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(54) **METHODS OF STRATIFYING ADOLESCENT IDIOPATHIC SCOLIOSIS, ISOLATED NUCLEIC ACID MOLECULES FOR USE IN SAME**

VERFAHREN ZUR STRATIFIZIERUNG VON ADOLESZENTER IDIOPATHISCHER SKOLIOSE, ISOLIERTE NUKLEINSÄUREMOLEKÜLE ZUR VERWENDUNG DAFÜR

PROCÉDÉS DE STRATIFICATION D'UNE SCOLIOSE IDIOPATHIQUE DE L'ADOLESCENCE, MOLÉCULES D'ACIDE NUCLÉIQUE ISOLÉES EN VUE D'UNE UTILISATION DANS LESDITS PROCÉDÉS

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Description

FIELD OF THE INVENTION

[0001] The present invention relates methods of stratifying adolescent idiopathic scoliosis, isolated nucleic acid molecules for use in same.

BACKGROUND OF THE INVENTION

[0002] Spinal deformities and scoliosis in particular, represent the most prevalent type of orthopaedic deformities in children and adolescents (0.2-6% of the population). Published studies suggest that one percent to six percent of the population will develop scoliosis. This condition leads to the formation of severe deformities of the spine affecting mainly adolescent girls in number and severity.

[0003] At present, the cause of adolescent idiopathic scoliosis (AIS), remains unclear (Connor JM, Conner AN, Connor RA, Tolmie JL, Yeung B, Goudie D. Genetic aspects of early childhood scoliosis. Am J Med Genet. 1987;27:419-424; and Machida M. Cause of idiopathic scoliosis. Spine. 1999;24:2576-2583) and there remains a need to stratify children or adolescents having AIS, identify children or adolescents at risk of developing AIS and identify which of the affected individuals are at risk of progression.

[0004] It has been showed that Pitx1 +/- mice developed severe spinal deformities after weaning. Paired-like homeodomain transcription factor 1 (Pitx1, previously called Ptx1) is a homeodomain transcription factor detected initially throughout pituitary development. The Pitx-family contains three related members, Pitx1, Pitx2 and Pitx3, which are members of the paired class of homeodomain proteins. The three Pitx factors have similar transcription properties (Drouin,J., Lanctôt,C., & Tremblay,J.J. La famille Ptx des facteurs de transcription à homéodomaine. Médecine/Sciences 14, 335-339 (1998); Drouin,J., Lamolet,B., Lamonerie,T., Lanctot,C., & Tremblay,J.J. The PTX family of homeodomain transcription factors during pituitary developments. Mol. Cell Endocrinol. 140, 31-36 (1998); and Lanctôt,C., Lamolet, B., & Drouin,J. The bicoid-related homeoprotein Ptx1 defines the most anterior domain of the embryo and differentiates posterior from anterior lateral mesoderm. Development 124, 2807-2817 (1997)). Among others, this transcription factor controls the development of craniofacial and hind limb specific structures in mammals. The *pitx1* gene is highly expressed in mouse hind limb long bones during development and accumulation of high levels of Pitx1 proteins were detected by immunohistochemistry on hind limb long bone sections mainly in the periarticular region, along the perichondrium (including at the hip and knee joints) and also in the nuclei of proliferative chondrocytes (Lanctôt,C., Lamolet,B., & Drouin, J. The bicoid-related homeoprotein Ptx1 defines the most anterior domain of the embryo and differentiates posterior from anterior lateral mesoderm. Development 124, 2807-2817 (1997)). Pitx1 expression was also detected in craniofacial structures such as the mandible and at the temporo-mandibular joints. It has been shown that targeted inactivation of the mouse pitx1 gene severely impairs craniofacial and hind limb development. While null mice died at birth, all PITX1 +/- mice which are normal at birth, developed severe spinal deformities (100% starting at 2 months).

[0005] The present description refers to a number of documents.

SUMMARY OF THE INVENTION

[0006] In accordance with the present invention, there is provided a method of stratifying a subject having adolescent idiopathic scoliosis (AIS) comprising: providing a cell sample isolated from the subject; detecting Paired-like homeodomain transcription factor 1 (Pitx1) expression in the cell sample; whereby the results of the detecting step enables the stratification of the subject having AIS as belonging to an AIS subclass.

[0007] In accordance with another aspect of the present invention, there is provided a method of stratifying a subject having adolescent idiopathic scoliosis (AIS) for a clinical trial comprising: providing a cell sample isolated from the subject; detecting Paired-like homeodomain transcription factor 1 (Pitx1) expression in the cell sample; and stratifying the subject for a clinical trial based on the results of the detecting step.

[0008] In accordance with another aspect of the present invention, there is provided a method for predicting a risk for developing adolescent idiopathic scoliosis (AIS) in a subject comprising providing a cell sample isolated from the subject; and detecting Paired-like homeodomain transcription factor 1 (Pitx1) expression in the cell sample; wherein an absence of Pitx1 expression is indicative that the subject is at risk for developing AIS.

[0009] In a specific embodiment of these methods, an absence of Pitx1 expression is indicative that the subject is at risk for developing a Cobb's angle of 45° and above. In another specific embodiment, the detecting step is performed with an isolated nucleic acid molecules specific to a Pitx1 transcription product. In another specific embodiment, the isolated nucleic acid molecule is detectably labeled. In another specific embodiment, the detecting step is performed with an antibody that binds specifically to Pitx1. In another specific embodiment, the cell sample is selected from the group consisting of an osteoblasts sample, a chondrocytes sample, a skeletal myoblasts sample and a blood sample.

In another specific embodiment, the cell sample is an osteoblasts sample. In another specific embodiment, the method further comprises a step of selecting a preventive action or a treatment in light of the results of the detecting step. In another specific embodiment, said subject is pre-diagnosed as being a likely candidate for developing adolescent idiopathic scoliosis.

[0010] In accordance with another aspect of the present invention, there is provided a method of selecting a compound potentially useful in the treatment of adolescent idiopathic scoliosis, said method comprising the steps of (a) contacting a test compound with at least one cell known to express Paired-like homeodomain transcription factor 1 (Pitx1); and (b) determining Pitx1 expression level; wherein the test compound is selected if Pitx1 expression level is increased in the presence of the test compound as compared to that in the absence thereof.

[0011] In a specific embodiment, said cell is an osteoblast. In another specific embodiment, said cell is from a subject having adolescent idiopathic scoliosis (AIS). In another specific embodiment, the subject is a human.

[0012] Disclosed is a comprising an isolated nucleic acid molecule specific to a transcription product of a Paired-like homeodomain transcription factor 1 (Pitx1) and instructions to use the probe to predict whether a subject is at risk for developing adolescent idiopathic scoliosis.

