J. Appl. Genet. 39(4), 1998, pp. 331-348

Molecular studies in *osteogenesis imperfecta* (OI) I. Clinical analysis of patients with *osteogenesis imperfecta*

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Abstract. The goal of this study is to develop optimal diagnostic methods for *osteogenesis imperfecta* (OI), which will allow to distinguish familial from spontaneous cases and can be used in prenatal diagnostics as well. The paper summarizes the clinical part of the study, in which 69 families were analyzed. The families with OI were registered, their pedigrees were studied, a clinical classification of the disease was carried out and the dermatoglyphics of the affected patients were analyzed. Based on the above results a diagnostic algorithm was elaborated.

Key words: clinical classification, collagen type I, *osteogenesis imperfecta* (OI).

Introduction

Osteogenesis imperfecta (OI) is a generalized connective tissue disorder characterized by a variable clinical picture. The disease is caused by a qualitative and quantitative impairment of the protein building the connective tissue framework of the bones – collagen type I (BYERS 1993). The incidence of all OI forms is about 1 : 10000, but there are only few population studies available. Danish authors ANDERSEN and HAUGE (1989) estimated OI prevalence at birth at 21.8 : 100000 and an incidence in a general population at 10.6 : 100000. There is no difference in OI incidence between ethnic groups (BYERS 1993). Excavations proved that the disease was known in ancient Egypt.

Received: October 1998.
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The family of collagen proteins consists of at least 28 different collagens, and their basic chains are encoded by 17 different genes (NINOMIYA, OLSEN 1984). Collagen type III, V and VI are present in virtually all tissues. The majority of other types are specific for certain tissues or structures; for example, collagens type II, IX, X and XI are found in the hyaline cartilage, and type IV in the basement membrane. Collagen type I is the main connective tissue compound of the skin, bones, tendons and vessel walls. The protein consists of fibers, which are built up of 300 nm trimeric domains composed of two α1 chains and one α2 chain. Each chain is made up of 1000 amino acids and forms a left-handed helix, and all three chains are joined together to form a right-handed superhelix.

Collagen type I synthesis begins within a cell, where procollagen type I is formed. Procollagen is then secreted to the extracellular space where it is modified to collagen. Procollagen chains: pro-α1 and pro-α2 are encoded by COL1A1 and COL1A2 genes localized, respectively, on chromosomes 17q21-q22 and 7q21-q22. The protein is synthesized in the well-known processes of transcription and translation. Then posttranslational modification takes place, which consists of:

– modifications of amino acids (e.g., hydroxylation of prolyl and lysyl residues),
– glycosylation of hydroxylysyl residues,
– proteolysis of the globular domains and cutting them off from procollagen,
– formation of collagen fibrils.

Point mutations, deletions, insertions, impaired posttranscriptional mRNA modification in one of the alleles of locus COL1A1 and COL1A2 lead to lack of expression ("null") or structural defects in α1 or α2 chains. The lack of expression is accompanied by a 50% reduction in collagen synthesis while a structural defect leads to changes in the quality of collagen and in the quantity as well. Both abnormalities are responsible for symptoms of OI. In 90% of cases, a defect in collagen synthesis is due to different mutations (substitution, deletion, insertion) in one of the previously mentioned genes. These mutations usually consist in substitution of glycine for another amino acid (STARMAN et al. 1989). Since 1/3 of collagen amino acids are glycyl residues, these mutations always cause changes in the structure of the protein (BYERS 1993).

The sequence of cDNA for the α1 collagen chain was determined for the first time in 1988 by TROMP et al. (1988), and for the α2 chain in the same year by KUIVANIEMI et al. (1988). Since that time molecular techniques have been used to identify different mutations within COL1A1 and COL1A2 genes and

to diagnose OI caused by mutations in genes other than collagen (WALLIS et al. 1993).

The clinical presentation of the disease depends on the type of defective chain and on the nature and localization of the defect. A defect in the α1 chain results in the presence of 25% of collagen molecules with two abnormal chains, 50% with one and 25% with two normal chains. The ratio of abnormal to normal collagen molecules is 1 : 1 in the case of an α2 chain defect. An identical codon change can give different clinical manifestations depending on the position in the α chain. Defects in the C-terminal of the protein, responsible for the endoplasmatic storage of the protein heterotrimer, result in more severe clinical manifestations than defects in the N-terminal. A defect leading to a decreased production of collagen gives milder clinical manifestations than a defect causing changes in collagen structure. There are four clinical OI types.

Heterogeneity in etiology and clinical manifestations of OI is caused by different types of mutations in the genes responsible for type I collagen production (COL1A1 and COL1A2). Practically, this indicates difficulties in defining the prognosis for individual patients (with the exception of OI type II), and especially problems in providing genetic counselling and defining indications for prenatal diagnostics.

The goal of this study is to elaborate optimal diagnostic methods of OI, which will allow to distinguish familial from spontaneous cases and can be used in prenatal diagnostics as well. A proper algorithm will lead to an early diagnosis, appropriate therapeutic measures and to a cost-effective diagnostic process. The study was divided into three stages:

1. Registration of the families with OI, analysis of their pedigrees, clinical classification and analysis of the dermatoglyphics of the affected patients.
2. Segregation analysis of the molecular polymorphic markers in the families:
   a) Restrictive Fragments Length Polymorphism (RFLP) of COL1A1 gene,
   b) AlleloSpecyfic Amplification (ASA) of COL1A1 gene,
   c) Variable Number of Tandem Repeats (VNTR) of COL1A2 gene.
3. Search for mutations of COL1A1 and COL1A2 genes in cDNA fragments.

Material and methods

A method of multiple ascertainment of children with OI was employed. The following sources of information were used:

1. