Recent advances in understanding the pathogenesis and management of reticular dysgenesis

Manfred Hoenig[1], Ulrich Pannicke[2,3], Hubert B. Gaspar[4], Klaus Schwarz[2,3]

[1] University Medical Center Ulm, Department of Pediatrics, Ulm, Germany

[2] Institute for Transfusion Medicine, University of Ulm, Germany

[3] Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service Baden-Wuerttemberg – Hessen, Germany

[4] UCL Great Ormond Street Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom

Corresponding Author: Manfred Hoenig, MD, University Medical Center Ulm, Department of Pediatrics, Eythstrasse 24, 89075 Ulm, Germany. Phone: 49 731 500 57262.

e-mail: manfred.hoenig@uniklinik-ulm.de

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Abstract

Reticular Dysgenesis is a rare immunodeficiency which is clinically characterized by the combination of Severe Combined Immunodeficiency (SCID) with agranulocytosis and sensorineural deafness. Mutations in the gene encoding adenylate kinase 2 (*AK2*) were identified to cause this phenotype. In this review, we will demonstrate important clinical differences between RD and other SCID entities and summarize recent concepts in the understanding of the pathophysiology of the disease and also the management strategies for this difficult condition.

Introduction

Severe combined Immunodeficiencies (SCID) comprise a heterogeneous group of genetic diseases with the common denominator of a profound deficiency in number or function of T cells. B-cell function is affected either primarily or secondarily – due to the lack of T-cell help. In addition to this combined defect of specific immune functions, patients with Reticular Dysgenesis (RD) have very low neutrophil counts in their peripheral blood and some a complete congenital aleukocytosis. With the absence of major pillars of specific and non-specific immune functions, RD patients present earlier and with different infectious patterns as compared to other SCID-entities.

The name “Reticular dysgenesia” given to the disease when it was first described by de Vaal Seynhaeve in 1959 (De Vaal and Seynhaeve 1959) can only be understood with a look back on the concepts and the understanding of hematopoiesis at that time. “Multipotent primitive reticular cells” in the bone marrow and lymphatic organs were thought to have the ability to differentiate in lymphocytes and granulocytes with a potential similar to what we nowadays attribute to hematopoietic stem cells. The monozygotic male twins described by the authors in this first publication died from suspected bacterial infections at the age of 5 and 8 days respectively. On postmortal examination, thymic tissue was nearly absent as were tonsils, lymph nodes and Peyer´s patches. In the bone marrow (BM) normal development of red cells and megakaryocytes was seen but myeloid elements were missing. Reticular cells were abundantly present in BM and lymphatic tissue. Therefore, the pathogenesis of the disease was thought to base on the inability of these reticular cells to develop “into the mother cells of the myeloid series” (De Vaal and Seynhaeve 1959).

Starting from 1983, successful bone marrow transplantation (Levinsky and Tiedeman 1983) for patients with RD clinically demonstrated that the immunological phenotype can be

reversed with the engraftment of bone marrow cells from healthy donors. The follow up of the survivors after successful transplantation revealed sensorineural hearing defects as a non-hematological additional component of the disease which was shared by all RD patients (Small*, et al* 1999).

With the definition of the underlying genetic defect there was a hope not only to explain a rare fatal immunodeficiency but also to gain insight in basic mechanisms in the development and differentiation of early bone marrow progenitors with the potential to give rise to the lymphatic and myeloid line and in the development of the inner ear.

In 2009 these efforts resulted in the description of mutations in the gene coding for adenylate kinase 2 (*AK2*) by two independent groups in 12 independent families (Lagresle-Peyrou*, et al* 2009, Pannicke*, et al* 2009).

Adenylate kinase 2 is mainly located in the mitochondrial intermembrane space and catalyzes the reaction ATP + AMP ⇌ 2ADP. AK2 is part of an enzymatic network which provides the transport of high-energy phosphate groups generated in the mitochondria by oxidative phosphorylation (Dzeja and Terzic 2003). Even though some important functional aspects were clarified in cell culture and animal models in recent years, the questions regarding organ (bone marrow and inner ear) and lineage (myeloid and lymphatic lines) specificity in RD patients remain poorly understood.

Beyond scientific considerations on the pathogenesis of the disease, the identification of the genetic defect enabled investigators to collect more homogeneous patient cohorts defined not only by a shared phenotype but by mutations in the same gene. To date, all patients identified with the clinical criteria of SCID, agranulocytosis and sensorineural deafness have been shown to have mutations in *AK2*. Comparing this cohort with other SCID entities, clear differences in clinical presentation and therapeutic needs have become evident and are

described in this review. These include the neonatal history, age at onset of infections, microbiobial agents responsible for these infections, presence of anemia and thrombocytopenia, unresponsiveness of neutrophil counts to GCSF and the sensorineural hearing deficit. Beyond that, recent advances regarding AK2 function contributing to the understanding of the pathophysiology of RD will be discussed.

Clinical presentation

As already described by de Vaal and Seynhaeve (De Vaal and Seynhaeve 1959) many successive patients with RD were born premature and small for gestational age (SGA) (Espanol*, et al* 1979, Haas*, et al* 1977, Hoenig*, et al* 2017, Levinsky and Tiedeman 1983, Ownby*, et al* 1976, Reubsaet*, et al* 2007). As AK2 is expressed in many tissues and plays a role in basic processes of cellular energy supply, a crucial role of AK2 in intrauterine growth could be postulated, although the mechanisms that translate these metabolic processes into premature birth are unknown. The penetrance of this phenomenon is incomplete and no clear genotype-phenotype correlation (see below) can be described for this or any other clinical signs or symptoms. After successful bone-marrow transplantation there are no indicators that growth or development of children is primarily influenced by the genetic defect (Hoenig*, et al* 2017). Another open question is to what extent hematopoietic cell lineages beyond lymphocytes and granulocytes are affected by the disease during intrauterine growth. Neonatal and even intrauterine anemia and thrombocytopenia are reported for some patients (in the absence of obvious secondary causes of infection) but others are born with normal hemoglobin levels and normal platelet numbers (Hoenig*, et al* 2017). Erythropoiesis and megakaryocytes are described without major abnormalities in bone marrow aspirates. In contrast to that, the developmental arrest of myeloid precursors at the promyelocyte stage is a very constant finding in almost all RD patients. The resulting neutropenia in the peripheral blood is a striking feature and almost all patients reported fulfil the criteria of agranulocytosis with less than 500 neutrophils /µl (Espanol*, et al* 1979, Gitlin*, et al* 1964, Heymer*, et al* 1990, Hoenig*, et al* 2017, Niehues*, et al* 1996, Ownby*, et al* 1976). In typical and representative bone marrow aspirates, promyelocytes are abundantly present and the cellularity is rather normal or increased than reduced. Administration of

GCSF to RD patients typically fails to increase peripheral blood neutrophil counts in contrast to other congenital neutropenias (Maheshwari 2014).