[0013] Disclosed is a kit comprising an isolated nucleic acid molecule specific to a transcription product of a Paired-like homeodomain transcription factor 1 (Pitx1) and instructions to use the probe to stratify a subject having adolescent idiopathic scoliosis.

[0014] Further disclosed is the kit which further comprises a container for a nucleotide sample from the subject.

[0015] Further disclosed is a kit comprising an antibody specific to a Paired-like homeodomain transcription factor 1 (Pitx1) and instructions to use the antibody to predict whether a subject is at risk for developing adolescent idiopathic scoliosis.

[0016] Further disclosed is a kit comprising an antibody specific to a Paired-like homeodomain transcription factor 1 (Pitx1) and instructions to use the antibody to stratify a subject having adolescent idiopathic scoliosis.

[0017] The articles "a," "an" and "the" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[0018] The term "including" and "comprising" are used herein to mean, and re used interchangeably with, the phrases "including but not limited to" and "comprising but not limited to".

[0019] The terms "such as" are used herein to mean, and is used interchangeably with, the phrase "such as but not limited to".

[0020] As used herein the terms "likely candidate for developing adolescent idiopathic scoliosis" include children of which a least one parent has adolescent idiopathic scoliosis. Among other factors, age (adolescence), gender and other family antecedent are factors that are known to contribute to the risk of developing a scoliosis and are used to a certain degree to assess the risk of developing AIS. In certain subjects, scoliosis develops rapidly over a short period of time to the point of requiring a corrective surgery (often when the deformity reaches a Cobb's angle $\geq 50°$). Current courses of action available from the moment AIS is diagnosed (when scoliosis is apparent) include observation (when Cobb's angle is around 10-25°), orthopaedic devices (when Cobb's angle is around 25-30°), and surgery (over 45°). A more reliable determination of the risk of progression could enable to 1) select an appropriate diet to remove certain food products identified as contributors to scoliosis; 2) select the best therapeutic agent; and/or 3) select the least invasive available treatment such as postural exercises, orthopaedic device, or less invasive surgeries or surgeries without fusions (a surgery that does not fuse vertebra and preserves column mobility). The present invention encompasses selecting the most efficient and least invasive known preventive actions or treatments in view of the determined risk of developing AIS. The present invention also encompasses stratifying AIS patients with methods of the present invention.

[0021] As used herein, the terms "severe AIS" refers to a scoliosis characterized by Cobb's angle of 45° or more.

[0022] As used herein, the term "Pitx1 expression" is used to refer Pitx1 transcription and/or Pitx1 translation. In a more specific embodiment, Pitx1 expression refers to Pitx1 transcription.

[0023] As used herein the terms "risk of developing AIS" and "risk of progression of AIS" are used interchangeably and refer to a genetic or metabolic predisposition of a subject to develop a scoliosis (i.e. spinal deformity) and/or a more severe scoliosis at a future time.

[0024] As used herein the term "subject" is meant to refer to any mammal including human, mice, rat, dog, cat, pig, monkey, horse, etc. In a particular embodiment, it refers to a human. In an other particular embodiment, it refers to a horse and more specifically a racing horse.

[0025] As used herein the terms "blood sample" is whole blood and it is a cell sample in that it comprises peripheral blood mononuclear cells.

[0026] Without being so limited, cells where Pitx1 is known to be expressed include cells from muscles, bone and cartilages such as osteoblasts, chondrocytes and skeletal myoblasts.

[0027] The present invention also relates to methods for the determination of the level of expression of transcripts or translation product of a single gene such as pitx1. The present invention therefore encompasses any known method for such determination including real time PCR and competitive PCR, Northern blots, nuclease protection, plaque hybridi-

zation and slot blots.

[0028] The present invention also concerns isolated nucleic acid molecules including probes and primers to detect Pitx1. In specific embodiments, the isolated nucleic acid molecules have no more than 300, or no more than 200, or no more than 100, or no more than 90, or no more than 80, or no more than 70, or no more than 60, or no more than 50, or no more than 40 or no more than 30 nucleotides. In specific embodiments, the isolated nucleic acid molecules have at least 17, or at least 18, or at least 19, or at least 20, or at least 30, or at least 40 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 300 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 200 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 100 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 90 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 80 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 70 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 60 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 50 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 40 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 17 and no more than 40 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 30 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 17 and no more than 30 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 300 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 200 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 100 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 90 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 80 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 70 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 60 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 50 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 40 nucleotides. It should be understood that in real-time PCR, primers also constitute probe without the traditional meaning of this term. Primers or probes appropriate to detect Pitx1 in the methods of the present invention can be designed with known methods using sequences distributed across the Pitx1 nucleotide sequence. (Buck et al. Design Strategies and Performance of Custom DNA Sequencing primers. Biotechniques 27:528-536 (September 1999)).

[0029] Although amino acid and nucleotide sequences for Pitx1 are included herein, the present invention is not so limited and encompasses the detection of any Pitx1 protein or nucleotides isolated from a subject. Without being so limited, the present invention encompasses the detection of the Pitx1 presented in Table 1.

[0030] TABLE 1. ACCESSION NUMBERS FOR PITX1 SEQUENCES

Nucleotide		**Protein**
Genomic	AC004764.1	AAC17733.1
Genomic	AC008406.7 (17530..24049, complement)	None
Genomic	AF009648.1	AAB65251.1
Genomic	AF009649.1	AAB65251.1
Genomic	AF009650.1	AAB65251.1
Genomic	CH471062.2	EAW62226.1
		EAW62227.1
Genomic	CS278249.1	CAJ86537.1
mRNA	AK290635.1	BAF83324.1
mRNA	AL578756.2	None
mRNA	BC003685.1	AAH03685.1
mRNA	BC009412.1	AAH09412.1
mRNA	BX362641.2	None

(continued)

Nucleotide		Protein
mRNA	CR601326.1	None
mRNA	CR603120.1	None
mRNA	CR610821.1	None
mRNA	U70370.1	AAC51126.1
Synthetic	EU446647.1	ABZ92176.1
		P78337.2
mRNA	NM_002653 version NM_002653.4	NP_002644 version NP_002644.4
Chromosome 5, reference assembly, complete sequence	NC_000005(134391323-134397863)	NC_000005.8
Genomic	NC_000005.8 Reference assembly 134397863..134391323, complement	
Genomic	NT_034772.5 36784977..36778437, complement	
Genomic	AC_000048.1 130493751..130487209, complement	
Genomic	NW_922784.1 8121767..8115225, complement	
Genomic	AC_000137.1 91265592..91277386	
Genomic	NW_001838952.2 4413502..4425296	
Genomic	NW_001838952.2 4413502..4425296	

[0031] Probes of the invention can be utilized with naturally occurring sugar-phosphate backbones as well as modified backbones including phosphorothioates, dithionates, alkyl phosphonates and α-nucleotides and the like. Modified sugar-phosphate backbones are generally known (Miller, 1988. Ann. Reports Med. Chem. 23:295; Moran et al., 1987. Nucleic Acids Res., 14:5019.). Probes of the invention can be constructed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), and preferably of DNA.

