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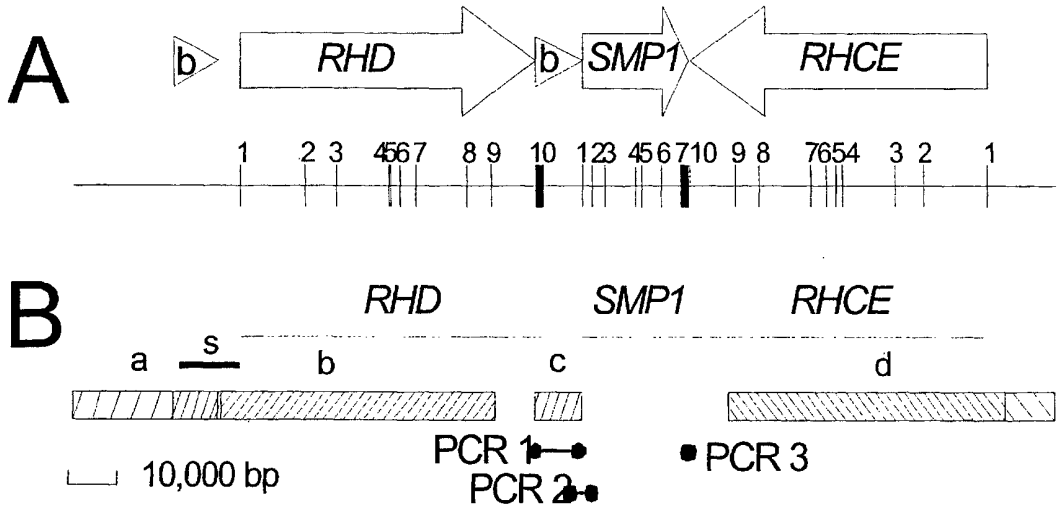
(54) Title: MOLECULAR STRUCTURE OF RHD NEGATIVE LOCUS



(57) **Abstract:** The present invention relates to a nucleic acid molecular structure representing the Rhesus genes locus comprising the RHD, SMP1 and RHCE genes and/or the Rhesus box(es), preferably the hybrid Rhesus box, the upstream Rhesus box and/or the downstream Rhesus box. Furthermore, the invention relates to a process for the specific detection of the common RHD negative haplotypes. The invention further relates to the detection of RHD positive haplotypes in D-negative individuals. Various mutations in the RHD gene have been identified that allow for the development of diagnostic tools. The invention also relates to oligonucleotides, that specifically hybridize to the hybrid box, preferably the breakpoint or breakpoint region or to the upstream and downstream Rhesus boxes. Additionally, the invention relates to kits comprising or employing the above recited compounds of the invention.

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Previous Correction:
see PCT Gazette No. 41/2001 of 11 October 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/EP 00/10745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47 C12N15/12 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, SEQUENCE SEARCH, WPI Data, PAJ, SCISEARCH, BIOTECHNOLOGY ABS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARRITT, B. ET AL.: "Evolution of the Rh (rhesus) blood group genes: a 50 year old predication (partially) fulfilled" HUMAN MOLECULAR GENETICS., vol. 6, no. 6, 1997, pages 843-850, XP000998694 OXFORD UNIVERSITY PRESS, SURREY., GB ISSN: 0964-6906 cited in the application page 846 page 847; figure 4 --- -/--	1,35,48