With these severely decreased neutrophil counts the majority of patients with RD come to medical attention within few days after birth. Septicemia is the most frequent clinical presentation, followed by omphalitis and other sites for bacterial infections. Invasive candidiasis and congenital CMV have been reported as non-bacterial infections causing serious and life-threatening infections. Opportunistic infections such as Pneumocystis jirovecii pneumonia (PCP), acquired cytomegalovirus (CMV) or recurrent viral infections of the respiratory tract which are typical for other SCID-entities are not reported for RD patients (Hoenig*, et al* 2017). The hypothetical but probably appropriate explanation for this phenomenon is that bacterial infections simply develop much faster after postnatal colonization with these ubiquitously present microbial agents before other acquired infections can develop.

Lymphocyte counts are considerably reduced in the majority of patients and even though some have some T, B or NK cells detectable these cells are non-functional and do not reliably contribute to antimicrobial defense of the host. In some patients with RD, lymphocyte counts of up to 3000 cells/µl are reported and can hamper an early diagnosis especially as these patients might have atypical clinical presentations with later onset of infections or signs of autoimmune reactions described with the term of Omenn syndrome (Henderson*, et al* 2013, Hoenig*, et al* 2017). Maternal T cells are found in about half of the patients with RD. These can cause clinical signs such as erythrodermia, hepatomegaly or non-infectious diarrhea which are very similar to the manifestations described in Omenn syndrome. The identification of maternal T cells can be very helpful as an unambiguous sign of severe T-cell deficiency.

In table 1 some clinical and laboratory findings are listed which should give rise to the suspicion of RD. The clinical constellation of leukopenia in a neonate is not specific for RD and secondary causes such as septicemia are far more frequent than RD – but if a child presents with combined neutropenia and lymphopenia, RD should be actively ruled out. This is important in RD as other SCID-entities, since early diagnosis and the decisive planning of the curative HSCT is crucial for the outcome of these patients.

Protein function of adenylate kinase 2

Mutations in the gene *AK2*, which encodes the adenylate kinase 2, have been shown to cause immunodeficiencies in humans of variable severity with a range from RD to a less severe combined immunodeficiency (Al-Mousa*, et al* 2016, Guilcher*, et al* 2017, Henderson*, et al* 2013, Lagresle-Peyrou*, et al* 2009, Pannicke*, et al* 2009). The AK2 enzyme is located in the mitochondrial intermembrane space and catalyzes reversibly the transfer of a phosphate group from an ATP to an AMP resulting in the production of two ADP molecules (Fig 1). These ADP molecules can be transported into the mitochondrial matrix by the adenine nucleotide translocator (ANT), also known as the ADP/ATP translocator. In the matrix, the ADP gets phosphorylated to ATP by the ATP synthase, using a proton gradient across the inner mitochondrial membrane which is built up by the mitochondrial oxidative phosphorylation reaction (OXPHOS). The ATP molecules are exported out of the matrix by the ANT and can serve as a central source of power for energy consuming processes in the cell. The human adenylate kinase 2 is expressed in a broad spectrum of tissues and occurs in two splicing variants, isoforms A and B (Noma 2005) (NCBI Entrez Gene ID 204). Differences of the two variants of AK2 are confined to the C-terminal region (Fig 2). Isoform A consists of 239 amino acids and has a molecular weight of 26.5 kDa, Isoform B is composed of 232 amino acids and weighs 25.6 kDa. Whether both isoforms differ in their subcellular localization, their enzymatic activities or other cellular functions beyond the kinase activities has not been investigated so far. Three domains of AK2 are essential for its enzymatic activity (Fig 3). The phosphate-binding loop (P-Loop) is composed of amino acids 22 to 30. The NMP_{bind} domain is spanning amino acids 45 to 74 and binds the adenosine monophosphate. During the catalytic cycle the LID domain, comprising amino acids 141 to 178, moves towards the NMP_{bind} domain in a highly dynamic manner and initiates the

transfer of one phosphate group between the two covered adenosine phosphates (Madej*, et al* 2014, Saraste*, et al* 1990, Schlauderer and Schulz 1996).

Pathophysiology of AK2 deficiency in cellular and animal models

To understand the developmental and cellular consequences of a lack of AK2 expression or of AK2 function(s) various animal and cellular models were generated.

In *Drosophila melanogaster* the adenylate kinase 2 gene (*Dak2*) was inactivated by P-element transposition. Homozygous embryos developed, but growth ceased before the third instar larval stage (Fujisawa*, et al* 2009). Initial embryonic survival was thought to depend on maternally provided Dak2 mRNA. The loss of Dak2 activity resulted in growth arrest and death. Tissue specific inactivations of the *Dak2* gene were all lethal during larval stages no matter whether only ectodermal, mesodermal or endodermal tissues were targeted or whether visceral and somatic muscles, ectodermal hindgut or the central nervous system were affected (Horiguchi*, et al* 2014). This suggests that Dak2 is essential for general cell survival and morphogenesis. Gene expression analyses implicated that the loss of Dak2 activity leads to the downregulation of genes of proteasomal subunits and the mitochondrial translation machinery thereby contributing to the pathogenesis.

When another insect model *Helicoverpa armigera* was investigated, AK2 knockdown by RNA interference (RNAi) resulted in larval growth arrest, reduction of body weight, a delay in developmental stage(s), and a decrease of circulating haemocytes, the vital components of the insect immune system (Chen*, et al* 2012). Because RNAi may not deplete AK2 completely as the mutation in Drosophila, the larvae advanced to pupae and to adult flies. In this study, mRNA levels of insect growth and developmental genes were reduced by AK2 downregulation.

