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Genetic and functional characteristics of the human in vivo LRP1/A2MR receptor suggested as a risk marker for Alzheimer's disease and other complex (degenerative) diseases

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Abstract

LDL receptor-related protein/alpha2-macroglobulin receptor (LRP1/A2MR) a multiligand receptor is considered as not only being a possible risk factor of neurodegenerative diseases like Alzheimer's disease but also as determining the progression of other complex diseases like atherosclerosis and cancer. Although a large number of in vitro studies have highlighted its functional importance, as yet not enough is known about the clinical importance of the genetic background of LRP1 in human diseases.

The aim of this ex vivo/in vivo study of 448 subjects was to present data on genetic LRP1 variants of healthy European Caucasians from Central Germany. Genotype-dependent LRP1 expression was analyzed in a representative subgroup (gene expression: $n = 127$, protein expression: $n = 44$). These data were evaluated in comparison to other published clinical LRP1 studies. For 15 functionally interesting genetic variants the genotype and allele distributions of the German Caucasians were presented in relation to their in vivo LRP1 gene and protein expression. A direct influence of the LRP1 promoter polymorphism c.1-25C>G on the human in vivo LRP1 expression level was demonstrated. In an analysis of 48 further studies genomic and functional results were evaluated. The analysis especially on Alzheimers's disease partly highlighted contradictory results, but suggested that ethnic as well as genomic characteristics determine LRP1 expression and must be considered in clinical investigations on human LRP1.

Keywords: Low density lipoprotein receptor-related protein 1/alpha2-macroglobulin receptor; Mutation; Expression; Alzheimer's disease; Atherosclerosis; Cancer

1. Introduction

1.1. Physiological background

The LDL receptor-related protein 1 (LRP1)/alpha-2 macroglobulin receptor (A2MR), CD 91, a member of the low density lipoprotein receptor superfamily (Herz and Strickland, 2001; Schneider and Nimpf, 2003; Strickland et al., 2002; Willnow, 1999) functions as a multifunctional receptor of more than 30 unrelated ligands (Hussain, 2001; Hussain et al., 1999; Birkenmeier et al., 1999) which are involved in complex diseases like, for example, neurodegenerative diseases (e.g. Alzheimer's disease (AD), Parkinson disease), atherosclerosis (e.g. coronary artery disease (CAD)) or cancer (Boucher et al., 2003; May and Herz, 2003; Myllykangas et al., 2000; Schellenberger et al., 2000; Strickland et al., 2002; Thal et al., 1997; Van Uden et al., 2000). This fact and its evolutionary conservation, proved by LRP1-like homologues in the nematode *C. elegans* and many other species, underline its importance as a fundamental cell receptor of animal life (Grimsley et al., 1999; Yochem and Greenwald, 1993). The internalization

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via LRP1 involves extracellular macromolecules as well as macromolecular complexes. Beside important ligands like lipoproteins, proteinases, proteinase-inhibitor complexes and extracellular matrix proteins (Kasza et al., 1997) the interplay with the heat shock protein gp96 (Binder et al., 2000) suggests that LRP1 is involved in the necrotic cell death leading to proinflammatory immune response. Being involved in the regulation of the extracellular proteolytic activity LRP1 also seems to play a key role in the cell growth and migration as well as in the remodelling and repair of tissue. At least, it functions as a signalling receptor in the Src and Ras activation and the inositol and Wnt signalling (Herz and Strickland, 2001; Van der Geer, 2002; Strickland and Ranganathan, 2003). The crucial role of LRP1 in both lipid metabolism and embryonic development has been confirmed in experiments with knockout mice (Herz et al., 1992; Willnow and Herz, 1995).

These multiligand properties and its wide tissue distribution (Handschug et al., 1998; Herz et al., 1988; Moestrup et al., 1992; Schulz et al., 1995) indicate that LRP1 is a fundamental cargo receptor ensuring the supply of vital components to cells and tissue. On the other hand, the pathological accumulation of LRP1 ligands, described in the case of several diseases (Gliemann, 1998), also suggest that it is an essential disposal vesicle. It may be speculated that both structural failure of LRP1 itself as well as a dysfunction of the multitude of ligands and/or other transport components causally determine such a degenerative accumulation of LRP1 ligands.

Even though in vitro studies could prove these facts (Beisiegel et al., 1996; Garner et al., 1997; Van Uden et al., 1999) little is known about the human in vivo regulation of LRP1. The dynamic regulation of the in vivo LRP1 gene and protein expression is not sufficiently understood up until now. In vitro, an increase in both LRP1 protein expression and LRP1 gene expression level could be demonstrated by stimulating cells, for example, with leptin, but first in vivo results show an inverse relation in a disease-related study on coronary atherosclerosis (Schulz et al., 2003). This data suggests a very complex regulation in vivo of this endocytosis receptor, influenced by a variety of different factors, including not only LRP1/A2MR ligands but also factors like age, gender, circadian rhythm, e.g. or other hitherto unnoticed factors (Field and Gibbons, 2000; Schulz et al., 2003). First results describe an association of human LRP1 expression with LRP1 genotypes (Schulz et al., 2002, 2003; Kang et al., 2000).

1.2. Genetic background

The human LRP1 (see also gene card, http://bioinfo.weizmann.ac.il/cards-bin/cardsearch.pl GC12PO57746) located on chromosome 12q13-14 consists of 4525 amino acids (31 ligand-binding repeats, 22 growth factor repeats, and 2 NPXY sequences, Herz et al., 1988) and has a cDNA of 14 896 bp (database accession numbers: mRNA, NM_002332; protein, NP_002323; genomic contig, NT_029419) arranged in 89 exons (Herz et al., 1992). The four functionally important clusters of complement type repeats are located in the following cDNA positions: cluster I: 524–790, cluster II: 3026–4012, cluster III: 8036–9283, cluster IV: 10 466–11 794 (Brown et al., 1991; Herz et al., 1988), the promoter from position −1318 to −1 (Gaeta et al., 1994), two domains responsible for clustering into coated pits 13 865–13 903 and 13 967–14 008), and a membrane-binding domain (13 725–13 798) (Herz et al., 1988). More than 100 genomic variations of the human LRP1 gene have been recorded in several gene regions, in the promoter sequence as well as in exons or introns and in the untranslated regions (Kang et al., 1997; Van Leuven et al., 1998, 2001; Zuliani and Hobbs, 1994; Schulz et al., 2002, see also dbSNP LRP1, incorporated into NCBI's Entrez system). Because of the fundamental physiological importance of LRP1, in several studies an attempt has been made (with varying results) to associate genetic variants with complex diseases like AD, CAD or cancer.

