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(54) NUCLEIC ACID SEQUENCES OF HYPERPLASIA AND TUMOURS OF THE THYROID

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(57) **ABSTRACT**

The invention relates to a nucleic acid which codes for a polypeptide. Said polypeptide comprises a KRAB domain and/or at least one zinc finger motif, whereby the KRAB domain contains the amino acid sequence of SEQ ID NO: 1 and the zinc finger motif is of the type contained in the zinc finger domain with the amino acid sequence of SEQ ID NO: 2.

Figure 1

Figure 2

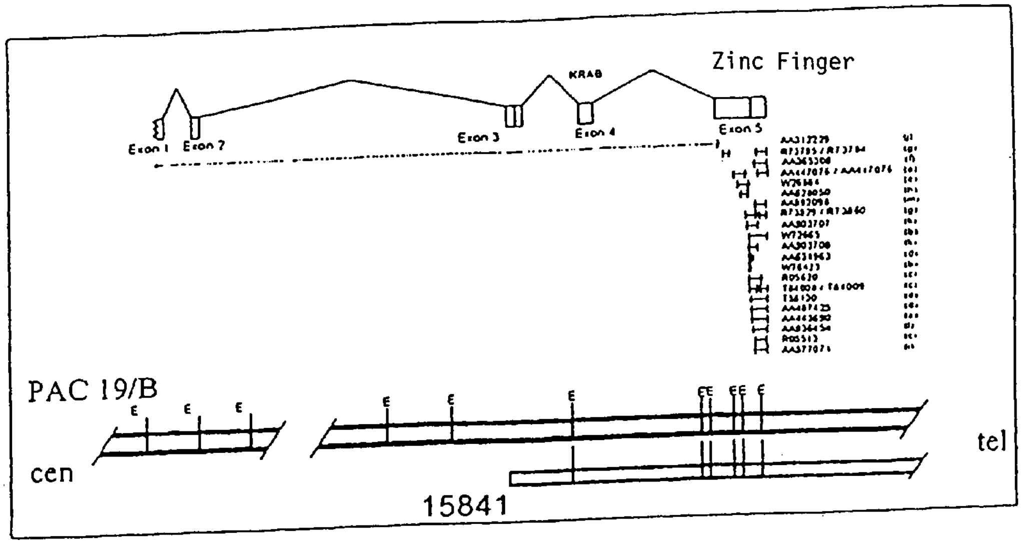

Zinc Finger
KRAB
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
PAC 19/B
cen
tel
15841

Figure 3

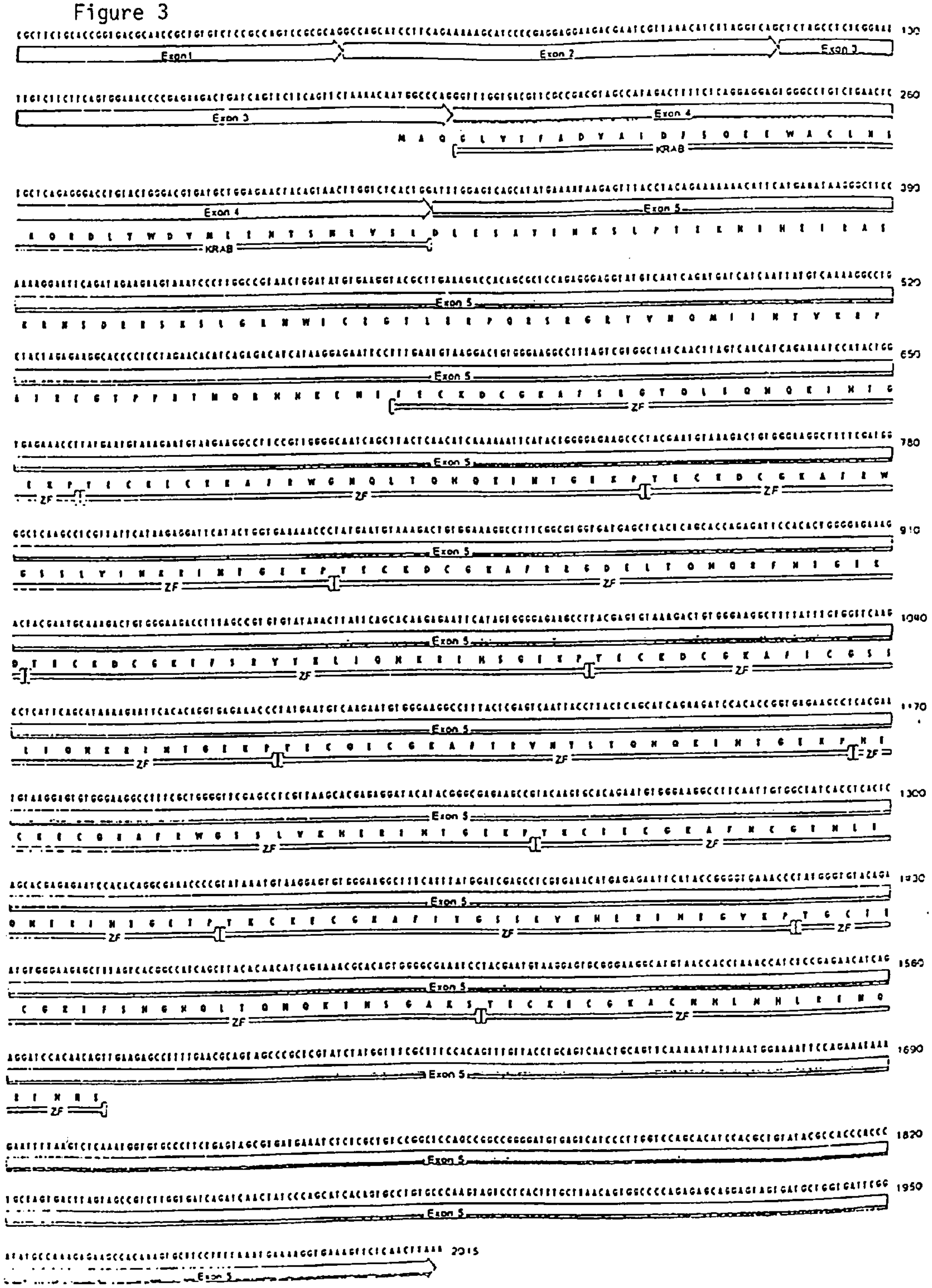
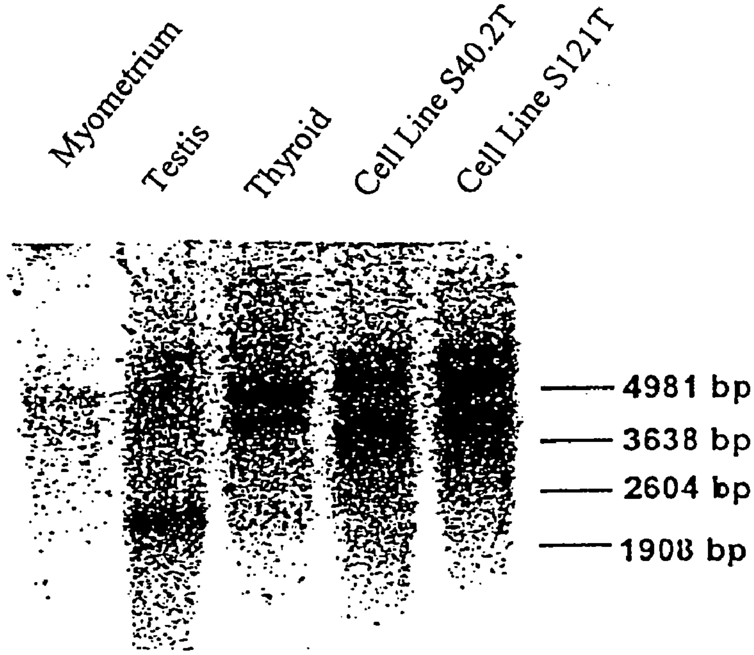


Figure 4


Myometrium
Testis
Thyroid
Cell Line S40.2T
Cell Line S121T
4981 bp
3638 bp
2604 bp
1908 bp

NUCLEIC ACID SEQUENCES OF HYPERPLASIA AND TUMOURS OF THE THYROID

[0001] The present invention is related to a nucleic acid which codes for a polypeptide, said polypeptide comprising a KRAB domain and/or at least one zinc finger motif. The invention is further related to vectors, polypeptides, cells, antibodies, ribozymes, their use to diagnose and/or treat functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours and their use to produce a medicament, pharmaceutical compositions and kits, as well as methods for detecting functional disorders of the thyroid, hyperplasia of the thyroid and/or tumours of the thyroid, primers for preparing the nucleic acid, methods for preparing the nucleic acid and a pharmaceutical composition.

[0002] It has been known for a long time in medicine that the hormone production of the thyroid is important for the control of growth and development of the body and the changes associated with a functional disorder of this gland that are at least in some cases conspicuous in the true sense of the word are also well known.

[0003] With regard to the pathogenesis of strumae and thyroid tumours in general it has not yet been possible to develop a comprehensive concept especially at the molecular level. At present two concepts are being discussed one of which assumes that hyperplastic tissue is a result of chronic stimulation by a trophic hormone which finally leads to the growth of polyclonal nodules. This concept is referred to as non- neoplastic endocrine hyperplasia (NNEH). The second concept assumes that the nodules are real clonal tumours.

[0004] In the case of the thyroid it has turned out that the simple concept that applies to other glands cannot be applied to non-neoplastic endocrine hyperplasia. A review of the current concepts in this field may be found in Studer, H. (1995); Endocrine Reviews, vol. 16. no. 4. pages 411-426.

[0005] Hence an early diagnosis is necessary for the treatment of strumae and thyroid tumours in order to allow the use of suitable therapeutic concepts.