An initial model of morpholino oligonucleotide (MO)–mediated knockdown of zebrafish (*Danio rerio*) *ak2* expression does not affect the overall development of embryos but the morphants exhibited defects in leukocyte development with absent T cells in the thymus anlage (Pannicke*, et al* 2009). In a refinement of the zebrafish studies, *ak2* mutant lines showed a profound impairment of lymphoid as well as myeloid cell development (Rissone*, et al* 2015). Additionally, a markedly reduced expression of the erythroid marker *hbae1* was noted supporting the idea that erythropoiesis defects are intrinsic to AK2 deficiency in this model. The observation of a large fraction of anemic RD patients at birth complements these observations (Hoenig*, et al* 2017). Furthermore, Rissone et al. (Rissone*, et al* 2015) showed that increased oxidative stress and reactive oxygen species (ROS) production results in cell death and apoptosis and that Ak2 deficiency causes a progressive reduction of hematopoietic stem and progenitor cells by this mechanism. These developmental blocks in Ak2 deficient zebrafish model could be reversed by antioxidative treatment. Unfortunately, homozygous Ak2 knockout mice die shortly after nidation before embryonic day 7 (Kim*, et al* 2014) (Waldmann, R. and Schwarz, K., unpublished), necessitating e.g. hematopoietic/lymphopoietic conditional Ak2 deletion for the modeling of AK2 defects. Similar to the observations made *in vivo* in zebrafish, increased ROS production and apoptosis initiation were also observed *in vitro* in patient fibroblasts (Pannicke*, et al* 2009). Upon differentiation fibroblast derived induced pluripotent stem cells (iPSCs) from an AK2 deficient patient recapitulated the promyeloid block that can be observed in the bone marrow of patients (Hoenig*, et al* 2017, Lagresle-Peyrou*, et al* 2009). This block is imitated in colony-forming assays with bone marrow PBMCs originating from RD patients (Lagresle-Peyrou*, et al* 2009). Red cell precursors derived of AK2 iPSC were dysplastic and erythroid colony building capacity was markedly reduced (Rissone*, et al* 2015). The myeloid

differentiation block of AK2 patient derived iPSC was overcome by antioxidative treatment (Rissone*, et al* 2015). In AK2 deficient iPSC derived myeloid cells the AMP/ADP ratio was deranged towards an increased AMP level whereas intracellular ADP levels were decreased. When purified CD34+ bone marrow cells from patients or AK2 CD34+ knockdown cells were differentiated *in vitro* to T and NK cells the survival and differentiation of these lymphoid cells was impaired (Six*, et al* 2015). In analogy to that, AK2 deficiency negatively affected the proliferative potential and the differentiation of the granuloytic lineage in colony-assays with CD34+ AK2 knockdown cells as starting point, while monocyte development was detectable (Six*, et al* 2015).

Transfection with vectors encoding the anti-apoptotic protein BCL2 or adenylate kinase 1 (AK1) did not reverse the lymphoid or myeloid differentiation block. These observations challenge the hypothesis that AK1 expression can compensate for an AK2 defect and for cell death in non-leukocytes (Pannicke*, et al* 2009). Eventually other mitochondrial inter-membrane space kinases may substitute for a loss of AK2 function dependent on their respective expression patterns (Tanimura*, et al* 2014). In an additional model, neutrophil generation from the HL-60 promyelocytic cell line with all-trans retinoic acid confirmed the AK2 dependence of neutrophil differentiation (Six*, et al* 2015, Tanimura*, et al* 2014).

Taken together, AK2 may play the critical role for the regulation of mitochondrial adenine nucleotide levels. As a result of AK2 deficiency, ATP-ADP mitochondrial matrix-intermembrane recycling could be impaired leading to increased ROS production. AK2 co-controls mitochondrial ADP and ATP levels as well as ROS production and regulates cell fates.

The data generated thus far presumes a mitochondrial intermembrane-space localization of AK2 driven by cotranslational import mechanisms requiring an innermembrane

electrochemical potential (Nobumoto*, et al* 1998) and involve AK2 in bioenergetic management. Yet, almost half of the AK2 protein in rat and porcine liver extracts (Nobumoto*, et al* 1998, Watanabe*, et al* 1979) was found in the cytosol. In *D. melanogaster* whole body extracts cytosolic Dak2 was present after subcellular fractionation (Noma*, et al* 2000). By competition between spontaneous protein folding and mitochondrial targeting/import the yeast AK2 homologue Adpk1p/Aky2p is also distributed between cytoplasm and mitochondria (Strobel*, et al* 2002). A significant amount of AK2 was also detected in the nuclear fraction of HeLa cells in fractionation experiments (Kim*, et al* 2014). These observations allow the generation of hypotheses that AK2 may cover additional cellular pathways, similar to the non-metabolic functions of glycolytic enzymes which not only function in the ATP generating canonical metabolism but are linked to epigenetic and transcription programs (Lee*, et al* 2007, Yu and Li 2017).

First data on AK2 non-energy related functions are emerging. During intrinsic apoptosis AK2 is caspase-independently simultaneously released from mitochondria together with cytochrome c (CYCS) , diablo IAP-binding mitochondrial protein (DIABOLO) und HtrA serine peptidase 2 (HTRA2) within a 3- to 10-min window after the induction of apoptosis by the incubation with staurosporine (Munoz-Pinedo*, et al* 2006). AK2 is suggested to mediate a novel mitochondrial apoptotic pathway through the formation of an AK2-FADD-CASP10 complex that may be involved in tumor surveillance (Lee*, et al* 2007). Additionally, an interaction of AK2 with dual-specific phosphatase 26 (DUSP26) was detected (Kim*, et al* 2014). DUSP26 is an atypical protein phosphatase that can dephosphorylate both phosphotyrosine and phosphoserine/phosphothreonine amino acids within one substrate. After binding, AK2 stimulates DUSP26 phosphatase activity in an AK2-kinase independent

reaction. The AK2-DUSP26 complex dephosphorylates FADD and is suppressing cell proliferation via this pathway.