The aim of this study was to investigate the genetic and functional in vivo properties of LRP1 in a representative sample of healthy European Caucasians from Central Germany, characterized by a well described state of health and to evaluate this data in an analysis involving 48 studies.

2. Subjects and methods

2.1. Subjects

Four hundred and forty-eight subjects of the same Caucasian origin from Central Germany were involved in this study on LRP1. All individuals were long-standing healthy blood donors (mean age 46.9 ± 10.4 years, 167 females). All were non-smokers, had undergone regular clinical check-ups by their family doctors and, for at least 3 years, had not experienced any symptoms relating to hypertension, diabetes mellitus, and other chronic illnesses including neurodegenerative or cardiovascular diseases or cancer.

The Ethics Committee of the Martin-Luther-University Halle-Wittenberg approved this study. The investigations were in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Methods

The investigations of genomic variants of LRP1 were performed using DNA from leucocytes separated from human venous blood (Old, 1986) and the methods (SSCP and sequencing analyses) described previously (Schulz et al., 2002; Van Leuven et al., 2001). The corresponding accession numbers of the sequence data of the human LRP1/A2MR gene are: NM_002332 and NT_029419. The genomic variants investigated are characterized in Table 1.

Table 1
Important LRP1 polymorphisms/mutations investigated in clinical case-control studies

Genomic variants	Localization	Functional region	Amino acid change or other effect	Importance	Database references (EMBL, GDB, SNP)	References
Tetranucleotide-repeat	5′upstream of the LRP1 gene	5′upstream of the promoter, at the 3′end of an *Alu* sequence (Zuliani and Hobbs, 1994)	$(TTTC)_n$ and $(GAAA)_n$, respectively	Length polymorphism		Zuliani and Hobbs (1994) and Van Leuven et al. (1998)
c.1-25C>G	5′upstream of the LRP1 gene	Promoter, near to a DNA binding site (X15424)	CCCCCC>CCCGCC	Polymorphism, creation of an additional binding site for the universal transcription factor SP 1	Y18524	Schulz et al. (2002)
c.766C>T	Exon 3	LDL-receptor domain class A, LDL receptor-like domain 1 (NM_002332)	GAC>GAT Asp100Asp	Polymorphism	NM_002332 dbSNP: 1799986	Kang et al. (1997)
c.1116C>T A217V	Exon 6		GCC>GTC Ala217Val	Polymorphism, amino acid substitution	NM_002332 dbSNP: 1800127	Van Leuven et al. (1998)
c.2789G>C A775P	Exon 14		GCA>CCA Ala775Pro	Mutation		Van Leuven et al. (2001)
c.4012C>T	Exon 22	LDL receptor-like domain 2 (NM_002332)	TGC>TGT Cys1182Cys	Polymorphism	Y13469 NM_002332 dbSNP: 2228186	Schulz et al. (2002)
IVS24+123C>A	Intron 24			Polymorphism	Y08236	Schulz et al. (2002)
IVS24+690G>A	Intron 24			Polymorphism	Y08236	Schulz et al. (2002)
c.6704G>A D2080N	Exon 39	LDL-receptor YWTD domain (NM_002332)	GAC>AAC Asp2080Asn	Mutation, amino acid substitution		Van Leuven et al. (2001)
c.8362C>A D2632E	Exon 48	LDL-receptor domain class A (NM_002332)	GAC>GAA Asp2632Glu	Mutation		Van Leuven et al. (2001)
c.10249G>A	Exon 61	60 bp downstream of the LDL-receptor domain class B (NM_002332)	ACG>ACA Thr3261Thr	Polymorphism	NM_002332 dbSNP: 1140648	Van Leuven et al. (1998)
c.12082C>T	Exon 76		ATC>ATT Ile 3872Ile	Polymorphism	dbSNP: 1800160	Van Leuven et al. (1998)
c.13601G>A G4379S	Exon 85		GGC>AGC Gly4379Ser	Mutation		Van Leuven et al. (2001)
c.13933C>T	Exon 88	Between two regions responsible for clustering into coated pits (Herz et al., 1988) (NM_002332)	GAC>GAT Asp4489Asp	Mutation	Y18525	Schulz et al. (2002)
IVS88+15G>A	Intron 88	Between two regions responsible for clustering into coated pits (Herz et al., 1988 (NM_002332))	GG>AG	Mutation, creation of a new theoretical acceptor splice site	Y11437	Schulz et al. (2002)

The study of human in vivo LRP1 gene (competitive RT-PCR) and protein (immuno-macroarray) expression was carried out with native monocytes, prepared immediately after blood withdrawal at 7:30–8:00 a.m. in order to ensure comparable differentiation states of cells as described previously (Schulz et al., 2003). By means of FACS-analyses, the quality of the monocytes was tested using the macrophage-specific antibody against the mannose-receptor CD 206 (positive signal with mannose-receptor specific antibody < 2%). The total RNA and the protein needed for the expression study were isolated from the counted monocytes by use of TriStar™-Reagent (AGS) in accordance with the producer's instruction for use.

2.2.1. Competitive PCR—measurement of the LRP1/A2MR gene expression

An amount of 0.5 μg of the total RNA was reversely transcribed into cDNA. By competitive PCR the expression measurement was carried out in accordance to Handschug et al. (1998) (RT-primer: 5′-CAG TGC GAT TGC CCT TGG GTG CTC A-3′, sense-primer: 5′-GCT GTG TGA TGG CAA CGA CGA CTG-3′, antisense-primer: 5′-CCA TCC AGC TTG GCC ACC TCG ATC-3′). A specific LRP1 cDNA standard of known concentration was used as a reference. Every proband sample was analyzed by use of a titration (eight independent and corresponding standard dilutions). Variations of reverse transcription and competitive PCR were minimized by a lab-internal pooled RNA standard constantly analysed as a control sample in every preparation series. Competition results were analyzed by a computer-based imaging system (Image Quant™, MOLECULAR Dynamics GMBH). The LRP/A2MR mRNA expression of every sample was calculated by linear regression of the eight corresponding dilution results.