[0006] The object of the present invention was to provide agents for diagnosing and treating functional disorders of the thyroid, hyperplasia of the thyroid and tumours of the thyroid at the molecular level, and in particular it is intended to provide nucleic acid sequences which are involved in the pathogenicity mechanisms and are suitable for examining these in more depth as well as to provide sequences which have an effect on or can influence the pathogenicity mechanisms.

[0007] Furthermore drugs which are based thereon and general pharmaceutical compositions should be provided.

[0008] The same also applies to polypeptides derived from the nucleic acid sequences and antibodies and ribozynies directed against them and pharmaceutical compositions which contain them.

[0009] Furthermore it is intended to provide kits for diagnosing and/or treating functional disorders of the thyroid, hyperplasia of the thyroid and tumours of the thyroid as well as methods for their detection.

[0010] The object is achieved according to the invention by a nucleic acid which codes for a polypeptide, wherein the polypeptide comprises a KRAB domain and/or at least one zinc finger motif and the KRAB domain comprises the amino acid sequence of SEQ ID NO: **1** and the zinc finger motif is one which is contained in the zinc finger domain having the amino acid sequence of SEQ ID NO:2.

[0011] The object is also achieved by a vector which contains the nucleic acid sequence according to the invention.

[0012] Finally the object is achieved by a polypeptide which comprises a KRAB domain and/or a zinc finger domain and is coded by a nucleic acid sequence according to the invention.

[0013] Furthermore the object is achieved by a cell which contains a vector according to the invention.

[0014] The object is additionally achieved by an antibody which is directed against a polypeptide according to the invention.

[0015] Moreover the object is achieved according to the invention by an antibody which is directed against a nucleic acid sequence according to the invention.

[0016] The object is additionally achieved according to the invention by a ribozyie which is directed against a nucleic acid sequence according to the invention.

[0017] In addition the object of the invention to provide medicaments and pharmaceutical compositions comprising such agents and in particular for the diagnosis and/or treatment of functional disorders of the thyroid, hyperplasia of the thyroid and/or tumours of the thyroid, is achieved by a nucleic acid according to the invention or its use, by a vector according to the invention or its use, by a polypeptide according to the invention or its use, by a cell according to the invention or its use, by an antibody according to the invention or its use, by a ribozyme according to the invention or its use and by a pharmaceutical composition which contains an agent which is selected from the group comprising a nucleic acid, a vector, a polypeptide, a cell, an antibody, a ribozyme, each of which are according to the invention, and combinations thereof and a pharmaceutically acceptable carrier.

[0018] The object is also achieved by a kit according to the invention for diagnosing functional disorders, hyperplasia and tumours of the thyroid, wherein the kit contains at least one element which is selected from the group comprising a nucleic acid, a vector, a polypeptide, a cell, an antibody and a ribozyme, each according to the invention.

[0019] The method according to the invention for detecting functional disorders, hyperplasia and/or tumours of the thyroid which comprises the steps contacting the thyroid material with an agent which is selected from the group comprising a nucleic acid sequence, a vector, a polypeptide, an antibody and a ribozyme each according to the present invention and determining whether functional disorders, hyperplasia and/or tumours are present also achieves the object according to the invention.

[0020] The object is furthermore achieved by using a nucleic acid sequence according to the invention as a primer.

[0021] Furthermore the object is achieved according to the invention by a primer for preparing and/or screening and/or detecting a nucleic acid which is complementary to a part of a nucleic acid sequence according to the invention. The sequence of this primer can also be derived by mutation or labelling of a nucleic acid sequence according to the invention.

[0022] The object is also achieved according to the invention by a method for preparing a nucleic acid which contains a sequence that can be detected in thyroid tumours in which a translocation with a breakage point is present in the chromosomal band 19q13, the said sequence being located within the chromosomal band 19q13, characterized in that the method comprises the steps:

[0023] preparing primers according to the invention for carrying out a polymerase chain reaction,

[0024] preparing a nucleic acid sequence taken from the band 19q13 of the human chromosome 19 or a nucleic acid according to the invention,

[0025] mixing the nucleic acid sequence or the nucleic acid with the primers,

[0026] carrying out a polymerase chain reaction.

[0027] One embodiment provides that the nucleic acid according to the invention comprises the nucleic acid sequence of SEQ ID NO: 3.

[0028] Another embodiment provides that the nucleic acid according to the invention can hybridize to the nucleic acid sequence of SEQ ID NO:3 and/or the nucleic acid sequence which codes for a polypeptide, said polypeptide comprising a KRAB domain and/or a zinc finger domain and the KRAB domain comprising the amino acid sequence of SEQ ID NO: 1 and the zinc finger domain comprising the amino acid sequence of SEQ ED NO: 2.

[0029] One embodiment provides that the nucleic acid according to the invention has at least one mutation.

[0030] One embodiment provides that the nucleic acid according to the invention is in the form of an antisense nucleic acid for one of the sequences according to the invention.

[0031] Furthermore provision can be made for the nucleic acid according to the invention to be modified.

[0032] The vector according to the invention can additionally contain one element which is selected from the group comprising promoters, terminators and enhancers.

[0033] A preferred embodiment provides that the vector is an expression vector.

[0034] A further preferred embodiment provides that at least one promoter is in-frame with a part of the nucleic acid sequence(s) according to the invention coding for a polypeptide.

[0035] A particularly preferred embodiment provides that the promoter is a eukaryotic promoter.

[0036] Furthermore the vector can additionally contain an additional nucleic acid sequence which is in the reading frame together with a part of a nucleic acid sequence according to the invention coding for a polypeptide, wherein the additional nucleic acid sequence is selected from the group of nucleic acid sequences comprising signal sequences and sequences which code for domains of proteins.

[0037] The polypeptides according to the invention can be modified.

[0038] The polypeptide according to the invention can also be derived from an amino acid sequence by conservative or silent amino acid mutation.

[0039] In the case of the cell according to the invention it can be provided that a nucleic acid sequence according to the invention is integrated into the genome of the cell.

[0040] In a further embodiment of the cell according to the invention it can be provided that a nucleic acid sequence according to the invention is present integrated into the human chromosome **19** and in particular into band 19q13.

[0041] Furthermore the cell which is at least diploid with regard to human chromosome **19** can be such that it only has one chromosome containing a nucleic acid sequence according to the invention.

[0042] A particularly preferred embodiment provides that the cell according to the invention is a eukaryotic cell.

[0043] The antibodies according to the invention can be monoclonal antibodies.

[0044] A further embodiment can provide that the antibodies according to the invention are modified.

[0045] In one embodiment the ribozyme according to the invention comprises at least a part of a nucleic acid sequence according to the invention.

[0046] A vector according to the invention and/or a cell according to the invention and/or a ribozyme according to the invention can be used to diagnose and/or treat functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours.

[0047] A vector according to the invention and/or a cell according to the invention and/or a ribozyme according to the invention can be used to prepare a medicinal drug.

[0048] When the kit according to the invention for diagnosing and/or treating functional disorders, hyperplasia and tumours of the thyroid is in particular used for diagnostic applications it can comprise one of the nucleic acid sequences according to the invention and/or an antibody according to the invention and especially one which is directed against the polypeptides according to the invention.

[0049] A further embodiment provides that the kit contains at least one cell according to the invention.

[0050] Furthermore the invention proposes that when the kit according to the invention is used for therapeutic applications, it contains a nucleic acid sequence, a polypeptide, an antibody and/or a ribozyme each according to the present invention.

[0051] The method according to the invention for detecting functional disorders, hyperplasia and/or tumours of the thyroid can be such that the thyroid material is present ex vivo.

[0052] In one embodiment of the use according to the invention of a nucleic acid according to the invention it can be provided that this nucleic acid is used as a primer. In this case a preferred embodiment is that, starting with a strand of a nucleic acid according to the invention, a nucleic acid sequence is determined that can hybridize with a part of the nucleic acid according to the invention and that this nucleic acid sequence is synthesized. Furthermore another preferred embodiment provides that starting with another strand which is complementary to the previously used strand, a nucleic acid sequence is selected which can hybridize with a part of this complementary strand and that this nucleic acid sequence is synthesized.

[0053] In the following tumours refers to malignant as well as benign tumours.

[0054] The basis of the invention was the surprising finding that the chromosome translocations on the human chromosome **19** observed in tissue specimens from follicular thyroid tumours, including thyroid carcinomas and strumae involve the chromosomal band 19q13 and that in particular all breakage points can be allocated to a particular nucleic acid sequence within band l9q13 as disclosed herein. In particular the inventive nucleic acids disclosed herein appear to be target nucleic acids for the aberrations 19q13 and in particular in epithelial tumours. In this connection target nucleic acid means that the observed chromosomal aberrations occur in the nucleic acid according to the invention or in its immediate vicinity. In this connection immediate vicinity means within a distance of about 200 kbp. The 3' end of the nucleic acid according to the invention as well as the 3' end of the cDNA shown in FIG. **3** (SEQ ID NO: 3) can be a reference point for this.

[0055] Furthermore it was surprisingly found that it is possible to produce cells which have the chromosomal change i.e. the chromosome translocations in band 19q13 as shown in example 2.

[0056] Finally it was also surprisingly found that the sequence within which the breakage points of the chromosome translocations are located, is expressed in thyroid tissue and in particular in thyroid tumour tissue.

[0057] In connection with the present invention zinc finger domain means a zinc finger motif and in particular a collection of at least two zinc finger motifs although each zinc finger motif per se satisfies the criterium of a domain i.e. the amino acid sequence of the zinc finger motif can already fold as such into a stable tertiary structure. In particular it should be noted in connection with the present invention that the structure referred to as the zinc finger domain contains 12 zinc finger motifs. The exact location of these zinc finger motifs is also shown in FIG. **3** (where the individual zinc finger motifs are labelled with ZF). In addition example 12 shows the exact nucleotide borders for the individual zinc finger motifs.