After the synthesis of transmembrane and secretory proteins these are translocated to the endoplasmatic reticulum (ER) for post-translational processing, including catalysis of protein-folding by chaperones. The unfolded protein response (UPR) coordinates the response to the need for a rising ER protein folding capacity. AK2 depletion compromises the UPR in adipocyte differentiation and most importantly in a cell model (BCL1 cells) of peripheral B-cell differentiation and secretory function (Burkart*, et al* 2011).

Of note, in a recent study to interrogate bioenergetic determinants regulating cellular ATP levels functional RNAi screens were performed under glycolytic and oxidative phosphorylation conditions. More than 1000 nucleus-encoded genes were analyzed which encode proteins that localize to mitochondria (Lanning*, et al* 2014). AK4, one of the three mitochondrial adenylate kinase isoforms, was identified as the protein most significantly associated with increased ATP production. AK2 was not involved in global effects on cellular energy levels in response to glucose, pyruvate, glutamine and galactose as fuel source.

Some of the presented results are contradictory to clinical and laboratory observations in RD patients. Thus, how these different observed biochemical functions finally integrate into the (immuno-) phenotype of RD patients remains to be established.

Mutations in *AK2* found in immunodeficient patients

The human *AK2* gene is located on the short arm of chromosome 1 (p35.1). The transcribed regions span 25,687 base pairs for isoform A (six exons) and 28,972 base pairs for isoform B (seven exons). In 38 immunodeficient patients suffering either from classical reticular dysgenesis (RD), from common variable immunodeficiency (CID) or from Omenn Syndrome

(OS) 21 different disease-causing recessive mutations have been detected so far (Al-Mousa*, et al* 2016, Guilcher*, et al* 2017, Henderson*, et al* 2013, Hoenig*, et al* 2017, Lagresle-Peyrou*, et al* 2009, Pannicke*, et al* 2009). The types of mutations encompass missense and nonsense mutations as well as small and large-scale deletions (Fig 4). All detected missense mutations, apart from the mutations of the start codon, affect residues which show a high degree of phylogenetic conservation and some contribute to the NMP_{bind} and LID domains (Fig 5). To date, only for a few mutations impact on RNA and protein stabilities has been investigated (Lagresle-Peyrou*, et al* 2009, Pannicke*, et al* 2009). The missense mutations Met1Val, Arg103Trp and Asp165Gly, the splice-site mutations Exon4-1G>A and Exon5+1G>A, the frame-shift mutations Cys40Valfs*5 and Tyr152Thrfs*12 as well as the large-scale deletion of 5kb involving exon 6, intron 6 and exon 7 do not strongly affect overall AK2 RNA stabilities but lead to a massive loss of AK2 proteins in patients' cells. As some of the patients with AK2 mutations present relevant numbers of leukocytes the compelling question remains open if these cells develop due to residual enzymatic activities of the mutated AK2 proteins or reversions in hematopoietic precursors. Other possible explanations include reversions of AK2 mutations in non-hematopoietic bystander cells in the bone marrow or genetic constellations beyond AK2 which can compensate partially for the AK2 deficiencies. Profound sensorineural hearing loss, which persisted after successful transplantations of hematopoietic stem cells, was documented for 20 patients with an RD phenotype (Guilcher*, et al* 2017, Hoenig*, et al* 2017). Al Mousa et al (Al-Mousa*, et al* 2016) report on four patients sharing an Ala182Asp mutation and the milder phenotype of a combined immunodeficiency with normal neutrophil and lymphocyte counts. Unfortunately, the authors do not report whether these patients suffer from sensorineural deafness.

Sensorineural deafness as non-haematological manifestation of RD

Clinical features of RD not associated with leukopoiesis are related to erythro- and thrombopoiesis (see above) and sensorineural deafness.

The hearing disability is an obligatory clinical feature of RD (Hoenig*, et al* 2017, Small*, et al* 1999) and has considerable impact on the development of RD patients especially after HSCT. Cochlear implants are nowadays chosen by many centers to improve the hearing disability but -before this option became available- non-invasive hearing supports were also applied successfully.

Presently, we lack information on the histopathology of the inner ear of RD patients and on the human anatomical structures which express AK2 during development and in adulthood. Thus, the cellular and molecular pathophysiology of the hearing loss is so far not understood, yet some preliminary hypotheses based on models have been forwarded. *Drosophila melanogaster* adenylate kinase 2 (*dak2*) knockdown microarray analyses revealed that Dak2 deficiency downregulates various genes, particularly those involved in proteasomal function and in the mitochondrial protein translation machinery (Horiguchi*, et al* 2014). Mutations in mitochondrial tRNA and rRNA (Guan 2004) as well as in the genes coding for mitochondrial aminoacyl tRNA synthetase (Pierce*, et al* 2011, Pierce*, et al* 2013) and mitochondrial ribosomal protein S12 (Toivonen*, et al* 2001) cause human deafness. Based on these associations, Horiguchi et al. (Horiguchi*, et al* 2014) suggested a contribution of dak2 to auditory function via mitochondrial translation.

To analyze RD hearing loss Lagresle-Peyrou et al. (Lagresle-Peyrou*, et al* 2009) performed immunohistology of the mouse inner ear to assess AK2 expression. AK2 was detectable only in the lumen of stria vascularis (SV) capillaries hinting at a potential ecto-enzyme function of

AK2. AK2 staining was neither seen in the vestibule at any developmental stage nor in the capillaries or vessels of the SV-adjacent connective tissue.

The scala media endolymph, the extracellular fluid surrounding the hair cell bundles is unusually rich in potassium ions. Potassium flows into the hair cells during their mechanic deflection. Subsequently, calcium channels at hair cell synapses open, synaptic vesicles fuse and neurotransmitters are released connecting the sound sensing hair cells to the central nervous system via spiral ganglion neurons (Nicolson 2017). Besides its functional necessity adequately high endolymph potassium levels contribute to hair cell health.

Most models for the maintenance of these high potassium concentrations include a potassium circuitry driven by transporters and ion channels from the hair cells to the supporting cells to the spiral ligament and eventually back to the SV, where potassium gets secreted back into the scala media endolymph (Mittal*, et al* 2017).