2.2.2. Macro array analysis—LRP1/A2MR protein expression

One microgram of the total protein was used as a starting point of the quantitative protein analysis. The determination of LRP/A2MR protein expression was carried out with the help of the Western blotting detection system ECL-Plus (Amersham) in accordance with the producer's instructions for use and with the high specific LRP1/A2MR antibody R777 (D.K. Strickland). For each proband sample six dilutions (factor 1.5, starting concentration: 5 ng/μl) of the TriStar™ protein were analyzed in comparison with a commercially available LRP/A2MR standard protein of human placenta (Biomac) of known concentration as a reference. Array results were analyzed by a computer-based imaging system (Image Quant™, MOLECULAR Dynamics GMBH). The LRP/A2MR protein expression of every sample was calculated by linear regression of the six corresponding dilution results.

2.2.3. Statistical analysis

Statistical analyses were carried out using SPSS 11.5. Values of $P < 0.05$ were considered to be significant. Data are reported as mean ± standard deviation. The genotype distributions were tested for deviation from Hardy–Weinberg predictions and analyzed in a co-dominant and/or a dominant/recessive model. In the case of a very rare frequency of the mutant allele only the dominant/recessive model (wild type carriers versus mutation carriers) was used. Categorical variables were plotted into contingency tables and evaluated using Pearson's Chi square analysis and Fisher's exact test. Metric parameters were analysed using the Kolmogorov–Smirnov test (test of normal distribution), and the one-way ANOVA (normal distributed values) and Kruskal–Wallis test (values not distributed normally) for independent samples.

3. Results

3.1. Genetic variants

In this study, 15 (six rare and nine more frequent) of the more than 100 genetic variations, selected in accordance with already published clinical studies and located in functionally interesting regions of the LRP1 gene, were evaluated in the sample of European Caucasians (Table 2) and tested for age and gender dependency. Polymorphisms were not related to age or gender in the healthy individuals investigated.

The genotype distributions determined were compared to the unaffected subjects (controls) of further 14 studies involving several ethnic populations from different areas (Table 2). The genotypes most frequently analyzed were the 5′tetranucleotide-repeat polymorphism and the c.766C>T polymorphism, located in exon 3 of the LRP1 gene. Because of the different aims of these 14 (mostly disease-related) studies, it was quite evident that their "control groups" would differ in terms of their state of health and ethnic background. Nevertheless, the healthy Caucasian blood donors from Central Germany exhibited genotype and allele frequencies comparable to those of white Americans and Canadians of European descent, Australian Caucasians, French Caucasians or Hispanics. Surprisingly, the 5′tetranucleotide-repeat polymorphism and the c.766C>T polymorphism highlighted distinctly different frequencies in two other European samples, namely in the group of Spanish Caucasians and probands from Northern Ireland. The Japanese, Chinese and Nigerian controls differed as was to be expected.

3.2. Functional studies

In this study, the investigation of the LRP1 expression of the long-standing healthy Caucasian blood donors from Central Germany revealed gender and age dependency. Whereas the LRP1 mRNA levels increased with age (≤49 years: 107.9

Table 2
Control subjects (unaffected probands): frequencies of important LRP1 polymorphisms in several ethnic populations

Genomic variants	Allele frequencies		Genotype frequencies			Number of cases	State of health	Ethnic population	Authors
Tetranucleotide-repeat, 5′upstream of the LRP1 gene	87	91	87/87	87/91	91/91	Only the 87 and the 91 alleles were considered in the table, not the very rare 83 and 95 alleles			
	0.408	0.592	0.163	0.491	0.346	448	Healthy blood donors	European German Caucasians	Halle, Germany
	0.407	0.593	0.163	0.488	0.349	516	Found cognitively intact	White Americans	Luedecking-Zimmer et al. (2003)
	0.406	0.594	0.157	0.498	0.344	508	Normoglycemics	Non-Hispanic	Harris et al. (1998)
	0.426	0.574	0.181	0.489	0.33	370		Hispanic white Americans from Southern Colorado	
	0.326	0.674	0.103	0.447	0.45	389		Sokoto Blacks, Nigeria	
	0.379	0.621	0.140	0.478	0.382	799		Benin Blacks, Nigeria	
	0.405	0.595	0.17	0.47	0.36	628		French Caucasians	Lambert et al. (1999)
	0.548	0.452	0.29	0.515	0.195	231	Healthy blood donors	Northern Irish population	McIlroy et al. (2001)
	0.347	0.653	0.13	0.43	0.44	226		Population from Central Spain	Bullido et al. (2000)
	0.21	0.79	0.02	0.36	0.61	200		Spanish Caucasian males	González et al. (2002)
	0.254	0.746	0.057	0.394	0.549	246		Japanese population	Hatanaka et al. (2000)
c.1-25C>G, 5′upstream of the LRP1 gene	C	G	CC	CG	GG				
	0.921	0.079	0.844	0.154	0.002	448	Healthy blood donors	European German Caucasians	Halle, Germany
c.766C>T, Exon 3	C	T	CC	CT	TT				
	0.848	0.152	0.719	0.259	0.022	224	Healthy blood donors	European German Caucasians	Halle, Germany
	0.858	0.142	0.74	0.23	0.027	622	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)
	0.821	0.179	0.668	0.305	0.027	187	Normal elderly	Canadian Caucasians	Beffert et al. (1999)
	0.807	0.193	0.67	0.273	0.057	106		White Americans of European descent	Kamboh et al. (1998)
	0.9	0.1				129		Chinese	Baum et al. (1998a,b)
	0.904	0.096	0.813	0.183	0.004	246		Japanese population	Hatanaka et al. (2000)
	0.914	0.086	0.836	0.156	0.008	237		Northern Ireland population	McIlroy et al. (2001)
	0.848	0.152	0.71	0.27	0.04	243		Population from Central Spain	Bullido et al. (2000)
	0.90	0.10	0.819	0.168	0.013	304		Spanisch population	Sanchez-Guerrra et al. (2001)
	0.852	0.148	0.738	0.228	0.034	290		French Caucasians	Verpillat et al. (2001)
A217V, Exon 6	C	T	CC	CT	TT				
	0.978	0.022	0.955	0.045	0	224	Healthy blood donors	European German Caucasians	Halle, Germany
	0.981	0.186	0.964	0.034	0.002	619	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)
	0.97	0.03	0.939	0.061	0	511	Found cognitively intact	White Americans	Luedecking-Zimmer et al. (2003)
A775P, Exon 14	G	C	GG	GC	CC				
	1	0	1	0	0	89	Healthy blood donors	European German Caucasians	Halle, Germany
	0.983	0.017				118	“Non-defined” patients		Van Leuven et al. (2001)
	1	0	1	0	0	689	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)