[0058] According to the present invention the nucleic acid according to the invention can be in various forms in the different embodiments. The following nucleic acids are listed purely for the purposes of illustration. One embodiment comprises the nucleic acid which codes for a KRAB domain of the amino acid sequence shown in SEQ ID NO: 1. Another embodiment of the nucleic acid according to the invention codes for a zinc finger motif as contained in the amino acid sequence of SEQ ID NO:2. More precisely such a nucleic acid sequence can contain one or more of the nucleic acid sequences that code for an amino acid sequence that is coded by a nucleic acid sequence of nucleotide positions 577 -660, 661 -744, 745 -828, 829 -912, 913 - 996, 997-1080, 1081 -1164, 1165 -1248, 1249-1332, 1333 -1416, 1417-1500 or 1501 -1575 (as also shown in example 12) of FIG. **3**. In other words the nucleic acid according to the invention can contain the nucleic acid from position 577 to 660 or other sequences defined by the aforementioned nucleotide positions. Furthermore it also encompasses those sequences that code for the amino acid sequences defined by the above-mentioned nucleic acid positions and which differ from the nucleic acid sequence shown in FIG. **3** as a result of the degeneracy of the genetic code. Finally the invention also encompasses those nucleic acid sequences which contain the nucleic acid sequence coding for the KRAB sequence of SEQ ID NO: **1** and the nucleic acid sequence corresponding to sequence positions **577** to **660** (the first zinc finger motif of the cDNA shown in FIG. **3** and hence the gene RITA). If the nucleic acid coding for the KRAB domain according to SEQ ID NO: 1 is referred to as A and the nucleic acids coding for the 12 zinc finger motifs shown in FIG. **3** are referred to as B**1**, B**2** etc. to B**12**, then the nucleic acid according to the invention especially includes those combinations of A with at least one element from the group comprising B**1** to B**12**. In this connection the group formed from the elements B**1** to B12 can also contain elements which are in turn combinations of at least two of the elements Bl to B12.

[0059] Hence the present nucleic acid sequences allow new approaches to diagnose and to treat or examine mechanisms associated with the occurrence of strumae and thyroid tumours or thyroid carcinomas. In particular knowledge of the sequence allows the direct or indirect use of diagnostic or therapeutic agents at a molecular level in the form of pharmaceutical compositions and drugs.

[0060] The nucleic acid sequences according to the invention can be used to design suitable diagnostic and therapeutic agents in the above sense as well as kits and methods in a manner known to a person skilled in this field.

[0061] Nucleic acid in this connection means DNA sequences as well as RNA sequences including hybrids thereof and also derivatives thereof with a modified backbone. It also includes single-stranded and double-stranded nucleic acids as well as those with a triple structure.

[0062] The strandedness of the DNA i.e. for example whether it is present in a single- stranded or double-stranded form, can change over the length of the nucleic acid sequence.

[0063] In particular the nucleic acid sequence according to the invention can also be present as a fragment rather than a complete sequence.

[0064] Furthermore within the scope of the present disclosure it is possible for the nucleic acid sequences to be present in a mutated form. Mutations as used herein means all mutations known to a person skilled in that art that can occur within a nucleic acid sequence including point mutations as well as non-point mutations i.e. inversions, insertions and deletions.

[0065] The criterion hybridization which is generally recognized in the prior art should be used herein especially with regard to the interaction of the nucleic acid according to the invention with other nucleic acids. It is known that the stringency of hybridization can be changed within a particular range by selecting suitable hybridization conditions and thus enable the nucleic acid sequences according to the invention to hybridize to nucleic acid sequences which can differ by varying degrees from the completely corresponding i.e. complementary sequences.

[0066] Nucleic acid sequence in the sense of the invention is also intended to mean any sequence that would hybridize with sequences according to the invention if it were not for the degeneracy of the genetic code. With regard to the open reading frame or the open reading frames present in the nucleic acid sequence according to the invention this means that it/they can be translated into an amino acid sequence using the genetic code. However, as a consequence of the degeneracy of the genetic code it is possible to obtain a nucleic acid sequence from an amino acid sequence obtained in this way again using the genetic code which is so different that it may no longer be able to hybridize with the nucleic acid sequence used to determine the amino acid sequence.

[0067] Starting from the nucleic acids disclosed herein it is possible for a person skilled in the art to produce a suitable antisense nucleic acid, in particular antisense RNA that can interact with the nucleic acid sequences according to the invention. This interaction can be the basis for directly influencing the processes involving the nucleic acid sequences. This interaction can for example occur at the level of transcription as well as at the level of translation.

[0068] Nucleic acid sequences are generally understood herein as nucleic acid sequences that can be isolated from the said tissue in situ or ex vivo for example from appropriate cell, tissue or organ cultures. However, it also refers to corresponding nucleic acid sequences that can be isolated from gene banks in particular human gene banks and more preferably gene banks of the human chromosome **19**. Furthermore the term nucleic acid sequences as used herein should also encompass those that can be produced by suitable synthetic techniques including the polymerase chain reaction and other biochemical and chemical synthetic processes known in the prior art.

[0069] In addition the sequences according to the invention can also be present in a modified form.

[0070] Modification is understood herein to include fragmentation, insertion, deletion and inversion of (partial) sequences of the nucleic acid sequences according to the invention. It also includes the insertion of other nucleic acid sequences. These nucleic acid sequences can for example code for particular domains, serve as spacers or serve as elements for translation and transcription regulation.

[0071] Furthermore the nucleic acids according to the invention can be modified such that they contain sequences or molecules that allow an interaction with other molecules. This can for example be in the form of a binding site on a solid support or a sequence that mediates binding to a nucleic acid binding protein.

[0072] In addition the nucleic acid sequences according to the invention can be labelled. Labelling is basically understood herein as direct as well as indirect labelling.

[0073] Labelling can be carried out using labels and labelling methods known in the prior art and includes radioactive, non-radioactive and fluorescent labels. Non-radioactive labels include among others digoxigenin, avidin, streptavidin and biotin.

[0074] A vector as used herein means in particular recombinant vectors that are known in the prior art. Such vectors include, among others, viral vectors such as adenoviral or retroviral vectors and phage systems, as well as plasmid vectors including cosmid vectors and artificial chromosomes that can be used in prokaryotic and/or eukaryotic systems.

[0075] In addition to the nucleic acid sequences according to the invention, the vectors according to the invention can contain additional elements that are known in the prior art. The respective elements such as promoters, terminators and enhancers are selected according to the respective host cell system in a manner known to a person skilled in the art. In particular this means the selection of a suitable eukaryotic promoter and of an inducible promoter. Apart from the said elements it is also possible that such vectors contain elements that result in the integration of at least the nucleic acid sequence according to the invention or a part thereof into the genome of the host cell system.

[0076] It is also possible that at least one of the said elements is connected in-frame with at least one open reading frame of the nucleic acid sequences according to the invention and it is especially advantageous when the transcription rate of the special open reading frame is regulated by an additionally inserted promoter in which case the promoter is then typically at a suitable distance to and in-frame with the open reading frame.

[0077] Furthermore provision can be made for an open reading frame of the nucleic acid sequences according to the invention to have a signal sequence preferably under the aforementioned conditions which allows a translocation of the gene product coded by the open reading frame which optionally allows a further modification of the gene product. Such signal sequences include those for the transport of the synthesized protein to the endoplasmic reticulum, Golgi apparatus, to lysosomes, to cell organelles such as mitochondria and chloroplasts and to the cell nucleus. Such a passage through various cell compartments allows a posttranslational modification and hence optionally a further advantageous structure of the gene product.

[0078] Apart from signal sequences, such a construct can also contain additional nucleic acid sequences which have the effect that the gene product of an open reading frame of the nucleic acid sequences according to the invention form a fusion product where the fused part can correspond to a domain of another protein and for example facilitate the detection of the gene product of the open reading frame of the sequences according to the invention or interaction with other molecules or structures in the biological system where the biological system is preferably the cell.

[0079] In addition to the aforementioned advantages of the nucleic acid sequences according to the invention and other advantages that a person skilled in the art can derive from the description, these are also inherent to the polypeptides derived from the said nucleic acid sequences. These polypeptides can be on the one hand derived directly from an open reading frame of the nucleic acid sequence according to the invention or they can be produced by a vector that is expressed in a host organism in a manner known to a person skilled in the art. In this case the host organism is typically firstly transformed with the vector according to the invention, the host organism is multiplied and the polypeptide is isolated from the host organism or from the medium if it is secreted into the medium.

[0080] In addition to a direct use of the polypeptides according to the invention to influence cellular processes in thyroid tissue, they can also be used to purify other components involved in the cellular processes for example by means of affinity chromatography or to produce suitable antibodies which can then in turn be used, among others, for therapeutic and/or diagnostic purposes.

[0081] Depending on the respective requirements with regard to post-translational modification or the desired degree of purity etc., either a prokaryotic or a eukaryotic host organism may be advantageous for producing the polypeptide according to the invention in a host organism.

[0082] The polypeptide according to the invention can be modified in a suitable manner. Modification is understood among others as a fragmentation and in particular as a shortening of the molecule. Modification in the sense used herein also includes the labelling of the polypeptide. The latter can be achieved by high molecular as well as low molecular compounds and includes radioactive, non-radioactive and fluorescent labelling. A label can for example also be present in the form of a phosphorylation or glycosylation of the protein. Suitable labelling methods and modification methods are known in the prior art (see e.g. Protein Methods, 2nd ed. D.M. Bollag et al., Wiley Liss, Inc., New York, N.Y., 1996) and are incorporated herein in their entirety.

[0083] Modification according to the invention also means any form of post-translational modification known to persons skilled in the art, in particular proteolytic processing of the polypeptide, attachment or separation of residues at the N-terminus, acetylation of the N-terminus, myristoylation of the N-terminus, attachment of glycosyl phosphatidyl residues, farnesyl residues or geranylgeranyl residues at the C-terminus, N-glycosylation, O-glycosylation, attachment of glycosaminoglycan, hydroxylation, phosphorylation, ADP-ribosylation and formation of disulfide bridges.