[0032] The types of detection methods in which probes can be used include Southern blots (DNA detection), dot or slot blots (DNA, RNA), and Northern blots (RNA detection). Although less preferred, labeled proteins could also be used to detect a particular nucleic acid sequence to which it binds. Other detection methods include kits containing probes on a dipstick setup and the like.

[0033] As used herein the terms "detectably labeled" refer to a marking of a probe or antibody in accordance with the presence invention that will allow the detection of the Pitx1 expression in methods and kits of the present invention. Although the present invention is not specifically dependent on the use of a label for the detection of a particular nucleic acid sequence, such a label might be beneficial, by increasing the sensitivity of the detection. Furthermore, it enables automation. Probes can be labeled according to numerous well known methods (Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Non-limiting examples of labels include 3H, 14C, 32P, and 35S. Non-limiting examples of detectable markers include ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radionucleotides. It will become evident to the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe or antibody.

[0034] As commonly known, radioactive nucleotides can be incorporated into probes of the invention by several methods. Non-limiting examples thereof include kinasing the 5' ends of the probes using gamma 32P ATP and polynucleotide kinase, using the Klenow fragment of Pol I of E. coli in the presence of radioactive dNTP (e.g. uniformly labeled DNA probe using random oligonucleotide primers in low-melt gels), using the SP6/T7 system to transcribe a DNA

segment in the presence of one or more radioactive NTP, and the like.

[0035] The present invention also relates to methods of selecting compounds. As used herein the term "compound" is meant to encompass natural, synthetic or semi-synthetic compounds, including without being so limited chemicals, macromolecules, cell or tissue extracts (from plants or animals), nucleic acid molecules, peptides, antibodies and proteins.

[0036] The present invention also relates to arrays. As used herein, an "array" is an intentionally created collection of molecules which can be prepared either synthetically or biosynthetically. The molecules in the array can be identical or different from each other. The array can assume a variety of formats, e.g., libraries of soluble molecules; libraries of compounds tethered to resin beads, silica chips, or other solid supports.

[0037] As used herein "array of nucleic acid molecules" is an intentionally created collection of nucleic acids which can be prepared either synthetically or biosynthetically in a variety of different formats (e.g., libraries of soluble molecules; and libraries of oligonucleotides tethered to resin beads, silica chips, or other solid supports). Additionally, the term "array" is meant to include those libraries of nucleic acids which can be prepared by spotting nucleic acids of essentially any length (e.g., from 1 to about 1000 nucleotide monomers in length) onto a substrate. The term "nucleic acid" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides, deoxyribonucleotides or peptide nucleic acids (PNAs), that comprise purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups, as may typically be found in RNA or DNA, or modified or substituted sugar or phosphate groups. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. Thus the terms nucleoside, nucleotide, deoxynucleoside and deoxynucleotide generally include analogs such as those described herein. These analogs are those molecules having some structural features in common with a naturally occurring nucleoside or nucleotide such that when incorporated into a nucleic acid or oligonucleotide sequence, they allow hybridization with a naturally occurring nucleic acid sequence in solution. Typically, these analogs are derived from naturally occurring nucleosides and nucleotides by replacing and/or modifying the base, the ribose or the phosphodiester moiety. The changes can be tailor made to stabilize or destabilize hybrid formation or enhance the specificity of hybridization with a complementary nucleic acid sequence as desired.

[0038] As used herein "solid support", "support", and "substrate" are used interchangeably and refer to a material or group of materials having a rigid or semi-rigid surface or surfaces. In many embodiments, at least one surface of the solid support will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. According to other embodiments, the solid support(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations.

[0039] Any known nucleic acid arrays can be used in accordance with the present invention. For instance, such arrays include those based on short or longer oligonucleotide probes or primers as well as cDNAs or polymerase chain reaction (PCR) products (Lyons P., 2003. Advances in spotted microarray ressources for expression profiling. Briefings in Functionnal Genomics and Proteomics 2, 21-30). Other methods include serial analysis of gene expression (SAGE), differential display, (Ding G. and Cantor C.R., 2004. Quantitative analysis of nucleic acids - the last few years of progress. J Biochem Biol 37, 1-10) as well as subtractive hybridization methods (Scheel J., Von Brevern M.C., Horlein A., Fisher A., Schneider A., Bach A. 2002. Yellow pages to the transcriptome. Pharmacogenomics 3, 791-807), differential screening (DS), RNA arbitrarily primer (RAP)-PCR, restriction endonucleolytic analysis of differentially expressed sequences (READS), amplified restriction fragment-length polymorphisms (AFLP).

[0040] "Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridization are sequence dependent, and are different under different environmental parameters. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, 1984; T_m 81.5°C + 16.6 (log M) +0.41 (%GC)-0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point I for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point I; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point I; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point I. Using the equation, hybridization and wash compositions, and desired T, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T of less than 45°C (aqueous solution) or 32°C (formamide solution), it

is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, 1993. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point T_m for the specific sequence at a defined ionic strength and pH.

[0041] An example of highly stringent wash conditions is 0.15 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2X SSC wash at 65°C for 15 minutes (see Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1X SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6X SSC at 40°C for 15 minutes. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.5 M, more preferably about 0.01 to 1.0 M, Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C and at least about 60°C for long robes (e.g., >50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2X (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

[0042] Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or Northern blot is 50% formamide, e.g., hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0. 1 X SSC at 60 to 65°C. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C.

[0043] Washing with a solution containing tetramethylammonium chloride (TeMAC) could allow the detection of a single mismatch using oligonucleotide hybridyzation since such mismatch could generate a 10°C difference in the annealing temperature. The formulation to determine the washing temperature is Tm (°C) =] -682 (L^{-1}) + 97 where L represents the length of the oligonucleotide that will be used for the hybridization.

[0044] Disclosed is a kit for stratifying AIS and/or predicting whether a subject is at risk of developing AIS comprising an isolated nucleic acid, a protein or a ligand such as an antibody.

For example, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the subject sample (DNA genomic nucleic acid, cell sample or blood samples), a container which contains in some kits of the present invention, the probes used in the methods of the present invention, containers which contain enzymes, containers which contain wash reagents, and containers which contain the reagents used to detect the extension products. Kits may also contain instructions to use these probes and or antibodies to stratify AIS or predict whether a subject is at risk of developing AIS.

[0045] As used herein, the term "purified" in the expression "purified antibody" is simply meant to distinguish man-made antibody from an antibody that may naturally be produced by an animal against its own antigens. Hence, raw serum and hybridoma culture medium containing anti-Pitx1 antibody are "purified antibodies" within the meaning of the present invention.