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2001	22. 10. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CARRITT, B. ET AL.: "Rh null phenotypes are not due to a gross deletion and can occur on different Rh genetic backgrounds" ANNALS OF HUMAN GENETICS, vol. 57, no. 4, 1993, pages 273-279, XP000926510 abstract page 274, paragraph 3 ---	1
X	OKUDA H ET AL: "SEQUENCE ANALYSIS OF THE SPACER REGION BETWEEN THE RHD AND RHCE GENES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 263, 24 September 1999 (1999-09-24), pages 378-383, XP000998148 ACADEMIC PRESS INC. ORLANDO, FL., US ISSN: 0006-291X cited in the application abstract page 378, column 2, last paragraph -page 380, column 1, last paragraph page 382, column 1 ---	1
X A	WO 99 37763 A (FLEGEL WILLY A ;WAGNER FRANZ F (DE); DRK BLUTSPENDEDIENST BADEN WU) 29 July 1999 (1999-07-29) page 1, paragraph 2 -page 27, paragraph 2 ---	6-9, 14-38, 40-45, 48,83 65-75, 77, 79-82,98
A	HU, G.: "Human small membrane protein 1 (SMP1) mRNA" EMBL SEQUENCE DATABASE, XP002170737 HEIDELBERG DE cited in the application Accession Nr.: AF081282 abstract ---	1,35,48, 96,97
A	US 5 972 602 A (SAUL ALLAN ET AL) 26 October 1999 (1999-10-26) the whole document ---	1,35-38, 40-45, 48, 67-75, 77,79-83
A	WO 99 47555 A (GENETICS INST) 23 September 1999 (1999-09-23) page 102 -page 103; claim 26 ---	1,35,96, 97
A	WO 98 45712 A (HUMAN GENOME SCIENCES INC) 15 October 1998 (1998-10-15) page 109 -page 111; claim 4 ---	1,35,96, 97
	-/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LE VAN KIM CAROLINE ET AL: "Molecular cloning and primary structure of the human blood group RhD polypeptide." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 22, 1992, pages 10925-10929, XP002134526 1992 ISSN: 0027-8424 the whole document	1,14-38, 48
A	CHERIF-ZAHAR B ET AL: "Organization of the gene (RHCE) encoding the human blood group RhCcEe antigens and characterization of the promoter region." GENOMICS, vol. 19, no. 1, 1994, pages 68-74, XP000926511 ISSN: 0888-7543 the whole document	1,35-38, 48
P,X	WAGNER, F.F. ET AL.: "RHD gene deletion occured in the Rhesus box" BLOOD, vol. 29, no. 12, 15 June 2000 (2000-06-15), pages 3662-3668, XP000926502 WASHINGTON US the whole document	1-5, 14-38, 40-45, 48, 67-75, 77, 79-83, 96,98
P,A	SINGLETON BELINDA K ET AL: "The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype." BLOOD, vol. 95, no. 1, 1 January 2000 (2000-01-01), pages 12-18, XP001011309 ISSN: 0006-4971 abstract	50-61
X	BLUNT, T. ET AL.: "Serotype switching in a partially deleted RHD gene" VOX SANGUINIS, vol. 67, no. 4, 1994, pages 397-401, XP000926512 abstract	6-13,39, 48,62, 99,100

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	AVENT ND ET AL: "Evidence of genetic diversity underlying Rh D-, weak D (Du), and partial D phenotypes as determined by multiplex polymerase chain reaction analysis of the RHD gene" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 89, no. 7, 1 April 1997 (1997-04-01), pages 2568-2577, XP002108680 ISSN: 0006-4971 the whole document ---	6-9, 14-36
X	DATABASE EM_HUM 'Online! EMBL; 13 August 1998 (1998-08-13) COBLEY. V. ET AL.: "Human DNA sequence clone RP3-469D22 on chromosome 1p35.1-36.13, contains the 5'part of the gene for RHCE." retrieved from EBI, accession no. HS469D22 Database accession no. AL031284 XP002178290 cited in the application abstract -----	6-13,39, 48,62

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/10745

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-95, 98-100 partially and 96, 97 completely.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 2-5, 36-38, 96, 97 completely and 1, 35, 40-45, 48, 50-61, 67-75, 77, 79-83, 98 partially

A nucleic acid molecular structure, representative of the common RHD negative haplotypes, comprising SMP1 and RHCE and the hybrid Rhesus box; vectors and hosts comprising said sequence; the use of said nucleic acid structure for the analysis of the Rhesus D phenotype and a process to specifically detect a RHD negative haplotype by utilizing the SMP1 gene and the Rhesus boxes, preferably the hybrid Rhesus box

2. Claims: 6-34, 39, 46, 47, 49, 62-66, 76, 78, 84-95, 99, 100 completely and 1, 35, 40-45, 48, 50-61, 67-75, 77, 79-83, 98 partially

A nucleic acid molecular structure, representative of the common RHD positive haplotypes, comprising RHD, SMP1 and RHCE and the upstream and downstream Rhesus boxes; vectors and hosts comprising said structure; the use of said nucleic acid structure for the analysis of the Rhesus D phenotype and a process to specifically detect a RHD negative haplotype; a method of producing a protein product of the RHD gene; an antibody or aptamer binding to said protein; a method for determining said protein; a method for characterizing monoclonal or polyclonal antisera; a method for identifying an antibody Vh or Vl chain and a process to determine the presence of antigen C.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-95, 98-100 partially and 96, 97 completely.

Present claim 1-5 and all the claims referring thereto relate to an extremely large number of possible nucleic acid structures. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion (i.e. only one) of the structures claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the upstream, downstream and hybrid Rhesus boxes, te sequence of which is represented by SEQ.ID's 19, 20 and 18 respectively as well as to the use of those sequences or probes derived therefrom for determining the Rhesus D haplotype and other uses of those sequences identified in the claims relating to invention I.

Present claims 6-34 and claims referring thereto relate to an extremely large number of possible nucleic acid structures. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion (i.e. only one) of the structures claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the subject-matter of claims 10-13.

Present claims 96 and 97 relate to an undefined number of possible SMP1 polymorhisms to determine RH haplotypes. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for NONE of the polymorphisms. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, NO the search has been carried out for those claims.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/10745

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9937763 A	29-07-1999	AU 2274699 A	09-08-1999
		BR 9815090 A	17-10-2000
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