[0084] It is also possible for the polypeptide according to the invention to be derived from a polypeptide according to the invention by amino acid mutation. Amino acid mutation means a mutation in which an amino acid is substituted for an amino acid with a similar side chain (conservative mutation) for example I by L or D by E as well as a mutation in which an amino acid is substituted by another amino acid without this substitution having a disadvantageous effect on the function of the coded polypeptide (silent mutation). The function of the coded polypeptide can be checked by a suitable assay. Such a functional assay for the KRAB domain and the zinc finger domain is for example a band-shift assay. Useful functional assays are disclosed in Margolin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4509-4513; Witzgall et al., 1994, Proc. Natl. Acad. Sci. USA 91:4514-4518, Andrews, N. C. and Faller, D. V. 1991, Nucleic Acids Res 19(9): 2499, Elser, B. et al., 1997, J. Biol. Chem. 272(44): 27908-12, Fan, C. M. and Maniatis, T., 1989, Embo J. 8(1): 101-110, Gamer, M. M. and Revzin, A. 1981, Nucleic.Acids Res 9(13):3047-3060, Kadonaga, J.T. et al., 1987, Cell 51(6):1079- 1090, Pierrou, S. et al., 1995, Anal. Biochem. 229(l):99-105, Singh, H. et al., 1986, Nature 319(6049): 154-158, Thiesen, H. J. and Bach, C., 1993, Ann N Y Acad. Sci. 684:246-249 and Vissing et al., 1995, FEBS 369:153-147 and are hereby incorporated by reference.

[0085] A polypeptide according to the invention can contain a KRAB domain or a zinc finger domain or a combination of both domains.

[0086] A polypeptide according to the invention can in particular contain the sequence shown in SEQ ID NO: 4.

[0087] The cells according to the invention can be used to yield additional advantages. These include among others the production of corresponding nucleic acid sequences and gene products derived therefrom.

[0088] In particular insertion of the nucleic acid sequences according to the invention into the genome of a cell is particularly important for example in order to further study the influence of such sequences especially in the cellular field. In this connection gene-dose effects or such like can be investigated and utilized for diagnostic and/or therapeutic purposes. In this connection a state appears to be especially advantageous in which, starting from diploid cells, only one chromosome contains the nucleic acid sequence according to the invention which is preferably inserted at its corresponding position in band 19q13 and the second chromosome has no chromosome translocation affecting the chromosomal band 19ql3. Such cells can for example be used as positive and/or negative controls in a diagnostic method.

[0089] In view of the technical teaching disclosed herein it is then also possible to produce antibodies against the gene product of the nucleic acid sequences according to the invention and also against the nucleic acid sequence itself. This also results in the advantages known to a person skilled in the art which derive from the presence of antibodies against chemical compounds. For example this would enable a purification or detection of the said compounds and also make it possible to influence the biological activity and bioavailability of the compounds against which the antibody is directed in situ as well as ex vivo or in vitro. More precisely monoclonal antibodies can be used to specifically detect gene products or to influence. at a cellular level an interaction of the gene products or nucleic acid sequence with other cellular components and thus to specifically influence the cellular processes. Depending on the effect of the respective compound against which the antibody is directed and how it effects the system in question, it is possible in principle to achieve stimulating as well as inhibiting effects.

[0090] Antibodies are understood herein to include polyclonal antibodies as well as monoclonal antibodies. Monoclonal antibodies are, however, particularly preferred due to their increased specificity. However, application cases are conceivable in which the purity and specificity of polyclonal antibodies is sufficient or in which the many other properties and specificities of polyclonal antibodies can be used in an advantageous manner. The production and use of antibodies is described in: A Laboratory Manual (E. Harlow & D. Lane, Cold Spring Harbor Laboratory, N.Y., 1988) and is incorporated herein.

[0091] Within the scope of this invention it is also possible for the antibodies to be single- chain antibodies.

[0092] The antibodies can also be fragmented and in particular shortened within the scope of the present invention. This includes a substantial truncation of the antibodies provided that the specific property of the antibody is retained i.e. binding to a defined epitope. Truncation is particularly advantageous when the corresponding antibody is to be used at a cellular level since it has improved permeation and diffusion properties compared to a complete antibody.

[0093] Furthermore other forms of modification are envisaged which are known to a person skilled in the field and are described for example in Antibodies: A Laboratory Manual (E. Harlow & D. Lane, Cold Spring Harbor Laboratory, N.Y., 1988). In general it can be stated that antibodies can in principle be modified in a similar manner to polypeptides and, to a certain extent, to nucleic acids and that the aforementioned also applies in the same sense to the antibodies according to the invention.

[0094] With regard to a pharmaceutical composition which contains a ribozyme in addition to a pharmaceutically acceptable carrier or the use of a ribozyme according to the invention as a drug and in particular to treat functional disorders, hyperplasia and tumours of the thyroid, it is also possible for the ribozyme to be constructed such that it specifically acts on the nucleic acid sequences according to the invention and hence in this case regulates expression and translation at a cellular level which is particularly important for the therapeutic and diagnostic aspect. Due to the fact that ribozymes have intramolecular as well as intermolecular catalytic effects, the nucleic acid sequences according to the invention can be changed such that they have regions which are themselves cleaved by the ribozyme activity of the changed region. In particular this also allows therapeutic methods that can be designed by a person skilled in the art in the light of the sequence information that is now available.

[0095] Like the nucleic acid sequences according to the invention themselves, ribozymes are of particular advantage when, but not only when, they are introduced in the effector cell for example by gene therapy. However, it is also conceivable that appropriate modifications are carried out ex vivo and such modified cells are then made available for reimplantation which is either allogenic or autogenic. The production and use of ribozymes is disclosed in Ribozyrne Protocols (Philip C. Turner, Ed. Humana Press, Totowa, N.Y., 1997) and is incorporated herein.

[0096] With regard to the design of the kit for diagnosing and/or treating functional disorders, hyperplasia and tumours of the thyroid it should be noted that the exact design of such a kit i.e. which specific components are contained therein are known to a person skilled in this field and in particular are possible in the light of the above-mentioned description of the effects and uses of the nucleic acid sequences disclosed herein. The kit according to the invention in any case contains at least one of the elements according to the invention which is selected from the group comprising the nucleic acid sequence, the vector, the polypeptide, the cell, the antibody and the ribozyme each in its inventive form. With regard to the use of the cells according to the invention they can in particular be used as a reimplant or as a positive and/or negative control in corresponding diagnostic or therapeutic methods.

[0097] In the method according to the invention for detecting functional disorders and hyperplasia and/or tumours of the thyroid also in the form of carcinomas and strumae, it is possible for the thyroid material to be present in situ, ex vivo or in vitro. The term"thyroid material" as used herein refers to material and in particular cellular material of the thyroid in its normal as well as in a pathological state. Hence the term thyroid material also includes material from thyroid glands with a functional disorder, from hyperplasia of the thyroid, from tumours of the thyroid, including carcinomas and strumae. The determination whether a functional disorder, hyperplasia and/or tumour is present is finally carried out by comparing the effect of the agent or agents according to the invention on the thyroid material to be examined with its/their effect on normal thyroid tissue. Other embodiments of the method according to the invention can be derived by persons skilled in the art on the basis of the disclosure contained herein.

[0098] The statements made above with regard to the various embodiments and to the achievable advantages also apply synonymously to the dnigs according to the invention and in particular to those for diagnosing and/or treating functional disorders, hyperplasia and/or tumours of the thyroid in the form of the sequence according to the invention or its use, in the form of a vector according to the invention or its use, in the form of the polypeptide according to the invention or its use, in the form of the cell according to the invention or its use, in the form of the antibody according to the invention or its use and in the formn of the ribozyme according to the invention or its use which are disclosed herein. The corresponding drugs can contain a quantity of one or several of the aforementioned agents.

[0099] The same also applies to pharmaceutical compositions according to the invention. In this case the pharmaceutical composition may contain only one of the compounds listed above in addition to the pharmaceutically acceptable carrier. Within the scope of the invention the pharmaceutical composition can also contain several of the said compounds in addition to the pharmaceutically acceptable carrier.

[0100] A pharmaceutically acceptable carrier is understood herein as all carriers known to a person skilled in the art that are typically selected depending on the form of administration. A pharmaceutically acceptable carrier can among others be water, buffer solutions, alcoholic solutions and such like.

[0101] With regard to the method according to the invention for constricting primers it should be noted that they are those which allow a specific preparation of the nucleic acids according to the invention or parts thereof.

[0102] The method according to the invention for preparing a nucleic acid sequence according to the invention can utilize, from which ever source, the nucleic acid sequences according to the invention or corresponding sequences using primers according to the invention as primers for a polymerase chain reaction in all its various embodiments. The design of suitable primers and the procedure for polymerase chain reactions is disclosed in PCR & PCR 2: A practical approach (M.J. McPherson, P. Quirke and G.R. Taylor, ed. IRL Press, Oxford, England 1991, & M.J. McPherson and B.D. Hamers, ed. IkL Press, Oxford, England 1995) and is incorporated herein.

[0103] Further features and advantages of the invention derive from the figures, the sequence protocol and the following examples.

[0104] FIG. **1***a*: shows a metaphase of the cell line S-**121**T/SV**40** with t(**5**;**19**)(q**13**:q**13**) after G band staining;

chromosome **19**, derivative chromosome **19** (="der(**19**))" and derivative chromosome **5** (="der(**5**)" are marked with arrows.