[0046] As used herein, the term "ligand" broadly refers to natural, synthetic or semi-synthetic molecules. The term "molecule" therefore denotes for example chemicals, macromolecules, cell or tissue extracts (from plants or animals) and the like. Non limiting examples of molecules include nucleic acid molecules, peptides, antibodies, carbohydrates and pharmaceutical agents. The ligand appropriate for the present invention can be selected and screened by a variety of means including random screening, rational selection and by rational design using for example protein or ligand modeling methods such as computer modeling. The terms "rationally selected" or "rationally designed" are meant to define compounds which have been chosen based on the configuration of interacting domains of the present invention. As will be understood by the person of ordinary skill, macromolecules having non-naturally occurring modifications are also within the scope of the term "ligand". For example, peptidomimetics, well known in the pharmaceutical industry and generally referred to as peptide analogs can be generated by modeling as mentioned above.

Antibodies

[0047] Both monoclonal and polyclonal antibodies directed to Pitx1 are included within the scope of this invention as

they can be produced by well established procedures known to those of skill in the art. Additionally, any secondary antibodies, either monoclonal or polyclonal, directed to the first antibodies would also be included within the scope of this invention.

[0048] As used herein, the term "anti-Pitx1 antibody" or "immunologically specific anti- Pitx1 antibody" refers to an antibody that specifically binds to (interacts with) a Pitx1 protein and displays no substantial binding to other naturally occurring proteins other than the ones sharing the same antigenic determinants as the Pitx1 protein. The term antibody or immunoglobulin is used in the broadest sense, and covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies, and antibody fragments so long as they exhibit the desired biological activity. Antibody fragments comprise a portion of a full length antibody, generally an antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, single domain antibodies (e.g., from camelids), shark NAR single domain antibodies, and multispecific antibodies formed from antibody fragments. Antibody fragments can also refer to binding moieties comprising CDRs or antigen binding domains including, but not limited to, VH regions (V_H, V_H-V_H), anticalins, PepBodies™, antibody-T-cell epitope fusions (Troybodies) or Peptibodies. Additionally, any secondary antibodies, either monoclonal or polyclonal, directed to the first antibodies would also be included within the scope of this invention.

[0049] In general, techniques for preparing antibodies (including monoclonal antibodies and hybridomas) and for detecting antigens using antibodies are well known in the art (Campbell, 1984, In "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", Elsevier Science Publisher, Amsterdam, The Netherlands) and in Harlow et al., 1988 (in: Antibody A Laboratory Manual, CSH Laboratories). The term antibody encompasses herein polyclonal, monoclonal antibodies and antibody variants such as single-chain antibodies, humanized antibodies, chimeric antibodies and immunologically active fragments of antibodies (e.g. Fab and Fab' fragments) which inhibit or neutralize their respective interaction domains in Hyphen and/or are specific thereto.

[0050] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc), intravenous (iv) or intraperitoneal (ip) injections of the relevant antigen with or without an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

[0051] Animals may be immunized against the antigen, immunogenic conjugates, or derivatives by combining the antigen or conjugate (e.g., 100 μg for rabbits or 5 μg for mice) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with the antigen or conjugate (*e.g.*, with 1/5 to 1/10 of the original amount used to immunize) in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, for conjugate immunizations, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[0052] Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256: 495 (1975), or may be made by recombinant DNA methods (e.g., U.S. Patent No. 6,204,023). Monoclonal antibodies may also be made using the techniques described in U.S. Patent Nos. 6,025,155 and 6,077,677 as well as U.S. Patent Application Publication Nos. 2002/0160970 and 2003/0083293 (see also, e.g., Lindenbaum et al., 2004).

[0053] In the hybridoma method, a mouse or other appropriate host animal, such as a rat, hamster or monkey, is immunized (e.g., as hereinabove described) to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the antigen used for immunization. Alternatively, lymphocytes may be immunized *in vitro.* Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell.

[0054] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0055] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] In the appended drawings:

[0057] Figure 1 compares Pitx1 expression in osteoblasts from severely affected AIS patients and matched control subjects. Reverse transcription-polymerase chain reaction for *pitx1* gene expression in human osteoblasts of control subjects (n=46) and patients with AIS (n=29). Pitx1 specific mRNA transcripts were detected in the control tissue. Loss of the *pitx1* gene expression was observed in all examined AIS samples and β-actin expression was used as internal control;

[0058] Figure 2 shows the sequence of a 10 kb pitx1 promoter region (SEQ ID NO: 1) and polymorphisms in that pitx1 promoter region between human subjects. The primers used to cover the different amplicons covering the 10 kb regions are provided in Table 4 below;

[0059] Figure 3 shows the sequence of the Pitx1 mRNA (SEQ ID NO: 2) (NM_002653); and

[0060] Figure 4 shows the Pitx1 amino acid sequence (SEQ ID NO: 3) (NP_002644).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0061] The present invention demonstrated by RT-PCR analysis a loss of pitx1 gene expression in osteoblasts derived from biopsies obtained intraoperatively of severely affected AIS patients (n= 46) while osteoblasts derived from non-scoliotic patients (trauma cases) still expressed Pitx1 (n= 29).

[0062] The present invention is illustrated in further details by the following non-limiting examples.

Human Specimens

[0063] Informed consent was obtained from all study participants as approved by each individual and collective Institutional Review Board (Ste-Justine University Hospital, Montreal's Children Hospital and The Shriners Hospital for Children all located in Montreal). All individuals were screened through a series of steps including history and clinical data, assuring the idiopathic nature of the problem. This was followed by a review of spinal radiographs. A person was deemed to be affected by AIS if history and physical examination were consistent with the diagnosis of idiopathic scoliosis and a minimum of a ten degree curvature in the coronal plane with vertebral rotation was found on the radiograph. Participants will also be screened as to the potential familial distribution of the disorder on a case by case basis. In the event that the disorder is found to be familial, family members will undergo parallel screening for the presence or absence of the condition. Other patients (without scoliosis) visiting our trauma clinics were used as controls.

[0064] The clinical characteristics of the examined AIS and control subjects are shown in Table 2 and Table 3 respectively. Scoliotic patients with a diagnostic other than AIS were tested as control subjects.

