortical visual impairment [confirmed by normal ERG (electroretinogram) and absent VEPs (visually evoked potentials)]. Brain abnormalities associated with Micro syndrome include postnatal microcephaly ($-4$ to $-6$ S.D.), with congenital microcephaly only rarely observed. Malformations, typically polymicrogyria and hypoplasia of the corpus callosum, are frequently seen upon MRI (magnetic resonance imaging), and may account for the seizures suffered by a subset of patients. Very severe developmental delay is evident, and, although they may develop early milestones such as smiling, the affected children usually achieve no developmental milestones beyond those at the 4 month level: they do not learn to crawl, pull up to a standing position, walk or talk. Characteristically, patients show congenital hypotonia and, from approximately 8–12 months, lower-limb spasticity leading to contractures. This spasticity is progressive, affecting the upper limbs and leading to paraplegia later in life. Hypogonadism is most common in male patients, and is thought to be hypothalamic in origin because, in one case, it was responsive to treatment with hCG (human chorionic gonadotropin) [8]. Facial dysmorphism is subtle and may not be observed in all patients.

Martsolf syndrome shares many of the characteristics of Micro syndrome, although it is less severe and is much less frequently reported [11–15]. The observed eye abnormalities are similar, but without optic atrophy and cortical visual impairment, they result in less functional visual impairment. Microcephaly and intellectual disability are also less pronounced, and the progressive spasticity may be confined to the lower limbs.

## *RAB3GAP1*

Mutations in *RAB3GAP1* were the first reported cause of Micro syndrome [3]. They are also the most frequently reported cause [3,9,10]. *RAB3GAP1* orthologues are present in plants as well as animals, indicating that it evolved from a common eukaryotic ancestor. However, despite being highly conserved, its physiological role may have changed or acquired new significance much more recently in evolution, as it is one of a small group of genes with elevated brain-expression levels in humans compared with non-human primates [16]. Rab3GAP1 has been characterized as a GAP (GTPase-activating protein) for Rab3 isoforms, although *RAB3* genes are thought to have originated in animals, later than *RAB3GAP1* [17]. It was first isolated as a 130 kDa Rab3A-binding protein from the synaptic soluble fraction of rat brain and was found to possess GAP activity towards Rab3, but not Rab2, Rab5A or Rab11 [18]. Although enriched in the synaptic soluble compartment, it is largely cytosolic and broadly expressed in a variety of tissues in rodents [3,18]. It remains the only identified RabGAP that lacks a characteristic TBC (Tre-2/Bub-2/Cdc16) GAP domain, although its novel C-terminal GAP domain is suggested to operate in the same way [19].

The physiological function of Rab3GAP1 has been explored in mouse and fruitfly models in which the corresponding gene has been disrupted [20,21]. In mice, disruption of *Rab3gap1* leads to enhanced STD (short-term depression) and increased ppf (paired-pulse facilitation) at CA1 hippocampal neurons [20]. In fruitflies, very similar effects are seen at the NMJ (neuromuscular junction) when

**Key words:** Martsolf syndrome, Micro syndrome, Rab protein, Rab3GAP1, Rab3GAP2, Rab18.

**Abbreviations used:** ER, endoplasmic reticulum; GAP, GTPase-activating protein; Gdi1/GDI1, GDP dissociation inhibitor 1; NMJ, neuromuscular junction; V-ATPase, vacuolar $H^+$-ATPase.

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*rab3-GAP* is disrupted, suggesting a general defect in neurotransmission [21]. Interestingly, very similar effects are also seen in mice lacking *Gdi1* (GDP dissociation inhibitor 1) [22]. This is significant, first because mutations in *GDI1* are associated with X-linked mental retardation (OMIM 300849), and secondly because Rab3GAP1 and GDI1 act sequentially to promote Rab3-GTP hydrolysis and the subsequent removal of Rab3-GDP from membranes, together promoting Rab3 recycling. Thus it is tempting to speculate that the animal models provide a correlate for the cognitive deficit seen in Micro syndrome and that this relates in some way to defective Rab3 recycling.