[**0105**] FIG. **1***b*: shows the same metaphase after FISH with the PAC clone **19**/B; the hybridization signals are located on chromosome **19**, der(**19**) and der(**5**);

[**0106**] FIG. **2**: shows the genomic structure of a gene which is referred to in the following as RITA (rearranged in thyroid adenomas) the gene that is involved in specific 19q13 translocations in thyroid tumours . Grey boxes represent the coding regions which belong to the open reading frame of exon **3** to exon **5**, whereas 5' or 3' non-translated regions are shown as white boxes. The arrows under the exon-intron structure represents cDNA clones from the data banks. The tips of the arrows show the sequencing direction for these clones. The source of the RNA/cDNA is indicated by a single letter in brackets behind the gene bank accession number of each clone (a: total foetus, b: foetal heart, c: foetal liver/spleen, d: lung, e: eye retina, f: epiphysis, g: breast, h: ovarial-cortical stroma; i: milk duct breast tumours, j: Jurkat T cells, k: germ cell tumour, **1**: neuroendocrine lung carcinoma, m: testis). Filled bars in the lower part of the figure represent the position of parts of cosmid **15841** and PAC**19**/B relative to RITA; the abbreviations "cen" and "tel" relate to the position of the centromer and the telomer relative to RITA; the orientation of the gene is from left to right in the figure in the direction of the telomer.

[**0107**] FIG. **3**: shows the cDNA sequence of RITA and also shows the size of the exons, the open reading frame and the amino acid sequence of the open reading frame derived therefrom. The regions corresponding to the KRAB domain and the zinc finger motifs (ZF) are indicated by bars with black borders;

[**0108**] FIG. **4**: shows expression studies of RITA: Northern blot analysis of poly(A)+RNA, which had been isolated from myometrium, testis, thyroid, the cell line S**40**.**2**T/SV**40** and the cell line S**121**T/SV**40** which reveals two transcripts of about 4.7 kbp and 5 kbp of different intensities in myometrium, testis and thyroid (lanes **1**-**3**). An additional transcript of about 2.1 kbp is only found in the testis (lane **2**). The cell lines S**40**.**2**T/SV**40** and S**121**T/SV**40** (lanes **4**-**5**) express two different transcripts of approximately 5.5 kbp and 6.2 kbp. More details of the expression studies are given, among others, in example 15.

Example 1

[**0109**] Cell cultures were set up from tissue specimens of follicular thyroid tumours (n=33), strumae (n=236) and of normal thyroid tissue (n=20) i.e. having the usual histological appearance and these cell cultures were processed for a chromosome analysis. The cell culture and chromosome preparation was carried out as described by Belge et al., (Belge, G., Thode, B., Bullerdiek, J., Bartnitzke, S., 1992, Cancer Genet. Cytogenet. 60, 23-26). 4 of 33 adenomas and 5 of 236 strumae had chromosome translocations involving the chromosomal band 19q13, whereas a corresponding chromosome change was not found in any specimen from the normal thyroid tissue.

Example 2

[**0110**] Cell cultures of tumours with aberrations of the band 19ql3 were used to prepare cell lines for further experiments. For this a part of the SV**40** genome was transfected into the cells using the calcium phosphate precipitation method as described by Kazmierczak et al. (Kazmierczak, B., Bartnitzke, S., Hartl, M., Bullerdiek, J., 1990; Cytogenet. Cell Genet. 53, 37-39). 3 cell lines were established which each contain the normal chromosome **19** and the resulting derivative chromosomes from the translocation (tab. 1)

TABLE 1

Overview of the cell lines derived from primary tumours

Lab. Code	Material	karyotype
S-121	adenoma	46, XX, t(5; 19)(q13; q13)
S-141.2	struma	46, XX, t(2; 19)(p13; q13)
S-40.2	struma	46, XX, t(1; 19)(p35 or p36, 1; q13)

Example 3

[**0111**] Cell lines with t(**5**;**19**)(q**13**;q**13**) and t(**1**;**19**(p**35**-p**36**.**1**;q**13**) were obtained by transfection with a construct which contained the SV**40** large T antigen as previously reported (Kazmierczak et al., 1990; Cytogenet Cell Genet 53:37-39); Belge et al., 1992, Cell Biol. Int. Rep., 16:339-347).

Example 4

[**0112**] Fluorescence in situ hybridization (FISH) analyses were carried out after GTG band staining of the same metaphase preparations. The treatment of the metaphases and subsequent FISH experiments were carried out using the protocol of Kievits et al., (1990, Cytogenet. Cell Genet. 53:134-136). Cosmid and PAC DNA was labelled with biotin-14-dATP by nick translation (Gibco BRL, Life Technology GmbH, Eggenstein, Germany). 20 metaphases were examined for each cosmid or PAC probe. Chromosomes were counterstained with propidium iodide, analysed on a Zeiss-Axiophot fluorescence microscope using a FITC filter (Zeiss) and recorded with a power gene karyotyping system (PSI, Halladale, UK).

Example 5

[**0113**] In order to obtain PAC clones which correspond to cosmid 15841 that had been isolated from the chromosome **19**-specific cosmid library of the Lawrence Livermore National Laboratory (Trask et al., 1992, Genomics 14:162-167; Ashworth et al., 1995, Nat. Genet. 11:422-427), the cosmid was subcloned and sequenced on a 373 DNA sequencer (Applied Biosystems, Weiterstadt, Germany) using the primer M**13** universal and M**13** reverse. PAC clones were then obtained by screening a human PAC library (Genome Systems, St. Louis, USA) using a set of primers which corresponded to the sequence of cosmid **15841**. PAC**19**/B was subcloned in three clone arrangements. As the first, *Eco*RI digested DNA fragments were cloned into the pGEM**11**zf(+) vector (Promega, Madison, USA). Two additional clone arrangements were prepared by shearing the DNA, in order to produce random fragments with an average size of 1 kbp and 3 kbp and cloned into the pTZ19R vector (MB**1** Fermentas, Amherst, USA). *Eco*RI- and *BamH*I-digested DNA fragments of cosmid **15841** (Trask et al., 1991, Somat. Cell Mol. Genet. 17:117-36)f; Trask et al., 1992, loc.cit.; Ashworth et al., 1995, loc.cit.) were also cloned into the pGEMllzf(+) vector. The sequencing was carried out with standard and internal primers using an AB**1** 373A sequencer.

Example 6

[**0114**] Alignment of the sequences and PCR primers was carried out with the Lasergene software package (DNA-Star, Madison, USA). Analyses of sequence homologies were carried out using the BLAST programme from NCB**1** (Altschul et al., 1997, Nucl. Acids Res. 25:3389-3402). GENSCAN was used for gene prediction and composition (Burge and Karlin, 1997, J. Mol. Biol. 268:78-94).

Example 7

[**0115**] Total RNA was isolated from two cell lines and from normal adult tissues using the TRIzol reagent (Gibco BRL, Life Technologies GmbH, Eggenstein, Germany) based on the one-step acid phenol RNA isolation method (Chomczynski and Sacchi, 1987, Anal. Biochem. 162:15-159). Poly(A)$^+$–RNAs were purified by oligotex $dC_{10}T_{30}$ adsorption (QIAGEN, Hilden, Germany). Approximately 5 μg poly(A)$^+$–RNAs were denatured and fractionated on a 1% agarose/6% formaldehyde gel and transferred to a Hybond–$^+$nylon membrane (Amersham Pharmacia, Freiburg, Germany). A partial cDNA clone (bp 17-1108) of the putative gene (RITA) (rearranged in thyroid adenomas) was used as a molecular probe. The probe was labelled with ^{32}P using a random primer extension protocol (Feinberg and Vogelstein, 1983, Anal. Biochem. 132:6-13). The ExpressHyb hybridization solution (Clontech Laboratories, Palo Alto, USA) was used for pre-hybridization as well as for hybridization. Pre-hybridization was carried out for 30 minutes and hybridization was carried out for one hour at 68° C. The membranes were washed twice for 20 minutes at room temperature in 2 ×SSC/0.05% SDS and twice for 20 minutes at 68° C. in 0.1×SSC/0.1% SDS. Signals were made visible using a STORM-Phosphorimager (Molecular Dynamics, Sunnyvale, USA) or by autoradiography.

Example 8

[**0116**] Standard PCR amplification was carried out in a final volume of 50 μl. Approximately 60 ng template was amplified in a standard PCR reaction containing 400 nM of each primer, 300 μM dNTPs, 1 ×PCR buffer (containing 1.5 mM $MgCl_2$) and 2.5 U AmpliTaq (Perkin Elmer Applied Biosystems, Weiterstadt, Germany).

[**0117**] Alu-PCR was used to obtain genomic sequences or closely overlapping fragments (close sequencing contigs). Gene-specific primers were designed using partial sequences of RITA and sequences of PAC**19**/B or cosmid **15841** subclones. 400 nM of the gene-specific primer was used for an Alu-PCR reaction together with 100 nM primer TCCCAAAGTGCTGGGATTACAG and CTGCACTC-CAGCCTGG. PCR products were separated on a 1.5% agarose gel, the bands of interest were cut out, purified from the gel sections using glass bead techniques (QIAExII gel isolation kit, QIAGEN, Hilden, Gennany), cloned into the pCR2.1 vector (Invitrogen, San Diego, USA) and subjected to DNA sequence analysis.

Example 9

[**0118**] In order to obtain a RITA-specific cDNA probe generated by RT-PCR, total RNA from normal fibroblast primary cultures was isolated using TRIzol reagent (Gibco BRL, Life Technologies GmbH, Eggenstein, Germany). Total RNA was denatured and fractionated on a 1% agarose/6% formaldehyde gel for quality control. 5 μg of the total RNA was reversely transcribed into a first strand cDNA using M-MLV reverse transcriptase (Gibco BRL, Life Technologies GmbH, Eggenstein, Germany) using the primer AAGGATCCGTCGACATCT (17) as a start for the transcription. The extension was carried out for 45 minutes at 42° C. in a volume of 20 gl. I ill of the reaction mixture was used as a template in a standard reaction using primers ACGCAACCGCTGTGTCTCC and AGGCCTTCCCA-CATTCTTGAC to amplify exon **1** to exon **5** of RITA. In order to control the cDNA quality, GAPDH expression was checked with a standard RT-PCR reaction using primers GGTGAAGACGCC AGTGGACTC and GTGAAGGTCG-GAGTCAACG.