[0065]

TABLE 2. CLINICAL CHARACTERISTIC **OF EXAMINED AIS SUBJECTS**

Number	GeN/Aer	Age	Curve type	Cobb angle	Heredity
1006	f	12,65	double major	61-46	No
1007	m	18,67	right thoracolumbar	61	Yes
1045	f	19,48	left thoracolumbar	38	No
1066	f	17,33	right thoracic	53	Yes
1137	f	20,54	double major	65-42	No
1167	m	14,58	left thoracic	49	No
1263	f	13,32	double major	53	No
1266	m	15,56	double major	52	No
1274	f	13,27	double major	42	No
1276	f	15,26	left thoracic	42	No
1277	f	12,73	double major	57-48	No
1280	f	14,4	double major	56-46	No
1294	f	16,92	left thoracic	N/A	Yes
1306	f	13,15	double major	77-48	Yes
1308	f	15,3	double major	77-20	No

(continued)

Number	GeN/Aer	Age	Curve type	Cobb angle	Heredity
1310	f	15,52	double major	55-42	No
1311	f	14,6	double major	78	Yes
1315	f	14,62	right thoracic	91	No
1317	f	13,97	right thoracic	53	Yes
1318	f	13,7	left thoracolumbar	49	No
1322	f	13,11	double major	51	No
1325	f	16,16	left thoracic	44	Yes
1329	m	14,02	right thoracolumbar	61	Yes
1335	f	17,6	double major	47-50	No
1337	f	14,13	double major	57-48	Yes
1339	f	14,28	right thoracic	31	No
1346	f	13,54	double major	50-34	Yes
1347	f	18,55	double major	56-45	No
1349	f	11,69	left thoracolumbar	74	No
1352	f	7,8	right thoracic	51	No
1360	f	9,92	double major	53-46	Yes
1385	f	16,06	double major	42-23	No
1390	f	15,61	left thoracolumbar	53	No
1391	f	15,01	left lumbar	54	No
1395	f	17,79	left thoracolumbar	84	Yes
1402	f	15,82	right thoracic	51	No
1406	f	14,89	double major	62-60	No
1409	f	13,62	right thoracic	40	No
1410	f	13,73	right thoracic	56	Yes
1417	f	13,24	right thoracic	59	Yes
1418	f	13,08	right thoracic	41	No
1420	f	13,42	double major	60-48	Yes
1422	f	12,44	double major	60-50	Yes
1425	f	13,42	right thoracic	68	Yes
1439	f	N/A	right thoracic	69	Yes
1442	f	N/A	right thoracic	60	No
N/A : not available					

[0066]

TABLE 3- CLINICAL CHARACTERISTICS OF EXAMINED CONTROL SUBJECTS

Number	Gender	Age	Health status	Curve type	Cobb angle	Heredity
C100	f	N/A	healthy	nd	nd	No
C101	N/A	N/A	healthy	nd	nd	No

(continued)

Number	Gender	Age	Health status	Curve type	Cobb angle	Heredity
C102	f	18	healthy	nd	nd	No
C103	N/A	N/A	healthy	nd	nd	No
C104	f	14	healthy	nd	nd	No
C105	f	11	healthy	nd	nd	No
C106	m	12	healthy	nd	nd	No
C107	f	13	healthy	nd	nd	No
C108	f	12	healthy	nd	nd	No
C109	m	14	healthy	nd	nd	No
C110	m	15	healthy	nd	nd	No
C111	m	14	healthy	nd	nd	No
C112	f	11	healthy	nd	nd	No
C113	m	14	healthy	nd	nd	No
C114	N/A	N/A	healthy	nd	nd	No
C115	m	17	healthy	nd	nd	No
C116	m	12	healthy	and	nd	No
C117	m	16	healthy	nd	nd	No
C118	m	12	healthy	nd	nd	No
C119	f	15	healthy	nd	nd	No
C120	f	15	healthy	nd	nd	No
C121	f	8	healthy	nd	nd	No
1285	f	15,87	paralytic scoliosis	N/A	72	Yes
1293	m	11,79	congenital scoliosis	left lumbar	38	No
1341	f	11,14	conqenital scoliosis	double major	61-65	No
1375	f	13,71	congenital scoliosis	right thoracolumbar	53	Yes
1431	m	19,13	neurological scoliosis	double major	90-90	No
1434	f	12,43	congenital scoliosis	double major	79-77	No
1436	f	13,93	kyphoscoliosis	kyphosis	120	No
N/A: not available and nd: not detected						

Osteoblasts cultures

[0067] Osteoblasts were obtained from bone specimens taken intraoperatively during spine surgeries of AIS patients and trauma surgeries in the case of control subjects. This cell type was chosen as cellular model for this study as described previously (Rodriguez MM, Ron D, Touhara K, Chen CH, Mochly-Rosen D. RACK1, a protein kinase C anchoring protein, coordinates the binding of activated protein kinase C and select pleckstrin homology domains in vitro. Biochemistry. 1999;38:13787-13794).

Isolation of human osteoblasts

[0068] In all cases, osteoblasts were obtained intraoperatively from bone specimens originating from vertebras (varying from T3 to L4 according to the surgical procedure performed). Bony fragments were mechanically reduced to smaller pieces with a bone cutter in sterile conditions and incubated at 37°C in 5% CO_2 in a 100 mm culture dish in presence

of DMEM medium containing 10% fetal bovine serum (FBS) (certified FBS, Invitrogen, Burlington, ON, Canada) and 1% penicillin/streptomycin (Invitrogen). After a 30-day period, the osteoblasts derived from the bone pieces were separated at confluence from the remaining bone fragments by trypsinization.

Total RNA isolation and RT-PCR

[0069] Extraction of RNA from osteoblasts was done using the standard Trisol Reagent method. (Invitrogen).

[0070] The RNA obtained from the osteoblasts was used for cDNA synthesis performed with the Invitrogen Thermoscript™ RT-PCR system and the respective protocol in the following conditions: Enzyme used: *Taq* DNA polymerase from Invitrogen™. PCR conditions: 95°C 5minutes, Hot start (1 cycle). Following three reactions (32 cycles) : 94°C, 45 Seconds Denaturation; 55°C 45 Seconds; Primer annealing; 72°C 1 minute Elongation; 72°C 2 minutes Last elongation (1 cycle); 4°C 20 minutes pause; Duration: 2 hours 42 minutes. The quality of the cDNA was tested by amplifying 233bp fragment of human beta-actin using the sense primer 5'-GGAAATCGTGCGTGACAT-3' (SEQ ID NO: 4) and antisense primer 5'-TCATGATGGAGTTGAATGTAGTT-3' (SEQ ID NO: 5). For quantitative analysis, all amplifications were normalized against that of the housekeeping gene β-actin. PCR amplified product were separated on 1.5% agarose gel and visualized by ethidium bromide staining.

Expression Analysis of Pitx1:

[0071] Coding region of Pitx1 501bp in length was amplified from the cDNA using the sense primer 5'- GACCCAGCCAAGAAGAAGAA-3' (SEQ 10 NO: 6) and the antisense primer 5'- GAGGTTGTTGATGTTGTTGAGG-3' (SEQ ID NO: 7) under the following PCR conditions: Enzyme used: Taq DNA polymerase from Invitrogen™. PCR conditions: 95°C 10 minutes hot start (1 cycle); Following three reactions (34 cycles): 94°C 45 Seconds Denaturation; 69°C 45 Seconds Primer annealing; 72°C 1 minute Elongation; 72°C 2 minutes Last elongation (1 cycle); 4°C 20 minutes; 4°C Pause; Duration: 2hours 34 minutes 11 seconds.