Table 2 (*Continued*)

Genomic variants	Allele frequencies		Genotype frequencies			Number of cases	State of health	Ethnic population	Authors
c.4012C>T, Exon 22	C	T	CC	CT	TT				
	0.683	0.317	0.478	0.411	0.112	224	Healthy blood donors	European German Caucasians	Halle, Germany
	0.659	0.341	0.43	0.47	0.11	690	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)
IVS24+123C>A, Intron 24	C	A	CC	CA	AA				
	0.688	0.312	0.482	0.412	0.106	226	Healthy blood donors	European German Caucasians	Halle, Germany
IVS24+690G>A, Intron 24	G	A	GG	GA	AA				
	0.693	0.307	0.491	0.403	0.106	226	Healthy blood donors	European German Caucasians	Halle, Germany
D2080N, Exon 39	G	A	GG	GA	AA				
	0.976	0.024	0.952	0.476	0	126	Healthy blood donors	European German Caucasians	Halle, Germany
	0.978	0.022	0.957	0.043	0	690	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)
D2632E, Exon 48	C	A	CC	CA	AA				
	1	0	1	0	0	89	Healthy blood donors	European German Caucasians	Halle, Germany
	0.999	0.001	0.999	0.001	0	692	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)
c.10249G>A, Exon 61	G	A	GG	GA	AA				
	0.685	0.315	0.482	0.406	0.112	224	Healthy blood donors	European German Caucasians	Halle, Germany
c.12082C>T, Exon 76	C	T	CC	CT	TT				
	0.993	0.067	0.987	0.013	0	224	Healthy blood donors	European German Caucasians	Halle, Germany
G4379S, Exon 85	G	A	GG	GA	AA				
	1	0	1	0	0	89	Healthy blood donors	European German Caucasians	Halle, Germany
	0.999	0.001	0.999	0.001	0	679	No history of CAD	Australian Caucasians	Pocathikorn et al. (2003)
c.13933C>T, Exon 88	C	T	CC	CT	TT				
	1	0	1	0	0	224	Healthy blood donors	European German Caucasians	Halle, Germany
IVS88+15G>A, Intron 88	G	A	GG	GA	AA				
	1	0	1	0	0	224	Healthy blood donors	European German Caucasians	Halle, Germany

± 58 ag/cell, ≥50 years: 138.1 ± 65.2 ag/cell, all: 114.6 ± 60.7 ag/cell, $P = 0.02$) no gender association was found ($P = 0.969$). The LRP1 protein expression demonstrated gender as well as age association. But, in contrast to the gene expression, the protein levels decreased with age (≤49 years: 8.7 ± 6 pg/cell, ≥50 years: 5.2 ± 3.7 pg/cell, all: 7.3 ± 5.4 pg/cell, $P < 0.037$). The females expressed more LRP1 than the males (12.9 ± 6.3 pg/cell versus 6.03 ± 4.4 pg/cell, $P < 0.002$).

The investigation of the genotype/phenotype relation (Table 3) revealed no significant association with genotype and LRP1 expression except for the c.1-25C>G promoter polymorphism: a distinct difference was found in the LRP1 gene (+28.8%, $P = 0.048$) and a weaker difference in the protein expression (+27.9%, n.s.) dependent on this genomic variant.

The other more frequently occurring LRP1 polymorphisms (A217V, A775P, c.4012C>T, IVS24+ 123C>A,

Table 3
Human ex vivo/in vivo LRP1 gene and protein expression related to frequent polymorphisms (PM) of the LRP1 gene

Polymorphism	Localization	Genotype	Gene expression			Protein expression		
			n	mRNA (ag/cell)	*P*	*n*	Protein (pg/cell)	*P*
Tetranukleotid repeat PM	5′upstream of the LRP1 gene	87/87	22	124.5 ± 61.3	0.362[a]	7	5.2 ± 3.8	0.27[a]
		87/91	65	118.9 ± 62.3		18	7.0 ± 5.0	
		91/91	38	103.8 ± 57.8		18	7.9 ± 6.0	
		All	125			43		
c.1-25C>G	**Promoter**	CC	110	109.6 ± 56.6	**0.048**[b]	31	6.8 ± 5.2	0.292[b]
		CG	16	141.2 ± 75.4		13	8.7 ± 5.9	
		GG	0			0		
		All	126			44		
c.766C>T	Exon 3	CC	93	120 ± 61.9	0.064[b]	34	7.5 ± 5.8	0.573[b]
		CT	33	97.9 ± 55.8		10	6.6 ± 3.8	
		TT	1	106.3		0		
		All	127			44		
A217V	Exon 6	CC	123	114.0 ± 60.6	0.548[b]	44	7.3 ± 5.4	
		CT	4	132.6 ± 71.0		0		
		TT	0			0		
		All	127			44		
c.4012C>T	Exon 22	CC	59	118.9 ± 61.8	0.493[a]	23	7.3 ± 4.7	0.633[a]
		CT	54	107.3 ± 59.6	(0.453[b])	18	7.7 ± 6.5	(0.844[b])
		TT	14	124.2 ± 61.5		3	4.4 ± 2.7	
		All	127			44		
IVS24+123C>A	Intron 24	CC	59	118.9 ± 61.8	0.212[a]	23	7.3 ± 4.7	0.59[a]
		AC	56	105.4 ± 59.6	(0.453[b])	19	7.6 ± 6.4	(0.844[b])
		AA	12	136.2 ± 57.5		2	3.5 ± 2.9	
		All	127			44		
IVS24+690G>A	Intron 24	GG	61	115.6 ± 63.5	0.361[a]	23	7.3 ± 4.7	0.59[a]
		AG	54	108.6 ± 58	(0.858[b])	19	7.6 ± 6.4	(0.844[b])
		AA	12	136.2 ± 57.5		2	3.5 ± 2.9	
		All	127			44		
D2080N	Exon 39	GG	120	115.2 ± 61.4	0.878[b]	42	7.5 ± 5.5	0.475[b]
		AG	6	119.1 ± 32.4		2	4.6 ± 4.3	
		AA	0			0		
		All	126			44		
c.10249G>A	Exon 61	GG	59	118.9 ± 61.8	0.374[a]	23	7.5 ± 4.9	0.836[a]
		AG	54	106.4 ± 59.9	(0.453[b])	17	7.5 ± 6.1	(0.844[b])
		AA	14	128 ± 59.1		4	5.8 ± 6.6	
		All	127			44		
c.12082C>T	Exon 76	CC	124	115.0 ± 61.4	0.652[b]	43	7.2 ± 5.4	-
		CT	3	98.9 ± 15.4		1	9.9	
		TT	0			0		
		All	127			44		

Significance calculation by use of [a]co-dominant (wild type/wild type vs. wild type/mutant vs. mutant/mutant) and/or [b]dominant/recessive model (wild type/wild type vs. wild type/mutant + mutant/mutant). In the case of a very rare frequency of the mutant allele only the dominant/recessive model (wild type carriers vs. mutation carriers) was used.