Example 10

[**0119**] The position of cosmid clones of the chromosome **19**-specific cosmid library of the Lawrence Livermore National Laboratory, Livermore, Calif. USA relative to the breakage point of the chromosomal translocation was determined with the aid of fluorescence in situ hybridization (FISH).

[**0120**] In two cell lines with the translocations t(5;19)(q**13**;q**13**) and t(**1**;**19**) (p**35**-p**36**.**1**; q**13**) the chromosome **19** breakage site was located by FISH analysis between cosmid clones **15841** and **29573**. These clones were isolated from the chromosome **19**-specific cosmid library of the Lawrence Livermore National Laboratory (Trask et al., 1992, Genomics 14: 162-167; Ashworth et al., 1995, Nat. Genet. 11: 422-427).

Example 11

[**0121**] Further tumours with aberrations of the chromosomal band **19q13** were selected from the tumour series referred to in example 1 (tab.2). Metaphase preparations of these tumours were used for fluorescence in situ hybridization. Also in this case it turned out that all breakage points were within the previously defined segment.

TABLE 2

Overview of the primary tumours used in the FISH

Lab. Code	Material	Karyotype
S-172	strumae	46, XX, t(2; 19)(p13; q13)
S-216	adenoma	46, XX, t(10; 19)(q24; q13)

Example 12

[**0122**] A set of primers was designed corresponding to the sequence of cosmid **15841** in order to obtain PAC clones which cover the region of the chromosomal breakage sites. This set of primers was used to screen a human PAC library (Genome Systems, St. Louis, USA). The screening led to two PAC clones which are referred to herein as PAC**19**/A and PAC**19**/B. These clones were firstly used for FISH studies on the cell line S-**121**T/SV**40** which exhibited a translocation t(**5**;**19**)(q**13**;q**13**). Signals on the normal chromosome **19** and the der(**19**) chromosome were detected with the PAC clone **19**/A. In contrast the PAC clone **19**/B exhibited signals on the normal chromosome **19**, on the der(**19**) chromosome and on the der(**5**) chromosome. Hence PAC**19**/B spans the breakage. site of this cell line. Additional FISH studies with PAC **19**/B on cells of the second cell line with the translocation t(**1**;**19**) (p**35** or p**36.1**; q**13**) disclosed hybridization signals which could be mapped on the normal chromosome **19** as well as on the two derivative chromosomes which indicates that the same breakage site region was involved in this translocation. DNA sequence analysis of one of the subclones which was derived from the PAC clone PAC**19**/B revealed a sequence homology with several cDNA clones from the databanks. A comparison of EST sequences with the genomic sequence of the PAC clone enabled a reconstruction of the structure of the gene including a determination of its exon-intron junctions. The total size of the cDNA cloned so far is 2015 bp, but this does not necessarily represent the total length of the cDNA.

[**0123**] With regard to the genomic structure, the gene has 5 exons. A part of exon **5** corresponds to the 3' non-translated region whereas its remaining part codes for a Krüppel-like zinc finger region (Schuh et al., 1986, Cell, 47: 1025-1032; Bellefroid et al., 1989, DNA, 8:377-387). Due to the high homology of exon **4** with parts of ZNF 140 (Vissing et al., 1995, FEBS, 369:153-157) and ZNF 91 (Bellefroid et al., 1993, EMBO J., 12:1363-1374) it can be concluded that this part of the gene codes for a KRAB-A domain (Bellefroid et al., 1991, Proc. Natl. Acad. Sci. USA, 88:3608-3612; Rosati et al., 1991, Nucl. Acids Res. 19: 5661-5667). Exon **3** contains a putative initiation codon which fits the consensus sequence A/GCCATGG (Kozak M. 1987, J. Mol. Biol. 196:947-950) and is why exon 1-3 cannot represent the 5=**40** non-translated region. The said literature references are incorporated by way of reference.

[**0124**] The position of the individual exons is as follows:

[**0125**] exon **1**: nucleotides **1-49**;

[**0126**] exon **2**: nucleotides **50-113**;

[**0127**] exon **3**: nucleotides **114-195**;

[**0128**] exon **4**: nucleotides **196-322**;

[**0129**] exon **5**: nucleotides **323-2014**

[**0130**] The sequence coding for RITA extends from nucleotide **187-1575**. A poly(A) signal is located between nucleotide **1683** and nucleotide **1689**. The KRAB domain is coded by nucleotides **196-322**.

[**0131**] A motif is understood herein as a section of a sequence at a DNA and/or protein level which is repeated in various modifications or in an identical form within the sequence. A protein domain is understood as a polypeptide chain or the part of a polypeptide chain that can independently fold into a stable tertiary structure. Zinc finger motifs may be found in the following nucleotides, as also shown in FIG. **3**.

[**0132**] 1. nucleotides **577-660**;

[**0133**] 2. nucleotides **661-744**;

[**0134**] 3. nucleotides **745-828**;

[**0135**] 4. nucleotides **829-912**;

[**0136**] 5. nucleotides **913-996**;

[**0137**] 6. nucleotides **997-1080**;

[**0138**] 7. nucleotides **1081-1164**;

[**0139**] 8. nucleotides **1165-1248**;

[**0140**] 9. nucleotides **1249-1332**;

[**0141**] 10. nucleotides **1333-1416**;

[**0142**] 11. nucleotides **1417-1500**;

[**0143**] 12. nucleotides **1591-1575**.

[**0144**] The said zinc finger motifs are contained in the zinc finger domain.

[**0145**] The polypeptide shown in SEQ ID NO: **4** which is coded by the cDNA shown in SEQ ID NO: **3** has a relative molecular mass of 53,737.79 Daltons, contains 436 amino acids of which 81 are K or R (basic amino acids), 54 amino acids are D or E (acidic amino acids), 96 are A or I or L or F or W or V (hydrophobic amino acids) and 144 amino acids are N or C or Q or S or T or Y (polar amino acids). The isoelectric point is 8.9.

[**0146**] The transcriptional orientation of the gene is towards the telomer which can be derived from the following results. Cosmid **15841** was only mapped on the der(**19**) chromosome with the aid of FISH, but in both cell lines signals of PAC**19**/B were allocated to the der(**19**) chromosome as well as to the der(**5**) chromosome or the der(**1**) chromosome. On the other hand, it was shown with the aid of PCR using specific primers for individual exons that cosmid **15841** only contains the exons **3-5**, whereas PAC**19**/B contains all so far identified exons. Hence the overlap between the two clones occurs in the 3' part of the gene.

[**0147**] A typical poly(A) signal is located at bp **1684**. An alternative poly(A) site AAATGAAAA is 30 bp upstream of the 3' end.

[**0148**] Northern blot hybridization of a probe that is specific for the gene (bp **17-1108**) on poly(A)+ Northern blots revealed a hybridization to two transcripts of approximately 4.7 kbp and 5 kbp in normal thyroid tissue and in myometrium. An additional strong band of 2.1 kbp was detected in RNA from testis. None of these transcripts was found in the two cell lines with 19 q aberrations which instead expressed two different transcripts of about 5.5 kbp and 6.2 kbp.

Example 13

[**0149**] RNA was isolated by standard methods from the above-mentioned cell lines and from material preserved in liquid nitrogen of the two primary tumours with the **19**q**13** aberrations and this RNA was used for Northern blots. After hybridization with the cDNA of the gene RITA, clear bands were found which allow the conclusion that sequences of this PAC clone belong to a gene that is expressed in follicular thyroid tumours.

Example 14

[**0150**] The methods described in examples 1 and 5 were used to carry out chromosomal analyses and molecular-cytogenetic examinations of five follicular thyroid carcinomas. Two of these tumours with complex karyotype changes also exhibited translocations with breakage points in **19**q**13**.

FISH that was also carried out yielded a location of the breakage points within the region covered by PAC (PAC19/B).

Example 15

[0151] The fact that the gene disclosed herein and disclosed nucleic acid sequences of the target gene with the 19q13 aberrations and in particular 19q13 translocations are present in thyroid tumours is suggested by three experimental findings.

[0152] 1. The breakage points are intragenic or no more than 200 kpb outside the 5' and 3' ends of the gene which either suggests the formation of a fusion gene or the transcriptional deregulation of the gene.

[0153] 2. With regard to the expression pattern of the gene it appears that the gene is expressed in many types of normal tissue as shown by the entries in the data bank and the Northern blot results described above. Nevertheless three different transcripts have been found in normal tissue of which the smallest is only detected in testicular tissue whereas the two larger transcripts are expressed ubiquitously. In contrast a unique, unusal expression pattern for the gene RITA was only detected in two cell lines with the translocations t(5;19)(q13;q13) and t(1;19)(p35-p36.1;q13).

[0154] 3. The structure of the gene suggests its involvement in transcription regulations. Its gene product, the RITA protein, belongs to the class of KRUPPEL-associated box(KRAB) zinc finger proteins (Bellefroid et al., 1991, loc.cit., Rosati et al., 1991, loc.cit.). These proteins form a large subfamily in which the KRAB-A domain has been identified as a potent transcription repressor domain (Margolin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4509-4513). It has been estimated that several hundred genes which code for Krüppel-like proteins exist in the mammalian genome (Bellefroid et al., 1991, loc.cit.). Genes which code for KRAB zinc finger proteins form about a third of these proteins. The binding of the transcription repressor protein kid-1, another KRAB zinc finger protein, to an individual DNA structure has recently been demonstrated (Elser et al., 1997, J. Bio. Chem. 272:279908-27912). At first sight this suggests that RITA has a function as a transcription regulator since the 19q13 translocations lead to a loss of function. On the other hand, the lack of deletions in this region in thyroid tumours and the specificity of some of these aberrations suggest a gain of function. The Wilms' tumour gene WTI codes for a classical zinc finger protein (Call et al., 1990, Cell 60:509-520; Gessler et al., 1990, Nature 343:774-778; Haber et al., 1990, Cell 61:1257-1269) and shares some common features with the KRAB zinc finger proteins with regard to its DNA binding characteristics (Elser et al., 1997, loc.cit.). There are many experimental findings that WT1 mutations have a recessive effect with regard to the development of Wilms' tumours. In contrast a specific translocation t(11;22)(p13;q12) that is observed in desmoplastic round cell tumours leads to the formation of chimeric transcripts of the gene EWS and TWI (Ladanyi and Gerald, 1994, Cancer Res. 54:2837-2840; Rauscher et al., 1994, Cold Spring Harb. Symp. Quant. Biol. 59:137-146). In the resulting protein the RNA binding domain of EWS is replaced by the zinc finger motif of WT1. There is compelling evidence that this fusion transcript is essential for the development of desmoplastic round cell tumours which is an example of how the gene can also be impaired by a dominant gain of function.