Pitx1 promoter sequencing

[0072] 10 kb region of the pitx1 promoter was amplified and sequenced to screen for mutations. Enzyme used: Platinum® Taq DNA polymerase High fidelity from Invitrogen™ under the following conditions:95°C 5 minutes hot start (1 cycle). Following three reactions 35 cycles: 94°C 45 Seconds Denaturation; 61.8°C 45 Seconds Primer annealing; 72°C 1 minute Elongation; 72°C 5 minutes Last elongation (1 cycle); 4°C 20 minutes; and 4°C Pause.

[0073] One hundred (100) ng of genomic DNA was mixed in a final volume of 25 μl containing 200 micromolar dNTPs, 1,5 mM $MgCl_2$, 10 pM of each primer (see Table 4 below for list of primers used), and 1 U Pfx DNA-polymerase (Invitrogen) or an other DNA polymerase.

[0074] PCR conditions: Regions PP1, PP2, PP3, PP6, PP7 were amplified using Platinum® pfx DNA polymerase from Invitrogen™ under the following PCR conditions: 95°C 5 minutes hot start (1 cycle); Following three reactions (35 cycles): 94°C 30 Seconds Denaturation; 60°C 30 Seconds; Primer annealing; 68°C 1 min 20 Sec Elongation; 68°C 2 minutes; Last elongation (1 cycle); 4°C 20 minutes; 4°C Pause.Duration : 2 hours 35 minutes 26 seconds

[0075] Regions PP4, PP5, PP8, PP9 and PP10 were amplified using Platinum®Taq DNA polymerase High fidelity from Invitrogen™ under the following conditions. 95°C 2 minutes hot start (1 cycle); Following three reactions (35 cycles); 94°C 45 Seconds Denaturation; 60°C 45 Seconds Primer annealing; 72°C 1 min 20 Sec Elongation; 72°C 5 minutes Last elongation (1 cycle); 4°C 20 minutes; 4°C Pause; Duration : 2 hours 53 minutes 4 seconds.

[0076]

TABLE 4: PITX PROMOTER PRIMERS

PP1 (962 bp)	forward primer 5'-CTGTTTGCTCAAGACGCTGA-3'; (SEQ ID NO: 8) reverse primer 5'-CTCGGCCTCACAAAAGAAAC-3' (SEQ ID NO: 9)
PP2 (966 bp)	forward primer 5'-TGTCTGCATTCAGGCTGTTC-3'; (SEQ ID NO: 10) reverse primer 5'-GATTCCCTCCTCGAGTCCTT-3' (SEQ ID NO: 11)
PP3 (1039 bp)	forward primer 5'-CAAGTGAGCTGGATGCTGAA-3'; (SEQ ID NO: 12) reverse primer 5'-AGGGAGTGTCCCTTCACAGA-3'(SEQ ID NO: 13)
PP4 (1085 bp)	forward primer 5'-GCTCAGCCATTCTCAGGAAC-3'; (SEQ ID NO: 14) reverse primer 5'-GCCATTGTCCCAGTCAAGAT-3'(SEQ ID NO: 15)
PP5 (1011 bp)	forward primer 5'-TCGCGTCAAGAGGGTATTTT-3'; (SEQ ID NO: 16)

(continued)

	reverse primer 5'-TAGGACCCATGGCTCTACCC-3'(SEQ ID NO: 17)
PP6 (1098 bp)	forward primer 5'-CACGAGTCAGGTGGGAAACT-3'; (SEQ ID NO: 18) reverse primer 5'-GACGTCTGCTGCTTTTCTGC-3'(SEQ ID NO: 19)
PP7 (963 bp)	forward primer 5'-AGGCACGGACTAGCAGGAC-3'; (SEQ ID NO: 20) reverse primer 5'-ATGCGGACGAAGCCAGAG-3'(SEQ ID NO: 21)
PP8 (986 bp)	forward primer 5'-TTAGCATTCAGCCCCTCTGT-3'; (SEQ ID NO: 22) reverse primer 5'-TTCATGAGATGCAGTCAGCAG-3'(SEQ ID NO: 23)
PP9 (951 bp)	forward primer 5'-ACAACTGGTAGGGGCAACAG-3'; (SEQ ID NO: 24 reverse primer 5'-TGTGTGGCTTTGGCAAATAA-3'(SEQ ID NO: 25)
PP10 (990 bp)	forward primer 5'-GCACTGTGCTCCAACTGTGT-3'; (SEQ ID NO: 26) reverse primer 5'-GGGGGAGTGTTCTTTTCCTT-3'(SEQ ID NO: 27)

EXAMPLE 1

Comparison of Pitx1 expression in osteoblasts of AIS subjects with that in osteoblast of matched controls

[0077] To determine whether pitx1 plays a role in the genetic control of AIS development and/or progression, an expression analysis of pitx1 gene using RNA prepared from osteoblasts cultures derived from biopsies obtained intra-operatively of severely affected AIS patients (n=46) and from non-scoliotic patients (trauma cases) control subjects (n=29) was performed.

[0078] As may be seen in Figure 1, all osteoblasts derived from the AIS patients showed a loss of pitx1 mRNA expression, while control subjects still expressed the mRNA.

EXAMPLE 2

Determination of Pitx1 expression in a subject sample

[0079] Tissue such as muscle (using for instance a needle in the paraspinal region), bone, cartilage, peripheral blood mononuclear cells (PBMCs such as T and B lymphocytes as well as macrophages) or any cells derived from tissues where Pitx1 is expressed is isolated from the patient. Extraction of RNA from these tissues is done using any standard RNA extraction method such as the standard Trisol Reagent method. Coding region of Pitx1 501 bp in length is amplified from the cDNA using for instance RT-PCR or real-time PCR. The sense primer 5'- GACCCAGCCAAGAAGAAGAA-3' (SEQ ID NO: 6) and the antisense primer 5'- GAGGTTGTTGATGTTGTTGAGG-3' (SEQ ID NO: 7) under the following PCR conditions: Enzyme used: Taq DNA polymerase from Invitrogen™. PCR conditions: 95°C 5minutes Hot start (1 cycle). Following three reactions (32 cycles): 94°C 45 Seconds Denaturation; 55°C 45 Seconds Primer annealing; 72°C 1 minute Elongation; 72°C 2 minutes Last elongation (1 cycle); and 4°C 20 minutes pause. Duration: 2 hours 42 minutes.