IVS24+690G>A, c.10249G>A) were neither associated with the gene nor with the protein expression. The c.766C>T polymorphism only showed a weak relation to the LRP gene expression (contrary to the c.1-25C>G promoter polymorphism: lower expression of the T carrier, $P = 0.064$) but also no association to the LRP1 protein expression ($P = 0.573$). A comparison between the genotype distribution of the c.766C>T and the c.1-25C>G polymorphism within the European German Caucasians investigated revealed no association but significantly different distribution pattern (CC and CT+TT versus CC and CG+GG, $P = 0.016$).

For the very rare polymorphisms/mutations (A217V, D2080N, D2632E, G4379S, c.13933C>T, IVS88+15G>A) no significant data for the expression were calculated or mutation carriers were not found within the subgroup investigated for LRP1 expression, respectively.

Seventeen further studies, all disease-related, on human in vivo/ex vivo LRP1 expression were included in an analysis (Table 4). Only studies with a larger (statistically relevant) number of samples were selected. Although the measurement method, the sample selection procedure, and/or the diseases investigated differed, most of the trials resulted in a disease-related change in human in vivo/ex vivo LRP1 expression. The age-dependent decrease in the LRP1 level, detected in this study, confirmed the results of Kang et al. (2000) and Handschug et al. (1998).

4. Discussion

LRP1 is one of the largest and most conserved human proteins known today. Even though a multitude of in vitro investigations proved its complex function as a multifunctional cell receptor (functioning as a cargo vesicle), little is known as yet about its in vivo regulation and importance to vital processes. Beside the genetic constellation of the LRP1, interaction with other genes and/or gene products, including numerous LRP1 ligands, should influence the in vivo function of this protein.

The aim of this study on healthy European Caucasians was to add to the data on human LRP1, genetic frequencies as well as in vivo functions, and to relate these results to further published studies of different or similar populations.

4.1. Genetic background

To evaluate ethnic or population-specific differences in the genotype distributions the controls in several studies were compared with the results from the present study. The different distribution patterns of the alleles and genotypes published could be influenced by different selection criteria for the control samples. For example, controls were described as being “cognitively intact” (Luedecking-Zimmer et al., 2003) or “normoglycemic” (Harris et al., 1998) or “without history of CAD” (Pocathikorn et al., 2003). Some control groups were not characterized in detail. In contrast, the long-standing German blood donors involved in this study represented a sample of healthy, well characterized probands continuously checked by their family doctor. But the similarities between the American, Australian, and Central-European Caucasians and the Eastern Asians’ varying data (but still comparable among themselves) suggests quite a distinct influence of ethnic background. This finding might support the thesis that the controversial results of published human disease-related studies on genomic LRP1 variants could well be caused by different ethnic backgrounds. Table 5 summarizes several clinical case-control studies dealing with complex diseases like Alzheimer’s disease, coronary artery disease, astrocytoma disease, Parkinson disease, argyrophilic grain disease, multiple sclerosis or familial hypercholesterolaemia and the genetic variants of LRP1. The most frequent data, found for AD, were consistent with the numerous proven in vitro functions of LRP1 in neurodegenerative processes. The 5′tetranucleotide-repeat polymorphism and the c.766C>T polymorphism of exon 3 but also the A217V-PM of exon 6 were investigated most frequently. It is very difficult to interpret the particularly contradictory data. In accordance with the results of the present study, in the context described above, the fact that ethnic differences also have a distinct impact on these disease-related studies should be indisputable. For example, the fact that the genetic background of the Spanish Caucasians and probands from Northern Ireland differed from other Europeans or North Americans of European descent (Table 2) suggests possible ethnic differences with regard to LRP1 function.

But differences in the studies analyzed could also stem from different clinical selection criteria for the cases as well as the controls. Moreover, it might also be possible that the polymorphisms investigated do not have an important influence on the structure and/or function of LRP1. The physiological function of LRP1 might be affected mainly by other genes that are not considered in these studies and/or the diseases described might not be related to the in vivo LRP1 in contrast to manifold in vitro studies. Finally, the controversial data from the 14 genetic disease-related studies evaluated could also underline the complex polygenetic background of the diseases investigated, including the LRP1 gene with its important cargo function.

4.2. Functional results

In this study, only 1 of the 10 genomic variants investigated was associated with the functional in vivo properties of LRP1. These results obtained from circulating human monocytes from venous blood, highlight the importance of this genomic LRP1 variant, the c.1-25C>G promoter polymorphism, which creates a new GC-box recognized by the constitutively expressed transcription factor SP1. This additional SP 1 binding motif might directly influence the basic LRP1 promoter activity leading to a higher transcription rate of the LRP1 gene. Likewise, we found significantly higher gene expression levels in the CG-carriers (+28.8%, $P <$

Table 4
Several clinical case-control studies investigating the human in vitro/ex vivo LRP1 gene and/or protein expression