The concentration of ATP in the endolymph is another factor which is essential for the homeostatic regulation of the electrochemical gradient for sound transduction (Housley 2000). Both, potassium and extracellular ATP concentrations, are potentially influenced by adenylate kinase function. By phosphotransfer, AKs communicate metabolic cellular signals from the mitochondria to ATP-sensitive potassium channels at the cellular membrane (Carrasco*, et al* 2001) and might influence the above mentioned potassium circulation by this mechanism. With their role in the cellular transport of high-energy phosphate groups, an influence of AK-function on extracellular ATP-levels could be suspected.

Hypothetically, AK2 deficiency in the inner ear might lead to a failure in the regulation of the potassium- and/ or ATP-homeostasis, a breakdown of the endocochlear potential which finally results in hearing loss.

An interesting issue for future investigations will be to work out whether hearing loss caused by *AK2* mutations is due to developmental or functional defects of the patient's inner ears. Inner ear development and auditory function are highly conserved in vertebrates (Nicolson 2017). Thus, a future definitive delineation of the developmental or functional defect(s) that cause(s) AK2- related RD deafness awaits the examination of either inner ear specific *Ak2* conditional murine knock-out models (Friedman*, et al* 2007) or the exploitation of zebrafish developmental experiments (Blanco-Sanchez*, et al* 2017).

Therapeutic options

In patients with SCID, attempts to perform HSCT without conditioning can be considered. This option can be important for seriously sick patients who are unable to tolerate any toxicity by chemotherapy. The establishment of a functional donor derived T-cell system is the main objective in this situation as this is the major requirement to overcome opportunistic or recurrent viral infections. Mature postthymic T cells have the ability of peripheral expansion. The establishment of a donor T-cell system by this pathway is independent of stem cell engraftment and can assure survival for years or even decades in SCID patients. With few exceptions, recipient derived B-cell function remains insufficient and has to be substituted by infusions with immunoglobulins.

This option of cure by peripheral T-cell engraftment is not an appropriate option for patients with RD. To overcome neutropenia in RD-patients, donor stem-cell engraftment is crucial. Accordingly, attempts without conditioning are reported as being successful in merely one single RD patient after transplantation with a T-cell replete graft from a matched family donor (Hoenig*, et al* 2017). In this setting the allo-effect of donor T-cells can support engraftment. After haploidentical, T-cell depleted transplantation, engraftment without conditioning was never achieved and even after conditioning a high rate of primary or secondary graft failures are reported for RD patients (Bertrand*, et al* 2002, Friedrich*, et al* 1985, Hoenig*, et al* 2017). For the therapeutic decisions, it is therefore important to keep in mind that myeloablative agents in the conditioning of patients with RD have a different meaning in comparison to other SCID entities and that myelaoablation contributes to long term cure from the disease. The intensity of myeloablation though has to be adapted to the situation of the individual patient.

Some patients develop isolated posttransplant neutropenia as an expression of primary or secondary graft failure. Donor T cells persist as their survival is independent of stem cell engraftment, platelets and red cells are derived from autologous cells but neutrophil counts will drop with the loss of donor-derived myelopoiesis. Attempts to treat granulocytopenia in these mixed chimera with GCSF cannot be recommended. Even if this may help to temporarily increase neutrophil counts, long term cure could never be achieved by this strategy. Two patients were treated with GCSF over periods of several months, finally developed myelodysplasia and died even after consecutive transplantations (Lagresle-Peyrou*, et al* 2011). For the constellation of neutropenia in mixed chimera, rapid retransplantation seems to be the only way to achieve long term cure. Why some patients loose donor stem cells and become neutropenic while others survive with a mixed chimerism and normal blood counts for years and decades is poorly understood (Hoenig*, et al* 2017). But from these mixed chimera some interesting facts regarding the pathophysiology of the disease can be deduced. Autologous neutrophils are rarely described in this situation and their development is difficult to explain and to understand as they seem to have a clear selective disadvantage and are unable to respond to GCSF. One could hypothesize a wide variety of conditions as GCSF-receptor mutations, clonal expansion with (pre-) leukemic mutations or genetic reversions of *AK2* mutations in an early progenitor cell, but this has never been followed systematically. The chimeric origin of platelets has never been studied but autologous erythrocytes are reported in a couple of patients and can even make up a higher percentage of peripheral red cells than CD34+ cells are found in the bone marrow (Hoenig*, et al* 2017). This finding is difficult to be brought in accordance with the observation of RD patients being born with anemia and with the speculations about a

primary influence of AK2 deficiency on erythropoiesis. One could hypothesize different roles of AK2 in fetal and adult erythropoiesis but this remains to be demonstrated.

Summary and Conclusions

RD is a complex and devastating rare disease that we are only now starting to understand. The identification of AK2 mutations emphasizes the important role of mitochondrial oxidative phosphorylation mechanisms on essential processes involved in early haematopoietic stem cell survival and function leading to the defects seen in RD. More systemically this is also an important mechanism in auditory functions as evidenced by the deafness experienced by all RD AK2 deficient patients. From a therapeutic perspective, HSCT clearly has an important role in treating the disease and can give rise to long term survival. However, the analysis of retrospective data highlights the importance of conditioning patients appropriately to allow robust myeloid and lymphoid engraftment. In time, these understandings of the underlying pathogenesis of the disease may lead to improved outcomes and novel therapeutic options for what is a complex and difficult disease.

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Tables

Table 1

Signs to suspect Reticular Dysgenesis:
• Bacterial infections within first weeks of life
• Low neutrophil counts
• Unresponsiveness of neutrophil counts to GCSF
• Low lymphocyte counts
• Bone marrow: promyelocyte developmental arrest of neutrophils
• Sensorineural deafness/hearing defect
additional less specific points:
• Evidence for materno-fetal engraftment (maternal T cells)
• Premature birth
• Small for gestational age (SGA)
• Anemia and/or thrombocytopenia
• Consanguineous parents

Figure legends

Fig 1. Localization and function of human adenylate kinase 2. The Human adenylate kinase 2 is located in the intermembrane space of mitochondria and catalyzes reversibly the conversion of one AMP and one ATP into two ADPs. In the mitochondrial matrix the ADPs are phosphorylated to ATP by the ATP synthase. This enzyme is triggered by a proton gradient across the inner mitochondrial membrane which is built up by the electron transport chain of oxidative phosphorylation. ANT, adenine nucleotide translocator.