SEQUENCE LISTING

[0080]

<110> CHU Sainte-Justine
Moreau, Alain

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Claims

1. A method of stratifying a subject having adolescent idiopathic scoliosis (AIS) comprising: providing a cell sample isolated from the subject; detecting Paired-like homeodomain transcription factor 1 (Pitx1) expression in the cell sample; whereby the results of the detecting step enables the stratification of the subject having AIS as belonging to an AIS subclass.

2. A method of stratifying a subject having adolescent idiopathic scoliosis (AIS) for a clinical trial comprising: providing a cell sample isolated from the subject; detecting Paired-like homeodomain transcription factor 1 (Pitx1) expression in the cell sample; and stratifying the subject for a clinical trial based on the results of the detecting step.

3. A method for predicting a risk for developing adolescent idiopathic scoliosis (AIS) in a subject comprising
providing a cell sample isolated from the subject; and
detecting Paired-like homeodomain transcription factor 1 (Pitx1) expression in the cell sample;
wherein an absence of Pitx1 expression is indicative that the subject is at risk for developing AIS.

4. The method of claim 3, wherein an absence of Pitx1 expression is indicative that the subject is at risk for developing a Cobb's angle of 45° and above.

5. The method of any one of claims 1 to 4 wherein the detecting step is performed with (a) an isolated nucleic acid molecules specific to a Pitx1 transcription product; or (b) an antibody that binds specifically to Pitx1.

6. The method of claim 5, wherein the isolated nucleic acid molecule is detectably labeled.

7. The method of any one of claims 1 to 6, wherein the cell sample is selected from the group consisting of an osteoblasts sample, a chondrocytes sample, a skeletal myoblasts sample and a blood sample.

8. The method of any one of claims 1 to 6, wherein the cell sample is an osteoblasts sample.

9. The method of any one of claims 1 to 8, further comprising a step of selecting a preventive action or a treatment in light of the results of the detecting step.

10. The method of claim 3 or 4, wherein said subject is pre-diagnosed as being a likely candidate for developing adolescent idiopathic scoliosis.

11. A method of selecting a compound potentially useful in the treatment of adolescent idiopathic scoliosis, said method comprising the steps of

(a) contacting a test compound with at least one cell known to express Paired-like homeodomain transcription factor 1 (Pitx1); and
(b) determining Pitx1 expression level;

wherein the test compound is selected if the Pitx1 expression level is increased in the presence of the test compound as compared to that in the absence thereof.

12. The method of claim 11, wherein said cell is an osteoblast.

13. The method of claim 11 or 12, wherein said cell is from a subject having adolescent idiopathic scoliosis (AIS).

14. The method of any one of claims 1 10 wherein the subject is a human.

Patentansprüche

1. Verfahren zur Stratifizierung eines Individuums mit adoleszenter idiopathischer Skoliose (AIS), das Folgendes umfasst: Bereitstellen einer Zellprobe, die von dem Individuum isoliert wurde; Nachweisen der Expression des Transkriptionsfaktors Pitx1 (paired-like homeodomain 1) in der Zellprobe; wobei die Ergebnisse des Nachweisschrittes die Stratifizierung des Individuums mit AIS bezüglich der Zugehörigkeit zu einer Subklasse der AIS ermöglichen.

2. Verfahren zur Stratifizierung eines Individuums mit adoleszenter idiopathischer Skoliose (AIS) für eine klinische Studie, das Folgendes umfasst: Bereitstellen einer Zellprobe, die von dem Individuum isoliert wurde; Nachweisen der Expression des Transkriptionsfaktors Pitx1 (paired-like homeodomain 1) in der Zellprobe und Stratifizierung des Individuums für eine klinische Studie auf der Grundlage der Ergebnisse von dem Nachweisschritt.

3. Verfahren zur Vorhersage eines Risikos für die Entwicklung von adoleszenter idiopathischer Skoliose (AIS) bei

einem Individuum, das Folgendes umfasst:

Bereitstellen einer Zellprobe, die von dem Individuum isoliert wurde; und
Nachweisen der Expression des Transkriptionsfaktors Pitx1 (paired-like homeodomain 1) in der Zellprobe;
wobei eine Abwesenheit der Pitx1-Expression darauf hinweist, dass ein Risiko für die Entwicklung von AIS für das Individuum vorhanden ist.

4. Verfahren nach Anspruch 3, wobei eine Abwesenheit der Pitx1-Expression darauf hinweist, dass ein Risiko für die Entwicklung eines Cobb-Winkels von 45° und größer für das Individuum vorhanden ist.

5. Verfahren nach einem der Ansprüche 1 bis 4, wobei der Nachweisschritt (a) mit isolierten Nukleinsäuremolekülen, die für ein Pitx1-Transkriptionsprodukt spezifisch sind, oder (b) mit einem Antikörper, der spezifisch an Pitx1 bindet, durchgeführt wird.

6. Verfahren nach Anspruch 5, wobei das isolierte Nukleinsäuremolekül nachweisbar markiert ist.

7. Verfahren nach einem der Ansprüche 1 bis 6, wobei die Zellprobe ausgewählt ist aus der Gruppe, die aus einer Osteoblasten-Probe, einer Chondrozyten-Probe, einer Myoblasten-Probe aus Skelettmuskulatur und einer Blutprobe besteht.

8. Verfahren nach einem der Ansprüche 1 bis 6, wobei die Zellprobe eine Osteoblasten-Probe ist.

9. Verfahren nach einem der Ansprüche 1 bis 8, das ferner einen Schritt des Auswählens einer präventiven Maßnahme oder einer Behandlung in Anbetracht der Ergebnisse des Nachweisschrittes umfasst.

10. Verfahren nach Anspruch 3 oder 4, wobei das Individuum bereits als ein möglicher Kandidat für die Entwicklung einer adoleszenten idiopathischen Skoliose diagnostiziert wurde.

11. Verfahren zum Auswählen einer Verbindung, die möglicherweise für die Behandlung der adoleszenten idiopathischen Skoliose zweckmäßig ist, wobei das Verfahren die Schritte von

(a) dem Inkontaktbringen einer Testverbindung mit mindestens einer Zelle, von der bekannt ist, dass sie den Transkriptionsfaktor Pitx1 (paired-like homeodomain 1) exprimiert; und
(b) dem Bestimmen der Expressionsstärke von Pitx1 umfasst;

wobei die Testverbindung ausgewählt wird, wenn die Pitx1-Expressionsstärke in Anwesenheit der Testverbindung im Vergleich zu derjenigen in Abwesenheit der Testverbindung erhöht ist.

12. Verfahren nach Anspruch 11, wobei die Zelle ein Osteoblast ist.

13. Verfahren nach Anspruch 11 oder 12, wobei die Zelle von einem Individuum mit adoleszenter idiopathischer Skoliose (AIS) ist.