The fruitfly *rab3-GAP* model was studied because of its identification in a screen for genes involved in one form of 'synaptic homoeostasis' (a term used to describe mechanisms that serve to maintain neurotransmission) [21]. In wild-type fruitflies, there is a compensatory increase in what is suggested to be presynaptic neurotransmitter release potential at the NMJ when postsynaptic responses are attenuated. This occurs both upon acute blockade of postsynaptic glutamate receptors with philanthotoxin-433 and upon chronic blockade in a GluRIIA mutant animal. However, in *rab3-GAP* mutants, the effect is lacking, indicating either that mutant neurons cannot increase quantal neurotransmitter release past a certain threshold, or that they are insensitive to homoeostatic signalling. Interestingly, the homoeostatic response is present in *rab3* mutants and also in *rab3*/*rab3-GAP* double mutants; again, this suggests that the repression of this response in the *rab3-GAP* mutants may be mediated via Rab3, its mislocalization or its defective recycling.

## *RAB3GAP2*

The first pathogenic mutation identified in *RAB3GAP2* was associated with a case of Martsolf syndrome [11]. This was a homozygous non-synonymous missense mutation, but also caused abnormal splicing of the transcript such that a truncated transcript was produced and levels of the full-length transcript were reduced. Thus it was initially unclear whether loss of functional Rab3GAP2 was less deleterious than loss of Rab3GAP1, or whether residual protein expression or function ameliorated the condition of Martsolf patients. More recently, an additional pathogenic *RAB3GAP2* mutation has been described as causative in a case of Micro syndrome and the details of this case suggest that loss-of-function mutations in *RAB3GAP2* and *RAB3GAP1* produce a clinically indistinguishable condition [6]. This finding supports the latter suggestion and therefore supports the hypothesis that Micro and Martsolf syndromes represent a heterogeneous condition, the severity of which is related to the severity of the causative mutation. However, as this hypothesis is currently based on the evidence of only two pathogenic mutations, further evidence would be useful to confirm it.

Rab3GAP2 was first reported as a 150 kDa protein that co-purified with Rab3GAP1 in the synaptic soluble fraction [18]. In later work, its cDNA was cloned and its tissue and subcellular distribution found to be similar to those of Rab3GAP1 [23]. Rab3GAP1 and Rab3GAP2 were found to form a complex *in vitro* and to co-immunoprecipitate *in vivo* and so Rab3GAP2 was characterized as a non-catalytic subunit of the Rab3GAP complex. Rab3GAP2 does not affect the *in vitro* GAP activity of Rab3GAP1 and so it is possible that it serves to stabilize, regulate or localize Rab3GAP1 correctly in cells [11,23]. However, two independent lines of evidence indicate that they do not always function as a complex. First, *in situ* hybridization experiments in zebrafish suggest that Rab3GAP2 expression is more restricted than that of Rab3GAP1, and, in particular, that each protein has an overlapping, but distinct, expression pattern in the brain and eye [11]. Secondly, immunoprecipitation studies suggest the formation of a mutually exclusive complex: Rabconnectin-3a (DMXL2) immunoprecipitates with Rab3GAP2, but not Rab3GAP1, and recombinant Rab3GAP2, but not Rab3GAP1, immunoprecipitates with rabconnectin-3a [24].

Rab3GAP2 has been found to interact with Rabconnectin-3b (WDR7) as well as Rabconnectin-3a, although in both cases, the interaction may be indirect as it was not shown *in vitro* [25]. Rabconnectin-3a and Rabconnectin-3b form a complex and are implicated in regulation of the V-ATPase (vacuolar $H^+$-ATPase), which is required for the acidification of organelles including late endosomes and secretory granules [25–27]. In the absence of either Rabconnectin or the ATPase, a number of transmembrane proteins accumulate in a late endosomal compartment and this is associated with a loss of Notch signalling, possibly due to disruption of $\gamma$-secretase activity [26]. Although Rab3GAP2 has not been linked to Notch signalling, the Rabconnectins are very highly expressed in brain and also localize to synaptic vesicles which require V-ATPase activity for their acidification and loading with neurotransmitters [24,25]. Thus, whereas Rab3GAP1 has been implicated in modulating quantal neurotransmitter release, Rab3GAP2 may be implicated in modulating the quantal content of individual synaptic vesicles.

## *RAB18*

*RAB18* is the most recently discovered disease gene for Micro syndrome and, as with *RAB3GAP1* and *RAB3GAP2*, the disease is associated with loss-of-function mutations [7]. These include a non-synonymous missense and a deletion mutation that each affect only a single amino acid, but abolish nucleotide binding of the mutant Rab18s and an anti-termination mutation that disrupts prenylation of the protein. As with *RAB3GAP1*, a *RAB18* orthologue is found in plants and so it is highly conserved. However, attempts to classify the encoded protein on the basis of phylogeny and active conformation have not provided any clues as to its function [17,28]. It has been suggested in the literature that Rab18 is one of a group of Rab proteins with a putative exocytotic role [29,30]. However, the systematic application of phylogenetic algorithms and principal component analysis

to the Rab protein family did not place the protein in this group [31]. Thus it remains to be shown whether Rab18 is part of some larger functional subgroup of Rab proteins or possesses a distinct role.