Fig 2. Comparison of human AK2 isoforms A and B. Both isoforms differ only at the very C-terminus. Isoform A (NCBI reference sequences cDNA NM_001625.3, protein NP_001616) is encoded by six exons whereas isoform B (NCBI reference sequences cDNA NM_013411.4, protein NP_037543) is assembled by seven exons. The alternative splice-donor site in exon 6, which is used for splicing of the isoform B RNA is given in bold letters.

Fig 3. Structure model of the human AK2 protein isoform A. Amino acids 15 to 232 are depicted (reference sequence NP_001616). The functionally important NMP_{bind} and LID domains are highlighted. The artificial substrate Di(adenosine-5') tetraphosphate is bound to the catalytic center. α-Helices are represented by barrels and β-sheets are shown as arrows. Figure modified from NCBI Structure Summary MMDM ID 37222 PB ID 2C9Y (www.ncbi.nlm.nih.gov/Structure/pdb/2C9Y).

Fig 4. Mutations in AK2 discovered in immunodeficient patients. In 38 patients 21 different mutations have been found. In the upper part the AK2 protein isoform B (NP_037543) and its functional domains P-Loop, NMP_{bind} and LID are depicted. Missense, nonsense and frame-shift mutations which compromise the AK2 amino-acid sequence are given. In the lower part the genomic structure of the *AK2* isoform B gene (NM_013411.4) and deletions as well as splice-site mutations are illustrated. The numbers of identified alleles for each specific mutation are given in brackets.

Fig 5. Phylogenetic conservation of AK2 amino acids affected by missense mutations. In immunodeficient patients AK2 amino acids 47, 100, 103, 165, 175, 182 and 186 have been found to be subjects of missense mutations. Amino acids which contribute to the NMP_{bind} domain are underlined in black, amino acids of the LID domain are underlined in gray. Reference sequences of compared AK2 proteins are NP_001616.1 (*H. sapiens*), XP_513289.2 (*P. troglodytes*), XP_005617670.1 (*C. lupus*), NP_001029138.1 (*M. musculus*), NP_112248.2 (*R. norvegicus*), NP_001004791.1 (*X. tropicalis*), XP_004947909.1 (*G. gallus*), NP_997761.1 (*D.rerio*) and NP_523839.2 (*D. melanogaster*).