Human disorder	Investigated subjects (number of subjects)	Measurement/samples	Results of clinical-functional study	Genetical-functional relations	Authors
AD	Americans (?): AD (?) and controls (?)	Immunohistochemistry; brain tissue	LRP1 in senile plaques can bind several LRP1 ligands but clearance can be impaired		Rebeck et al. (1995)
CAD	Finns (?): postmortem renal transplant donors (9)	In situ hybridization, immunocytochemistry; human atherosclerotic lesions	LRP1 mRNA and protein expression in SMC and macrophages in early and advanced lesions in human aortas; LRP1 is the only lipoprotein receptor expressed in lesions SMC in vivo		Luoma et al. (1994)
CAD	Swiss (?): patients undergoing cardiovascular surgery (12), mammary donors (5)	In situ hybridization, immunocytochemistry; healthy (mammary artery) and atherosclerotic arteries	Controls: LRP1 mRNA and protein detected in SMC, fibroblasts; endothelial cells negative for protein but positive for mRNA Cases: strong labelling for mRNA and protein in SMC, foam cells; high expression in macrophages located in the cap of the lipid-rich core		Lupu et al. (1994)
Haemodialysis patients	Japanese (?): (?) (chronically ill?) patients with haemodialysis (18) and controls (7)	RT-PCR, Southern blot; peripheral blood monocytes	LPR gene expression shows no significant differences between cases and controls		Konishi et al. (1997)
Liver disease; NID diabetes mellitus	Australians: healthy blood donors (50), liver disease patients (45), NIDDM patients (49)	Immunoassay; human plasma	LRP1 concentration in controls: 6.1 $\pm$ 1.2 μg/ml; significantly increased LRP1 plasma-levels in patients with liver disease		Quinn et al. (1997)
AD	German Caucasians (?): AD patients (16) and controls (7)	Immunocytochemistry; brain tissue	Detection of LRP1 transmembranous ß-subunit in plaque cores in AD, extramembranous alpha-subunit was localized in activated plaque-associated astrocytes and extracellulary in plaques		Thal et al. (1997)
Malignant astrocytoma	Japanese (?): patients with 15 astrocytomas, 12 with glioblastoma and 8 controls	RT-PCR, immunohistochemistry; astrocytoma tissues	LRP1 overexpressed in malignant astrocytomas especially glioblastomas		Yamamoto et al. (1997)
Astrozytoma	Chinese: patients with gliomas (25), with other brain tumors (23) and controls (heart tissue/10)	Differential PCR phosphoimaging; genomic DNA from paraffin-embedded brain tumors and normal heart tissue	LRP1 amplification occurred more frequently in 4 out of 25 high-grade gliomas but 0 out of 23 other brain tumors	Exon 3, c.766C>T. PM does not alter the LRP1 DNA-amplification	Baum et al. (1998a,b)
CAD	German Caucasians: CAD patients (100) and controls (110)	RT-PCR; macrophages from venous blood	Correlation of increased LRP1 mRNA levels to CAD; nearly constant intra-individual level over a period of 4 weeks; increase in age-dependent level		Handschug et al. (1998)

Table 4 (*Continued*)

Human disorder	Investigated subjects (number of subjects)	Measurement/samples	Results of clinical-functional study	Genetical-functional relations	Authors
Carcinoma	Australians (?): endometrial adenocarcinoma patients (33) and controls (14)	RT-PCR; endometrium	Reduced LRP1 gene expression in carcinomas, in normal endometrium, gene expression was significantly increased in secretory compared with proliferative phase		Foca et al. (2000)
AD	Americans of European descent with AD (37) and controls (103)	Immunoblotting, phosphoimaging; frozen brain tissue with senile plaques in some cases	Decrease in age-dependent LRP1 level in controls, significantly decreased levels in AD patients compared with controls, reduced LRP1 levels correlated with enhanced AD susceptibility	Exon 3, c.766C>T-PM: T-allele carriers of AD patients have significantly higher LRP1 levels	Kang et al. (2000)
AD	Americans (?): AD patients (8), controls (9 + 7)	Brain frontal cortex	85% increase in LRP1 levels along with an increased level of the LRP1 ligands, apo E and alpha2M		Qiu et al. (2001)
CVD	Frenchmen (?): Healthy subjects (7)	RT-PCR; venous blood cells	On a moderately high-carbohydrate/low-fat diet the LRP1 mRNA level decreased		Vidon et al. (2001)
AD	Americans (?): AD patients (5), controls (3)	Immunohistochemistry; human brain tissue	LRP1 was only present on cored, apo E-containing senile plaques; strong staining in neurons and reactive astrocytes		Arélin et al. (2002)
Type 2 diabetes mellitus, adipositas	Frenchmen (?): type 2 diabetes patients (8) and lean controls (8) and obese non-diabetic (8)	RT-PCR; skeletal muscle biopsies	Adipositas controls and healthy controls with comparable LRP1 mRNA expression, reduced LRP1 mRNA-expression in skeletal muscle of type 2 diabetic patients		Boucher et al. (2002)
Type 2 diabetes mellitus	Frenchmen (?): hyperlipidemic type 2 diabetes patients (9) and controls (10)	RT-PCR; circulating mononuclear cells	LRP1-mRNA concentration did not differ in patients and controls and were unaffected by fenofibrate		Forcheron et al. (2002)
CAD	German Caucasian males: CAD patients (89) and controls (108)	RT-PCR; monocytes from venous blood	Significant increase in the LRP1 mRNA in MI patients, normotensive patients exhibited the highest levels	Promoter-c.1-25C>G-PM: G carriers show increased LRP1 gene expression	Schulz et al. (2002)
CAD	German Caucasian males: CAD patients (36) and controls (36)	RT-PCR, immunomacroarray: monocytes from venous blood	Circadian and gender-specific rhythm of gene and protein expression; increased LRP1 gene expression but decreased LRP1 protein expression in MI patients compared to controls		Schulz et al. (2003)

AD: Alzheimer's disease, CAD: coronary artery disease, MI: myocardial infarction, CVD: cardiovascular disease.

Table 5
Several clinical case studies investigating the influence of LRP1 mutations/polymorphisms