Rab18 was partially cloned in 1992 [32], fully cloned in 1993 [33], and shown to be widely expressed in different tissues [34]. Initial characterization showed that it localized to endosomes in polarized epithelia [34]. Subsequent research has suggested, however, that it adopts various different subcellular localizations and can function in a cell-type-specific manner [35–40]. It appears to be expressed at different levels in the different tissues examined, with it being highly expressed in the brain and the heart. Furthermore, in a number of situations, its expression is reported to be inducible. For example, it is induced in endothelial cells stimulated with histamine [41], in differentiating adipocytes [42] and in the brains of alloxan-treated rats [43]. The most convincing evidence that the protein serves discrete cellular roles, however, comes from a series of divergent reports on its cellular localization and function.

Rab18 has been reported to associate with endosomes in polarized epithelia where it has been suggested to function in endocytosis [34]. In a macrophage cell line, it localized to a specialized phagocytic compartment and was suggested to function in immune evasion [36]. Several reports have found that the protein can localize to lipid droplets in adipocyte, fibroblast and epithelial cell lines [38,39,42], and this has been convincingly linked to roles in lipogenesis [42] and lipolysis [38,42]. However, in the same cells, it can localize to ER (endoplasmic reticulum) and Golgi under some circumstances [35,38], and one report has suggested that it functions in Golgi–ER trafficking [35]. In endocrine cell lines and in pituitary melanotropes, it has been found to be associated with secretory granules and suggested to function in modulation of the secretory response [40]. These reports are difficult to reconcile. However, a common feature of several of them is that the recruitment of Rab18 to intracellular organelles can be enhanced by cellular stimulation. In adipocytes, for example, recruitment of Rab18 to lipid droplets was promoted by treatment with insulin, which stimulates lipogenesis [42], or by the $\beta$-adrenoceptor agonist isoprenaline (isoproterenol), which stimulates lipolysis [38,42]. Similarly, in PC12 and AtT20 cells, stimulation with KCl led to redistribution of Rab18 from the cytosol to a subpopulation of secretory granules [40].

The above demonstrates that the physiological roles of Rab18 may in fact be much broader than can be inferred from the effects of its absence in Micro syndrome. Rab proteins can show various degrees of functional redundancy in different tissues, either as a result of the different expression patterns of Rabs with overlapping cellular roles, or because a given Rab may mediate responses through tissue-specific effector proteins. In the case of Rab18, the examination of its roles in the eye and nervous system, and in particular its functional relationship to Rab3GAP1 and Rab3GAP2, will be important.

## Summary

*RAB3GAP1*, *RAB3GAP2* and *RAB18* have broad expression patterns, but a key question for future research into Micro and Martsolf syndromes is how the proteins encoded by these genes act together in neurons. Rab3GAP1 is clearly implicated in regulating presynaptic neurotransmitter release in a Rab3-dependent manner [21]. Furthermore, this involvement may centre on the recycling of Rab3 between the membrane and cytosolic compartments, and donor and target membranes, an accepted function of all RabGAPs that may be mediated by other non-substrate Rabs acting in 'cascades' [44]. The loss of Rab3GAP1 does not equate to a loss of Rab3, as animal models without Rab3 display a very different phenotype [45]. Rab3GAP2 has been linked to the Rabconnectins, interacting partners that may function in the loading of synaptic vesicles with neurotransmitter, although at this point it is unclear whether it regulates this process [24–27]. If so, it is also unclear how it functions with respect to Rab3GAP1, which appears to be excluded from the Rab3GAP2–Rabconnectin complex [24]. Finally, Rab18 has been linked to a range of physiological processes, most convincingly with regulation of lipolysis and lipogenesis, but its roles in the nervous system have not yet been adequately explored. Its expression and localization may be responsive to physiological stimulation and cell-type-specific and interestingly, it can be recruited to secretory granules in some cell types [40]. Secretory granules share much of the same molecular machinery as synaptic vesicles, and it seems possible that at synaptic vesicles, the functions of Rab3GAP1, Rab3GAP2 and Rab18 could coincide.

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