[0155] Although the results of the Northern blot analyses suggest that the structure of RITA and its expression pattern have not yet been completely elucidated, presumably due to its close proximity to the breakage site region, the present application nevertheless discloses that RITA is a target gene for specific 19q aberrations in thyroid tumours which can be used as such for the diagnosis, prevention and treatment of functional disorders of the thyroid, hyperplasia of the thyroid and tumours of the thyroid.

[0156] The features disclosed in the previous description including the sequence protocols and in the figures and the claims can be important for the realization of the invention in its various embodiments either individually or also in any combination.

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                          SEQUENCE LISTING

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Glu Cys Lys Lys Ala Phe Arg Trp Gly Asn Gln Leu Thr Gln His Gln
        35                  40                  45

Lys Ile His Thr Gly Glu Lys Pro Tyr Glu Cys Lys Asp Cys Gly Lys
     50                  55                  60

Ala Phe Arg Trp Gly Ser Ser Leu Val Ile His Lys Arg Ile His Thr
 65                  70                  75                  80

Gly Glu Lys Pro Tyr Glu Cys Lys Asp Cys Gly Lys Ala Phe Arg Arg
                 85                  90                  95

Gly Asp Glu Leu Thr Gln His Gln Arg Phe His Thr Gly Glu Lys Asp
            100                 105                 110

Tyr Glu Cys Lys Asp Cys Gly Lys Thr Phe Ser Arg Val Tyr Lys Leu
        115                 120                 125

Ile Gln His Lys Arg Ile His Ser Gly Glu Lys Pro Tyr Glu Cys Lys
    130                 135                 140

Asp Cys Gly Lys Ala Phe Ile Cys Gly Ser Ser Leu Ile Gln His Lys
145                 150                 155                 160

Arg Ile His Thr Gly Glu Lys Pro Tyr Glu Cys Gln Glu Cys Gly Lys
                165                 170                 175

Ala Phe Thr Arg Val Asn Tyr Leu Thr Gln His Gln Lys Ile His Thr
            180                 185                 190

Gly Glu Lys Pro His Glu Cys Lys Glu Cys Gly Lys Ala Phe Arg Trp
        195                 200                 205

Gly Ser Ser Leu Val Lys His Glu Arg Ile His Thr Gly Glu Lys Pro
    210                 215                 220

Tyr Lys Cys Thr Glu Cys Gly Lys Ala Phe Asn Cys Gly Tyr His Leu
225                 230                 235                 240

Thr Gln His Glu Arg Ile His Thr Gly Glu Thr Pro Tyr Lys Cys Lys
                245                 250                 255

Glu Cys Gly Lys Ala Phe Ile Tyr Gly Ser Ser Leu Val Lys His Glu
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<213> ORGANISM: Homo sapiens
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<221> NAME/KEY: CDS
<222> LOCATION: (187)..(1575)
<223> OTHER INFORMATION: coding region for RITA

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ctctcggaat ttgtcttctt cagtggaaac cccgagaaga ctgatcagtt cttcagttct 180

aaaaca atg gcc cag ggt ttg gtg acg ttc gcc gac gta gcc ata gac 228
Met Ala Gln Gly Leu Val Thr Phe Ala Asp Val Ala Ile Asp
1 5 10

ttt tct cag gag gag tgg gcc tgt ctg aac tct gct cag agg gac ctg 276
Phe Ser Gln Glu Glu Trp Ala Cys Leu Asn Ser Ala Gln Arg Asp Leu
15 20 25 30

tac tgg gac gtg atg ctg gag aac tac agt aac ttg gtc tca ctg gat 324
Tyr Trp Asp Val Met Leu Glu Asn Tyr Ser Asn Leu Val Ser Leu Asp
35 40 45

ttg gag tca gca tat gaa aat aag agt tta cct aca gaa aaa aac att 372
Leu Glu Ser Ala Tyr Glu Asn Lys Ser Leu Pro Thr Glu Lys Asn Ile
50 55 60

cat gaa ata agg gct tcc aaa agg aat tca gat aga aga agt aaa tcc 420
His Glu Ile Arg Ala Ser Lys Arg Asn Ser Asp Arg Arg Ser Lys Ser
65 70 75

ctt ggc cgt aac tgg ata tgt gaa ggt acg ctt gaa aga cca cag cgc 468
Leu Gly Arg Asn Trp Ile Cys Glu Gly Thr Leu Glu Arg Pro Gln Arg
80 85 90

tcc aga ggg agg tat gtc aat cag atg atc atc aat tat gtc aaa agg 516
Ser Arg Gly Arg Tyr Val Asn Gln Met Ile Ile Asn Tyr Val Lys Arg
95 100 105 110

cct gct act aga gaa ggc acc cct cct aga aca cat cag aga cat cat 564
Pro Ala Thr Arg Glu Gly Thr Pro Pro Arg Thr His Gln Arg His His
115 120 125

aag gag aat tcc ttt gaa tgt aag gac tgt ggg aag gcc ttt agt cgt 612
Lys Glu Asn Ser Phe Glu Cys Lys Asp Cys Gly Lys Ala Phe Ser Arg
130 135 140

ggc tat caa ctt agt caa cat cag aaa atc cat act ggt gag aaa cct 660
Gly Tyr Gln Leu Ser Gln His Gln Lys Ile His Thr Gly Glu Lys Pro
145 150 155

tat gaa tgt aaa gaa tgt aag aag gcc ttc cgt tgg ggc aat cag ctt 708
Tyr Glu Cys Lys Glu Cys Lys Lys Ala Phe Arg Trp Gly Asn Gln Leu
160 165 170

act caa cat caa aaa att cat act ggg gag aag ccc tac gaa tgt aaa 756
Thr Gln His Gln Lys Ile His Thr Gly Glu Lys Pro Tyr Glu Cys Lys
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Asp Cys Gly Lys Ala Phe Arg Trp Gly Ser Ser Leu Val Ile His Lys

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gcc ttt cgg cgt ggt gat gag ctc act cag cac cag aga ttc cac act      900
Ala Phe Arg Arg Gly Asp Glu Leu Thr Gln His Gln Arg Phe His Thr
        225                 230                 235

ggg gag aaa gac tac gaa tgc aaa gac tgt ggg aag acc ttt agc cgt      948
Gly Glu Lys Asp Tyr Glu Cys Lys Asp Cys Gly Lys Thr Phe Ser Arg
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gtg tat aaa ctt att cag cac aag aga att cat agt ggg gag aag cct      996
Val Tyr Lys Leu Ile Gln His Lys Arg Ile His Ser Gly Glu Lys Pro
255                 260                 265                 270

tac gag tgt aaa gac tgt ggg aag gct ttt att tgt ggt tca agc ctc     1044
Tyr Glu Cys Lys Asp Cys Gly Lys Ala Phe Ile Cys Gly Ser Ser Leu
                275                 280                 285

att cag cat aaa aga att cac aca ggt gag aaa ccc tat gaa tgt caa     1092
Ile Gln His Lys Arg Ile His Thr Gly Glu Lys Pro Tyr Glu Cys Gln
            290                 295                 300

gaa tgt ggg aag gcc ttt act cga gtc aat tac ctt act cag cat cag     1140
Glu Cys Gly Lys Ala Phe Thr Arg Val Asn Tyr Leu Thr Gln His Gln
        305                 310                 315

aag atc cac acc ggt gag aag cct cac gaa tgt aag gag tgt ggg aag     1188
Lys Ile His Thr Gly Glu Lys Pro His Glu Cys Lys Glu Cys Gly Lys
    320                 325                 330

gcc ttt cgc tgg ggt tcg agc ctc gtt aag cac gag agg ata cat acg     1236
Ala Phe Arg Trp Gly Ser Ser Leu Val Lys His Glu Arg Ile His Thr
335                 340                 345                 350

ggc gag aag ccg tac aag tgc aca gaa tgt ggg aag gcc ttc aat tgt     1284
Gly Glu Lys Pro Tyr Lys Cys Thr Glu Cys Gly Lys Ala Phe Asn Cys
                355                 360                 365

ggc tat cac ctc act cag cac gag aga atc cac aca ggc gaa acc ccg     1332
Gly Tyr His Leu Thr Gln His Glu Arg Ile His Thr Gly Glu Thr Pro
            370                 375                 380

tat aaa tgt aag gag tgt ggg aag gct ttc att tat gga tcg agc ctc     1380
Tyr Lys Cys Lys Glu Cys Gly Lys Ala Phe Ile Tyr Gly Ser Ser Leu
        385                 390                 395

gtg aaa cat gag aga att cat acc ggg gtg aaa ccc tat ggg tgt aca     1428
Val Lys His Glu Arg Ile His Thr Gly Val Lys Pro Tyr Gly Cys Thr
    400                 405                 410

gaa tgt ggg aag agc ttt agt cac ggc cat cag ctt aca caa cat cag     1476
Glu Cys Gly Lys Ser Phe Ser His Gly His Gln Leu Thr Gln His Gln
415                 420                 425                 430

aaa acg cac agt ggg gcg aaa tcc tac gaa tgt aag gag tgc ggg aag     1524
Lys Thr His Ser Gly Ala Lys Ser Tyr Glu Cys Lys Glu Cys Gly Lys
                435                 440                 445

gca tgt aac cac cta aac cat ctc cga gaa cat cag agg atc cac aac     1572
Ala Cys Asn His Leu Asn His Leu Arg Glu His Gln Arg Ile His Asn
            450                 455                 460

agt tgaagagcct tttgaacgca gtagcccgct cgtatctatg gtttcgcttt          1625
Ser

ccacagtttg ttacctgcag tcaactgcag ttcaaaaata ttaaatggaa aattccagaa   1685

ataaagaatt ttaagtctca aatggtgtgc ccttctgagt agcgtgatga aatctctcgc   1745

tgtccggctc cagccggccg gggatgtgag tcatcccttg gtccagcaca tccacgctgt   1805

atacgccacc caccctgcta gtgacttagt agccgtcttg gtgatcagat caactatccc   1865
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aaatgaaaag gtgaaagttc tcaacttaaa                                     2015