Genomic variant	Human disorder	Ethnic origin (number of individuals/heterozygous carriers investigated/homozygous carriers)	Case report/result of the clinical association study	Authors
Tetranukleotid-PM 5′upstream of the LRP1 gene (for this PM only the number of investigated cases is shown in this table)	AD	AD (130 + "38") vs. controls (64 + 93)	No correlation	Clatworthy et al. (1997)
	AD	French population: sporadic late-onset AD patients (144) vs. controls (153)	Significant decrease in the 87 bp-allele	Wavrant-De Vrièze et al. (1997)
	AD	American Caucasians: AD patients (62) vs. Controls (282)	No association between LRP1 variants and occurrence of AD	Fallin et al. (1997)
	AD	Caucasians: AD (182) vs. controls (118)	Association of the 87 bp-allele to AD	Lendon et al. (1997)
	Population analyses	Healthy US whites: Hispanic (373) and non-Hispanic (522), Nigerian Blacks: Sokoto (390), Benin (800)	Analyses including biochemical parameters: origin-dependent genotype distribution, association with lipid profile and menopausal state	Harris et al. (1998)
	AD	American whites: sporadic late onset AD patients (216) vs. controls (106)	No correlation	Kamboh et al. (1998)
	AD	Late onset multiplex familial AD (179) and sporadic AD (436) vs. controls (240)	No association	Scott et al. (1998)
	AD	French Caucasians: AD patients (600) vs. age matched controls (646)	91 bp-allele is associated with AD	Lambert et al. (1999)
	AD	Spanish population: late-onset sporadic AD patients (188) vs. controls (226)	No association but gender modulation	Bullido et al. (2000)
	AD	Japanese population: AD patients (100) vs. Controls (246)	No association	Hatanaka et al. (2000)
	AD	Northern Ireland population: AD (219) vs. controls (237)	No significant differences in genotype and allele distribution	McIlroy et al. (2001)
	AD	French Caucasians: AD (274) vs. controls (290)	No association but strong linkage disequilibrium between alleles of two LRP1-PM (Tetranukl. and c.766C>T-PM)	Verpillat et al. (2001)
	CAD	Male Spanish Caucasians: juvenile MI patients (210) vs. controls (200)	No association	González et al. (2002)
	AD	US whites: late-onset AD (505) vs. controls (522)	No association	Luedecking-Zimmer et al. (2003)
c.1-25C>G-PM of the promoter region	CAD	German Caucasians: MI patients (214/28/0) vs. healthy controls (223/30/0)	Association with the severity of coronary obstruction (number of affected vessels), association with the ex vivo human LRP1 gene-expression in male individuals	Schulz et al. (2002)
c.766C>T-PM of Exon 3	AD	US European whites: late onset AD patients (157/26/4) vs. controls (102/34/3)	Association with AD (CC-carrier), this difference was highly accentuated among AD cases with positive family history of senile dementia	Kang et al. (1997)
	Astrocytoma disease	Chinese population: patients with astrocytoma (108/?/?) vs. breast tumor patients (89/?/?) vs. controls (227/?/?)	No correlation	Baum et al. (1998b)
	AD	Chinese population: several AD patients (143/?/?) and controls (129/?/?)	Population-specific differences to Caucasians, significantly decreased T-allele frequency in the pathologically diagnosed patient group, but not in the clinically diagnosed patients	Baum et al. (1998a)

Table 5 (*Continued*)

Genomic variant	Human disorder	Ethnic origin (number of individuals/heterozygous carriers investigated/homozygous carriers)	Case report/result of the clinical association study	Authors
	AD	Primarily whites: AD (234/53/4) vs. controls (103/40/1)	Significantly more CC carrier in the AD group, no correlation to age at onset	Hollenbach et al. (1998)
	AD	White Americans: AD (432/111/11) vs. controls (106/29/6)	TT carrier significantly increased in the control group	Kamboh et al. (1998)
	AD	Whites: late-onset sporadic AD (558/119/11) vs. controls (596/168/21)	CC genotype and C allele far more frequent in the AD patient group	Lambert et al. (1998)
	AD	UK?: Late onset AD (133) vs. controls (70) (only allele frequencies: C 0.84 vs. 0.85)	No correlation	Woodward et al. (1998)
	Parkinson disease	Chinese population: patients with Parkinson disease (186/?/?) vs. age-matched controls (187/?/?) vs. newborns (227/?/?)	No correlation	Baum et al. (1999)
	AD	Caucasians: neuropathological confirmed AD (225/58/9) vs. controls (187/57/5)	Genotype CC weak increased (n.s.) in patients	Beffert et al. (1999)
	AD	Spanish population: late-onset sporadic AD patients (199/47/1) vs. controls (243/66/4)	No association but gender modulation	Bullido et al. (2000)
	AD	Japanese population: AD (100/17/0) vs. controls (246/45/1)	No significant differences of genotype and phenotype distribution; association with age at onset	Hatanaka et al. (2000)
	AD	Taiwan Chinese population: AD (82/?/?) vs. controls (110/12/0)	No correlation	Hu et al. (2000)
	AD	Northern Ireland population: AD (219/24/2) vs. controls (237/37/2)	No significances	McIlroy et al. (2001)
	CAD	Czech population: CAD (654/180/12) vs. controls (525/136/12)	Lower T-allele frequencies in subjects aged 60 years or over	Benes et al. (2001)
	AD	Spanish population: sporadic AD (305/65/3)) vs. controls (304/51/4)	No association; weak correlation of CC genotype with AD in meta-analysis of previous studies	Sánchez-Guerra et al. (2001)
	AD	French Caucasians: AD (274/71/5) vs. controls (290/66/10)	No association but strong linkage disequilibrium between alleles of two LRP1-PM (Tetran. PM and c.766C>T-PM)	Verpillat et al. (2001)
	AGD	German population?: AGD (109/14/0) vs. controls (170/54/1)	CC genotype is associated with AGD	Ghebremedhin et al. (2002)
	CAD	Caucasians: CAD (598/128/6) vs. controls (622/143/17)	T homozygotes under-represented in CAD patients	Pocathikorn et al. (2003)
	Breast cancer	Czech Caucasians: female patients (164/62/4) vs. female controls (183/47/4)	T allele is associated with increased risk of breast cancer development	Benes et al. (2003)
	AD	Caucasians: AD (212/59/8) vs. controls (337/84/3)	C allele carrier with lower AD risk, higher age at onset	Kolsch et al. (2003)
A217V-PM of exon 6	AD	Northern French Caucasians: AD (648/19/0) vs. controls (670/33/2)	Weakly protective effect of the T-allele	Wavrant-De Vrièze et al. (1999)
	MS	German population?: MS (326/28/0) vs. controls (290/21/0)	No correlation	Schweer et al. (2001)
	AD, FH	AD patients (29/1/0), FH patients (110/4/0) vs. healthy controls (22/2/0) vs. non-defined probands (118/4/0)	Carrier: only 11 out of 279	Van Leuven et al. (2001)
	CAD	German Caucasian males: MI patients (107/10/0) vs. healthy controls (89/1/0)	No association with the LRP1-mRNA expression	Schulz et al. (2002)

Table 5 (*Continued*)