Figures

Figure 1

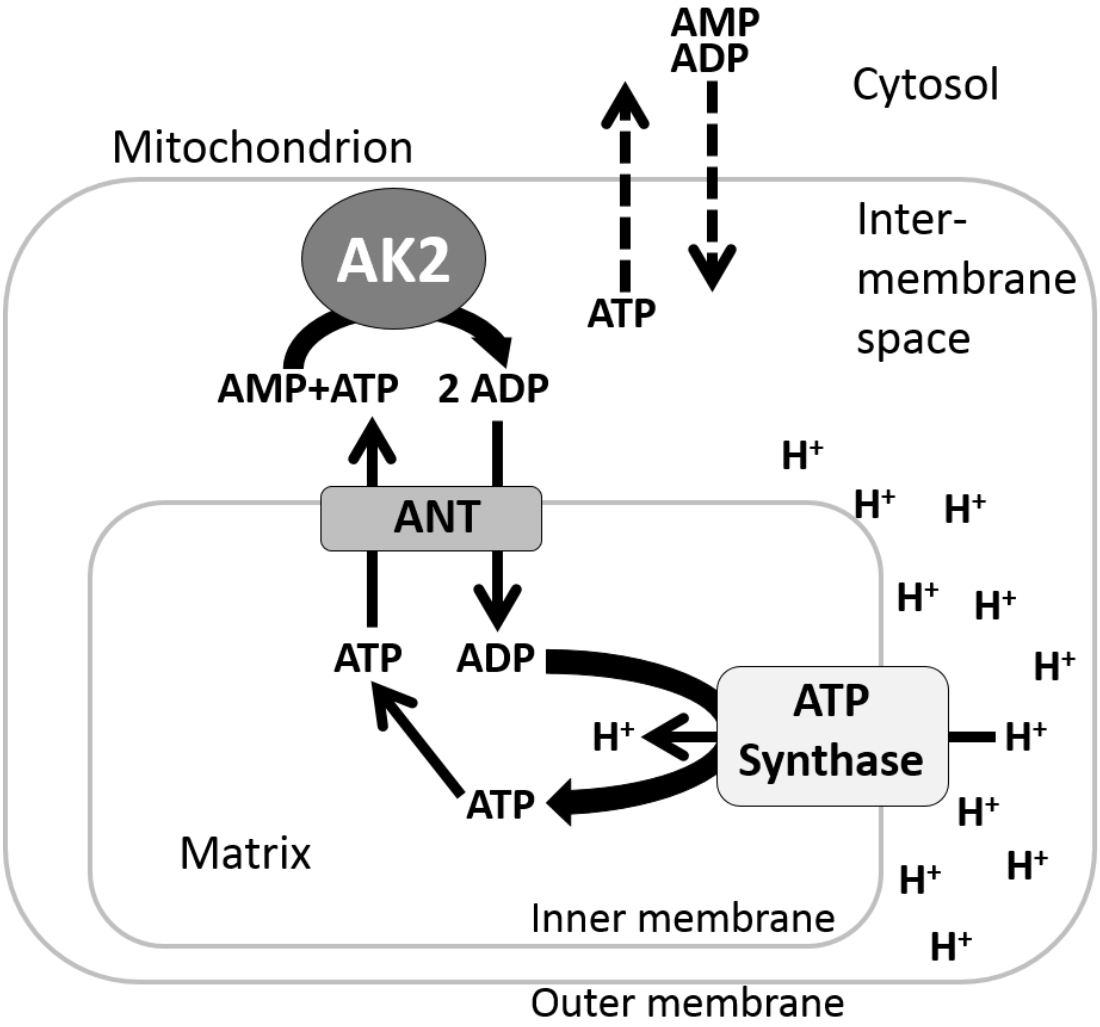


Figure 2

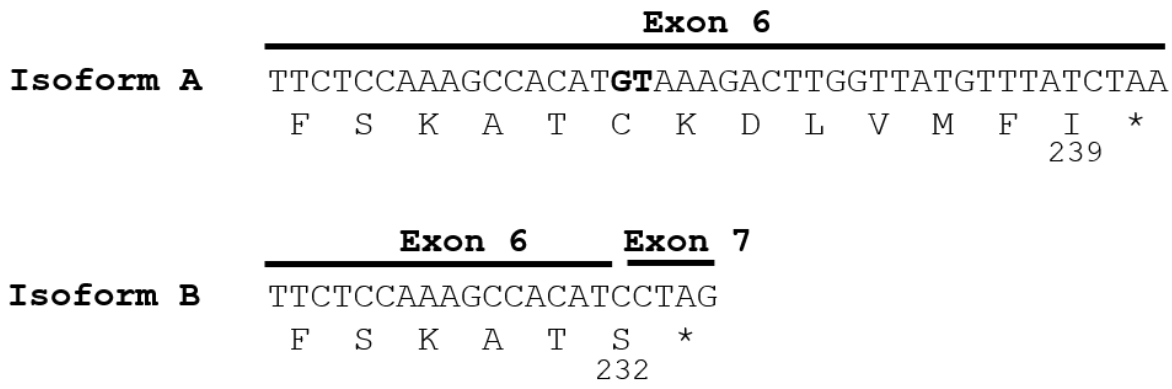


Figure 3

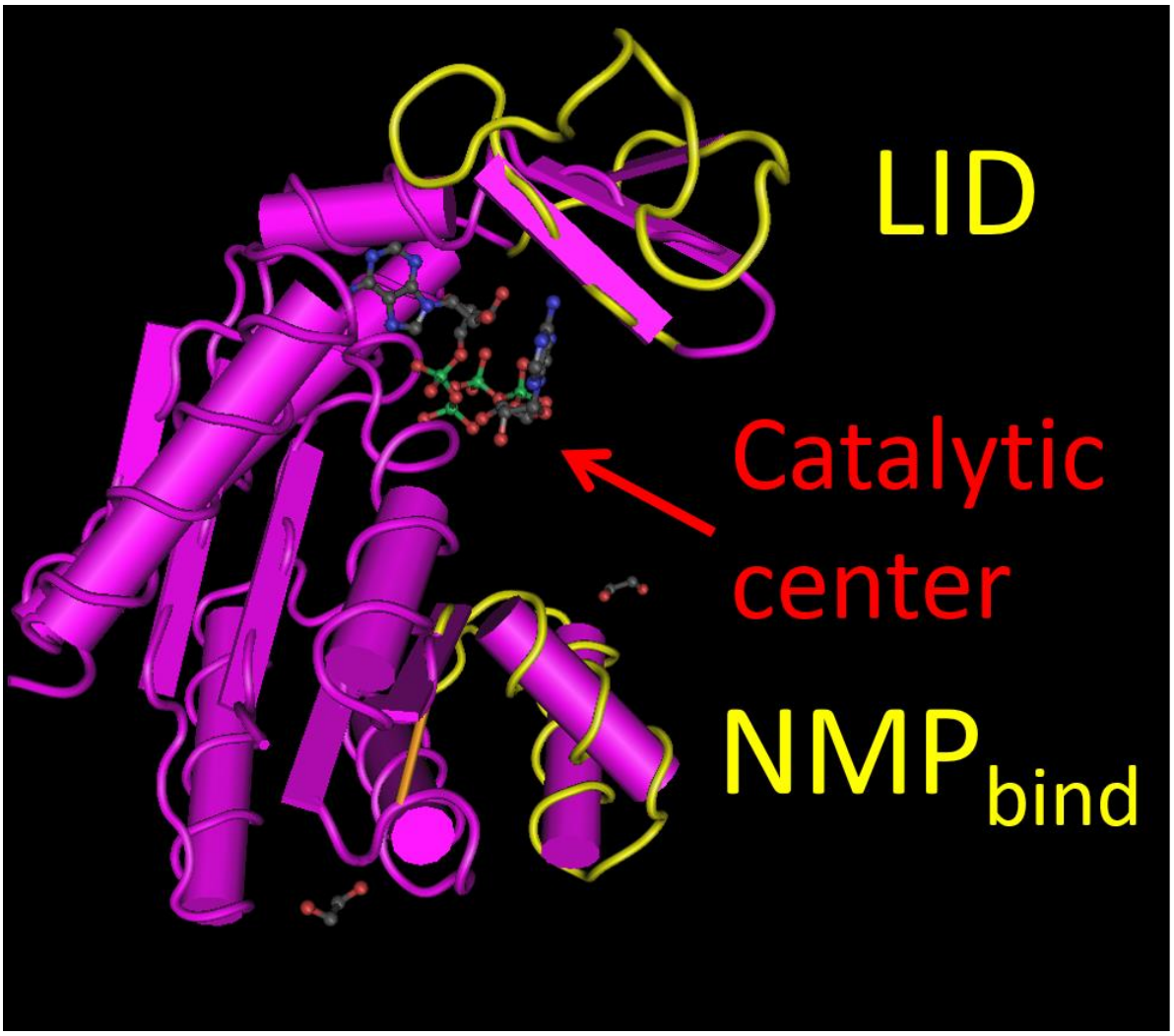
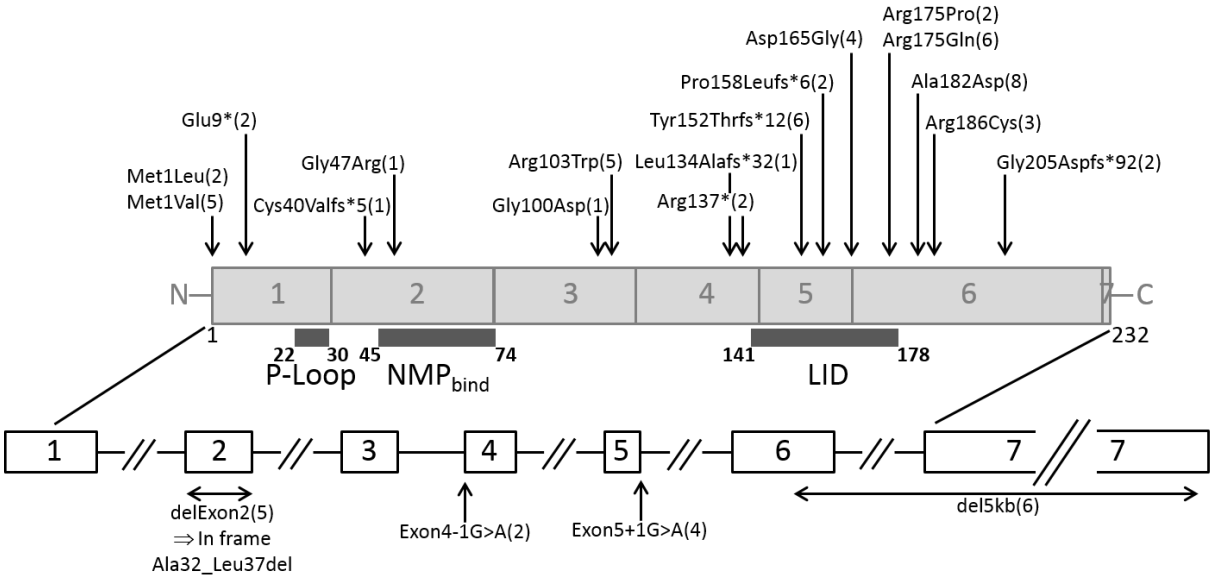