14. Verfahren nach einem der Ansprüche 1 bis 10, wobei das Individuum ein Mensch ist.

Revendications

1. Méthode de stratification d'un sujet ayant une scoliose idiopathique de l'adolescent (SIA) comprenant : l'obtention d'un échantillon cellulaire isolé chez le sujet ; la détection de l'expression du Facteur de transcription à homéodomaine de type Paired 1 (Pitx1) dans l'échantillon cellulaire ; dans laquelle les résultats de l'étape de détection permettent la stratification du sujet ayant une SIA comme faisant partie d'une sous-classe des SIA.

2. Méthode de stratification d'un sujet ayant une scoliose idiopathique de l'adolescent (SIA) pour un essai clinique comprenant : l'obtention d'un échantillon cellulaire isolé chez le sujet ; la détection de l'expression du Facteur de transcription à homéodomaine de type Paired 1 (Pitx1) dans l'échantillon cellulaire ; et la stratification du sujet pour un essai clinique basé sur les résultats de l'étape de détection.

3. Méthode de prédiction des risques pour un sujet de développer une scoliose idiopathique de l'adolescent (SIA) comprenant
l'obtention d'un échantillon cellulaire isolé chez le sujet ; et
la détection de l'expression du Facteur de transcription à homéodomaine de type Paired 1 (Pitx1) dans l'échantillon cellulaire ;
dans laquelle l'absence d'expression de Pitx1 est une indication que le sujet est à risque pour le développement d'une SIA.

4. Méthode selon la revendication 3, dans laquelle une absence d'expression de Pitx1 est une indication que le sujet est à risque pour le développement d'un angle de Cobb de 45° et plus.

5. Méthode selon l'une des revendications 1 à 4, dans laquelle l'étape de détection est réalisée avec (a) une molécule d'acide nucléique isolée spécifique d'un produit de transcription de Pitx1 ; ou (b) un anticorps qui se lie spécifiquement à Pitx1.

6. Méthode selon la revendication 5, dans laquelle la molécule d'acide nucléique isolée est marquée de manière détectable.

7. Méthode selon l'une des revendications 1 à 6, dans laquelle l'échantillon cellulaire est choisi dans le groupe constitué d'un échantillon d'ostéoblastes, d'un échantillon de chondrocytes, d'un échantillon de myoblastes squelettique et d'un échantillon sanguin.

8. Méthode selon l'une des revendications 1 à 6, dans laquelle l'échantillon cellulaire est un échantillon d'ostéoblastes.

9. Méthode selon l'une des revendications 1 à 8, comprenant en outre une étape de sélection d'une action préventive ou un traitement à la lumière des résultats de l'étape de détection.

10. Méthode selon la revendication 3 ou 4, dans laquelle ledit sujet est prédiagnostiqué comme étant un candidat probable pour développer une scoliose idiopathique de l'adolescent.

11. Méthode de sélection d'un composant potentiellement utile dans le traitement d'une scoliose idiopathique de l'adolescent, ladite méthode comprenant les étapes de

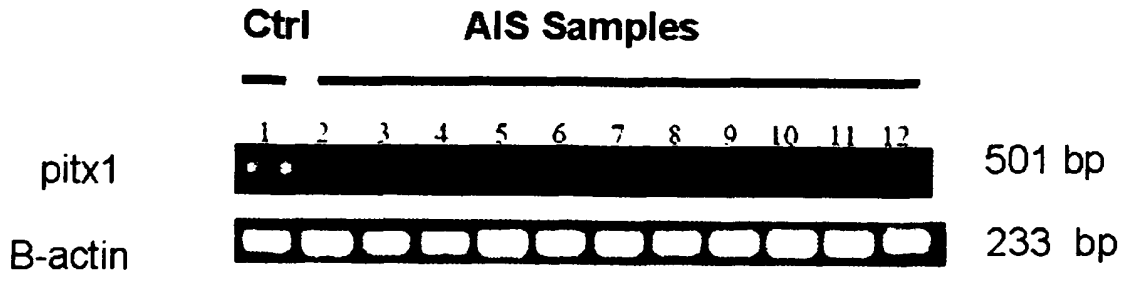
(a) la mise en contact d'un composé test avec au moins une cellule connue pour exprimer le Facteur de transcription à homéodomaine de type Paired 1 (Pitx1) ; et
(b) la détermination du niveau d'expression de Pitx1 ;

dans laquelle le composé test est choisi si le niveau d'expression de Pitx1 est augmenté en présence du composé test par rapport au niveau en son absence.

12. Méthode selon la revendication 11, dans laquelle ladite cellule est un ostéoblaste.

13. Méthode selon la revendication 11 ou 12, dans laquelle ladite cellule provient d'un sujet ayant une scoliose idiopathique de l'adolescent (SIA).

14. Méthode selon l'une des revendications 1 à 10, dans laquelle le sujet est un humain.



AIS	Controls
N= 46	N= 29

Figure 1

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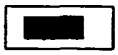

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PP3

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GG
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-497 **(-460)G RS7711062**
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-284
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GG
-213

PP1

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-142
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-71
-1

Figure 2

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121 cgcgagccgg agccggagcc ggagccgggg ccggccgggc tgctgaggcc cgagcggcag
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ggatccgtgt
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2341 tctgtctata tcgactaaaa aaaaaaaaaa aaa
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Figure 3

MDAFKGGMSLERLPEGLRPPPPPPHDMGPAFHLARPADPREPLENSASESSDTELPEKERGGEPKGPED
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NRRAKWRKRERNQQLDLCKGGYVPQFSGLVQPYEDVYAAGYSYNNWAAKSLAPAPLSTKSFTFFNSMSP
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TCNSSLASLRLKSKQHSSFGYGALQGPASGLNACQYNS

Figure 4

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	分层青少年特发性脊柱侧凸的方法，用于相同的分离的核酸分子		
公开(公告)号	EP2129799B1	公开(公告)日	2012-06-20
申请号	EP2008733628	申请日	2008-03-19
申请(专利权)人(译)	褚SAINTE-JUSTINE		
当前申请(专利权)人(译)	褚SAINTE-JUSTINE		
[标]发明人	MOREAU ALAIN		
发明人	MOREAU, ALAIN		
IPC分类号	C12Q1/68 C12Q1/02 G01N33/53 G01N33/68 C07K16/18 C07K14/47 C12N15/12		
CPC分类号	G01N33/6872 G01N2800/10		
优先权	60/908417 2007-03-28 US 60/895490 2007-03-19 US		
其他公开文献	EP2129799A4 EP2129799A1		
外部链接	Espacenet		

摘要(译)

一种对患有青少年特发性脊柱侧凸（AIS）的受试者进行分层的方法，包括：提供从受试者分离的细胞样品;检测细胞样本中的配对样同源域转录因子1（Pitxi）表达;由此，检测步骤的结果使得能够将具有AIS的受试者分层为属于AIS亚类。


```
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