Genomic variant	Human disorder	Ethnic origin (number of individuals/heterozygous carriers investigated/homozygous carriers)	Case report/result of the clinical association study	Authors
	AD	US whites: late-onset AD (536/22/8) vs. controls (511/31/0)	No association	Luedecking-Zimmer et al. (2003)
	CAD	Caucasians: CAD (594/37/1) vs. controls (619/21/1)	1.9 higher coronary risk for CT carriers	Pocathikorn et al. (2003)
A775P-mutation of exon 14	AD, FH	AD patients (29/0/0), FH patients (110/1/0) vs. healthy controls (22/0/0) vs. not specified probands (118/4/0)	Carrier: only 5 of 279	Van Leuven et al. (2001)
	CAD	German Caucasian males: MI patients (107/0/0) vs. healthy controls (89/0/0)	Undetected in 196 individuals	Schulz et al. (2002)
	CAD	Caucasians: CAD (587/4/0) vs. controls (689/0/0)	Rare mutation carriers (CT), only found in CAD	Pocathikorn et al. (2003)
c.4012C>T-PM of exon 22	CAD	German Caucasians: MI patients (207/100[a]/7[a]) vs. healthy controls (216/86[a]/23[a])	MI protective effect of the homozygous mutant genotype constellation of the four variants[a], more common heterozygous carriers in the patient group	Schulz et al. (2002)
	CAD	Caucasians: CAD (595/232/55) vs. controls (690/321/75)	CC carrier and C allele are associated with CAD	Pocathikorn et al. (2003)
IVS24+123 C>A-PM of intron 24	CAD	[a]	[a]	Schulz et al. (2002)
IVS24+690 G>A-PM of intron 24	CAD	[a]	[a]	Schulz et al. (2002)
D2080N-PM of exon 39	AD, FH	AD patients (29/0/0), FH patients (110/3/0) vs. healthy controls (22/0/0) vs. non-defined probands (118/2/0)	Carrier: total: only 5 out of 279	Van Leuven et al. (2001)
	CAD	German Caucasian males: MI patients (105/6/0) vs. healthy controls (88/5/0)	No association with the LRP1-mRNA expression	Schulz et al. (2002)
	CAD	Caucasians: CAD (596/29/0) and controls (690/30/0)	–	Pocathikorn et al. (2003)
D2632E-mutation of exon 48	AD, FH	AD patients (29/0/0), FH patients (110/0/0) vs. healthy controls (22/1/0) vs. Non-defined probands (118/0/0)	Carrier: only 1 of 279	Van Leuven et al. (2001)
D2632E-mutation of exon 48 continued	CAD	German Caucasian males: MI patients (107/0/0) vs. healthy controls (89/0/0)	Undetected in 196 individuals	Schulz et al. (2002)
	CAD	Caucasians: CAD (588/1/0) and controls (692/1/0)	–	Pocathikorn et al. (2003)
c.10249 G>A-PM of exon 61	CAD	[a]	[a]	Schulz et al. (2002)
G4379S-mutation of exon 85	AD, FH	AD patients (29/0/0), FH patients (110/1/0) vs. healthy controls (22/0/0) vs. Non-defined probands (118/1/0)	Carrier: only 2 out of 279	Van Leuven et al. (2001)
	CAD	German Caucasian males: MI patients (107/0/0) vs. healthy controls (89/0/0)	Undetected in 196 individuals	Schulz et al. (2002)
	CAD	Caucasians: CAD (568/1/0) and controls (679/1/0)	–	Pocathikorn et al. (2003)
c.13933C>T-mutation of exon 88	CAD	German Caucasians: MI patients (214/2/0) vs. healthy controls (224/0/0)	Only detected in (unrelated) MI patients	Schulz et al. (2002)
IVS88+15G>A-mutation of intron 88	CAD	German Caucasians: MI patients (214/2/0) vs. healthy controls (224/0/0)	Only detected in (unrelated) MI patients	Schulz et al. (2002)

AD: Alheimer's disease; CAD: coronary artery disease; FH: familial hypercholesterolaemia; MS: multiple sclerosis; MI: myocardial infarction; AGD: Argyrophilic grain disease.

[a] Four associated genetic variants.

0.049) (Table 3). The significant increase in the gene expression was followed by a possible increase in the protein expression level in the CG-carriers (+27.9%, n.s.). Only a second polymorphism, namely c766C>T, showed a slight (but insignificant) relation to functional characteristics: the T carrier had a considerably reduced mRNA gene expression ($P = 0.064$). Even though this genetic variant does not result in an amino acid exchange (Table 1) the base pair substitution T>C, situated in the LDL-receptor domain class A, could contribute to a change in the molecule structure and, thus, to different expression levels. A further indication of the possible importance of this polymorphism was highlighted by the results of Kang et al. (2000) who found that T-allele carriers of AD patients had significantly higher LRP1 levels, but these results were obtained with other methods and other human cells. These findings suggest the need to include genetic background in functional human studies on LRP1 and might indicate the possible cause of the differences in the expression studies published so far.

Clinical studies involving a (larger) number of probands that can be evaluated, dealing with human ex vivo/in vivo LRP1 gene and/or protein expression, are rare up until now. Table 4 summarizes several clinical case-control studies on AD, diabetes mellitus, liver disease, cancer, affected kidneys, and coronary vessel disease. Most of the trials resulted in detectable expression changes on a transcriptional and/or translational level, suggesting a disease-related regulation of this multifunctional receptor. Only three of these studies dealt with the genetic background, too. The polymorphisms investigated, namely the c.766C>T-PM of exon 3 and the promoter-PM c.1-25C>G could be associated with modified expression levels in the case of AD and CAD, respectively.

An evaluation of these case-control studies is very difficult because not only the ethnic background but also the measurement procedures (lab methods and cells/tissue used) are very different in most cases. But distinct human tissue specific LRP1 expression is indisputable (see also gene card, http://bioinfo.weizmann.ac.il/cards-bin/cardsearch.pl, GC12PO57746) and could be an important explanation for the somewhat controversial expression results published up until now. Moreover, Schulz et al. (2003) could also prove that gender-specific circadian variations in human in vivo LRP1 expression can be observed. Finally, the varying quality of the measurement procedures and the differences in the statistical verification of the results (sufficient number of samples) directly influence the quality and comparability of the results.

4.3. In conclusion

There is no question as to the importance of the multifunctional cell receptor LRP1 as a vital cargo transporter in a large number of human cells and tissues. The complexity of all of the largely unrelated ligands and their in vivo "voyage" via LRP1 are only partially understood at present. The human expression of LRP1 does not only seem to be tissue specific but also disease related as is suggested by several initial clinical studies, most of which deal with complex (degenerative) diseases. At present, the first results indicate a direct influence of genetic LRP1 variants on the expression levels. The human in vivo regulation and function of this protein must be further investigated in other disease-related studies.

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