Figure 4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Glu9*(2)
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Arg186Cys(3)
Met1Leu(2)
Gly47Arg(1)
Arg103Trp(5)
Leu134Alafs*32(1)
Gly205Aspfs*92(2)
Met1Val(5)
Cys40Valfs*5(1)
Gly100Asp(1)
Arg137*(2)
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1
2
3
4
5
6
7
C
1
232
22
30
45
74
141
178
P-Loop
NMP bind
LID
1
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3
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7
7
delExon2(5)
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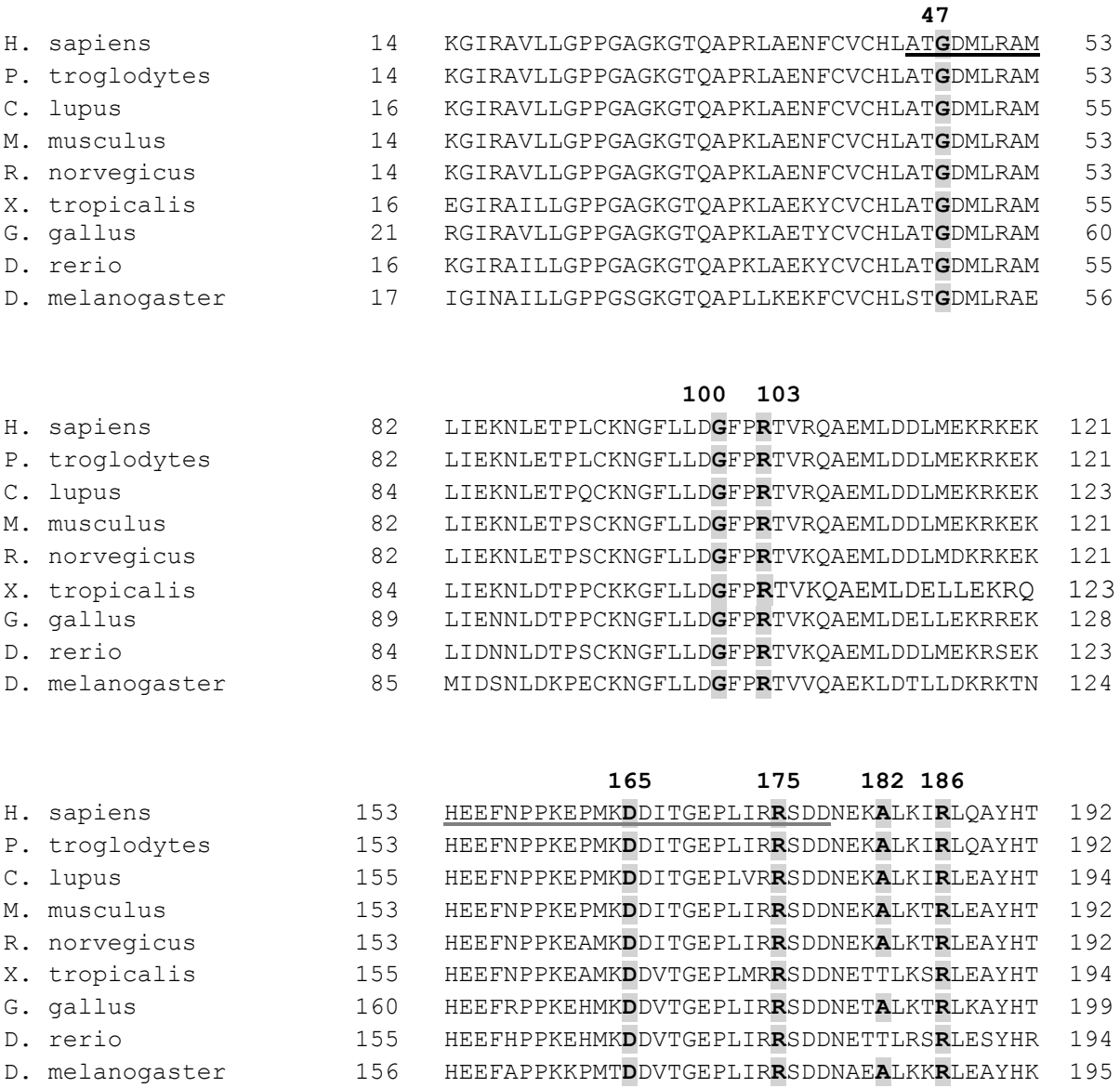
Exon5+1G>A(4)
del5kb(6)

Figure 5


47
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P. troglodytes 14 KGIRAVLLGPPGAGKGTQAPRLAENFCVCHLATGDMLRAM 53
C. lupus 16 KGIRAVLLGPPGAGKGTQAPKLAENFCVCHLATGDMLRAM 55
M. musculus 14 KGIRAVLLGPPGAGKGTQAPKLAENFCVCHLATGDMLRAM 53
R. norvegicus 14 KGIRAVLLGPPGAGKGTQAPKLAENFCVCHLATGDMLRAM 53
X. tropicalis 16 EGIRAILLGPPGAGKGTQAPKLAEKYCVCHLATGDMLRAM 55
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165 175 182 186
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