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<211> LENGTH: 463
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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Asp Val Met Leu Glu Asn Tyr Ser Asn Leu Val Ser Leu Asp Leu Glu
         35                  40                  45

Ser Ala Tyr Glu Asn Lys Ser Leu Pro Thr Glu Lys Asn Ile His Glu
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Ile Arg Ala Ser Lys Arg Asn Ser Asp Arg Arg Ser Lys Ser Leu Gly
 65                  70                  75                  80

Arg Asn Trp Ile Cys Glu Gly Thr Leu Glu Arg Pro Gln Arg Ser Arg
                 85                  90                  95

Gly Arg Tyr Val Asn Gln Met Ile Ile Asn Tyr Val Lys Arg Pro Ala
            100                 105                 110

Thr Arg Glu Gly Thr Pro Pro Arg Thr His Gln Arg His His Lys Glu
        115                 120                 125

Asn Ser Phe Glu Cys Lys Asp Cys Gly Lys Ala Phe Ser Arg Gly Tyr
    130                 135                 140

Gln Leu Ser Gln His Gln Lys Ile His Thr Gly Glu Lys Pro Tyr Glu
145                 150                 155                 160

Cys Lys Glu Cys Lys Lys Ala Phe Arg Trp Gly Asn Gln Leu Thr Gln
                165                 170                 175

His Gln Lys Ile His Thr Gly Glu Lys Pro Tyr Glu Cys Lys Asp Cys
            180                 185                 190

Gly Lys Ala Phe Arg Trp Gly Ser Ser Leu Val Ile His Lys Arg Ile
        195                 200                 205

His Thr Gly Glu Lys Pro Tyr Glu Cys Lys Asp Cys Gly Lys Ala Phe
    210                 215                 220

Arg Arg Gly Asp Glu Leu Thr Gln His Gln Arg Phe His Thr Gly Glu
225                 230                 235                 240

Lys Asp Tyr Glu Cys Lys Asp Cys Gly Lys Thr Phe Ser Arg Val Tyr
                245                 250                 255

Lys Leu Ile Gln His Lys Arg Ile His Ser Gly Glu Lys Pro Tyr Glu
            260                 265                 270

Cys Lys Asp Cys Gly Lys Ala Phe Ile Cys Gly Ser Ser Leu Ile Gln
        275                 280                 285

His Lys Arg Ile His Thr Gly Glu Lys Pro Tyr Glu Cys Gln Glu Cys
    290                 295                 300

Gly Lys Ala Phe Thr Arg Val Asn Tyr Leu Thr Gln His Gln Lys Ile
305                 310                 315                 320

His Thr Gly Glu Lys Pro His Glu Cys Lys Glu Cys Gly Lys Ala Phe
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                325                 330                 335

Arg Trp Gly Ser Ser Leu Val Lys His Glu Arg Ile His Thr Gly Glu
            340                 345                 350

Lys Pro Tyr Lys Cys Thr Glu Cys Gly Lys Ala Phe Asn Cys Gly Tyr
        355                 360                 365

His Leu Thr Gln His Glu Arg Ile His Thr Gly Glu Thr Pro Tyr Lys
    370                 375                 380

Cys Lys Glu Cys Gly Lys Ala Phe Ile Tyr Gly Ser Ser Leu Val Lys
385                 390                 395                 400

His Glu Arg Ile His Thr Gly Val Lys Pro Tyr Gly Cys Thr Glu Cys
                405                 410                 415

Gly Lys Ser Phe Ser His Gly His Gln Leu Thr Gln His Gln Lys Thr
            420                 425                 430

His Ser Gly Ala Lys Ser Tyr Glu Cys Lys Glu Cys Gly Lys Ala Cys
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Asn His Leu Asn His Leu Arg Glu His Gln Arg Ile His Asn Ser
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3. (canceled)
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25. (canceled)
26. (canceled)
27. (canceled)
28. (canceled)

29. A nucleic acid which codes for a polypeptide that influences the cellular process in thyroid tissue, wherein the polypeptide contains a KRAB domain and at least one zinc finger motif and the KRAB domain comprises the amino acid sequence of SEQ ID NO: 1 and the zinc finger motif is one which is comprised in the zinc finger domain having the amino acid sequence of SEQ ID NO: 2.

30. The nucleic acid of claim 29 comprising the nucleic acid sequence of SEQ ID NO: 3.

31. A nucleic acid coding for a polypeptide which influences the cellular process in thyroid tissue, wherein the nucleic acid hybridizes with a nucleic acid of claims **29** or **30**.

32. A nucleic acid of claims **29**, **30**, or **31** further comprising at least one mutation.

33. A vector comprising the nucleic acid sequence of claims **29**, **30**, **31** or **32**.

34. The vector of claim 33, further comprising at least one element which is selected from the group comprising promoters, terminators and enhancers.

35. The vector of claims **33** or **34**, wherein said vector is an expression vector.

36. The vector claims **33**, **34**, or **35**, wherein at least one promoter is in-frame with at least one part of a nucleic acid sequence according to claims **29**, **30**, **31** or **32** that codes for a polypeptide.

37. A polypeptide that influences the cellular process in thyroid tissue and comprises a KRAB domain and a zinc finger domain wherein the polypeptide is coded by a nucleic acid sequence according to claims **29**, **30**, **31** or **32**.

38. The polypeptide of claim 37, wherein said polypeptide is modified.

39. A cell containing a vector of claims **33**, **34**, **35** or **36**.

40. An antibody directed against a polypeptide according to claims **37** or **38**.

41. An antibody directed against a nucleic acid according to any of claims **29**, **30**, **31** or **32**.

42. A ribozyme directed against a nucleic acid according to claims **29**, **30**, **31** or **32**.

43. The ribozyme of claim 42, wherein said ribozyme contains at least a part of a nucleic acid as claimed according to any of claims **29**, **30**, **31**, or **32**.

44. Use of a nucleic acid according to claims **29**, **30**, **31** or **32** for diagnosis and/or treatment of functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours.

45. Use of a nucleic acid according to claims **29**, **30**, **31** or **32** for the manufacture of a medicament.

46. Use of a polypeptide of claims **37** or **38** for the diagnosis and/or treatment of functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours.

47. Use of a polypeptide according to claims **37** or **38** for the manufacture of a medicament.

48. Use of an antibody according to claims **40** or **41** for diagnosis and/or treatment of functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours.

49. Use of an antibody according to claims **40** or **41** for the manufacture of a medicament.

50. A kit for the diagnosis of functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours comprising: at least one element which is selected from the group comprising a nucleic acid, a vector, a polypeptide, a cell, an antibody and a ribozyme, from any of claims, **29**, **30**, **31**, **32**, **33**, **34**, **35**, **36**, **37**, **38**, **39**, **40**, **41**, **42** or **43**.

51. Method for detecting functional disorders of the thyroid and/or hyperplasia of the thyroid and/or tumours of the thyroid, the method comprising the steps of:

contacting the thyroid materials with an agent which is selected from the group comprising a nucleic acid, a vector, a polypeptide, an antibody, a ribozyme and a cell, from any of claims **29**, **30**, **31**, **32**, **33**, **34**, **35**, **36**, **37**,**38**, **39**, **40**, **41**, **42** or **43**, and

determining whether functional disorders of the thyroid and/or hyperplasia of the thyroid and/or tumours of the thyroid are present.

52. The method of claim 51 wherein the thyroid material is present ex vivo.

53. Use of a nucleic acid according to any of claims **29**, **30**, **31**, or **32** as a primer.

54. A primer for preparing and/or screening and/or detecting a nucleic acid, wherein said primer is complementary to a part of a nucleic acid sequence according to claims **29**, **30**, **31** or **32**.

55. A method for preparing a nucleic acid comprising a sequence that can be detected in thyroid tumours or strumae in which a translocation with a breakage point in the chromosomal band 19q13 is present, said sequence being located within the chromosomal band 19q13 , the method comprising the steps of:

preparing primers of claim 54 to carry out a polymerase chain reaction,

preparing a nucleic acid sequence taken from the band 19q13 of human chromosome **19** or a nucleic acid according to claims **29**, **30**, **31** or **32**;

mixing the nucleic acid sequence or the nucleic acid with the primers; and

carrying out a polymerase chain reaction.

56. A pharmaceutical composition comprising:

at least one agent which is selected from the group comprising a nucleic acid, a vector, a polypeptide, a cell, an antibody, a ribozyme, from claims **29**, **30**, **31**, **32**, **33**, **34**, **35**, **36**, **37**, **38**, **39**, **40**, **41**, **42** or **43**, or combinations thereof and at least one pharmaceutically acceptable carrier.

57. Use of a nucleic acid which can hybridize with a nucleic acid according to claims **29** or **30** for the diagnosis and/or treatment of functional disorders of the thyroid and/or hyperplasia of the thyroid and/or thyroid tumours.

58. Use of a nucleic acid which can hybridize with a nucleic acid according to claims **29** or **30** for the manufacture of a medicament for the treatment of functional disorders, hyperplasia and/or tumours of the thyroid.

* * * * *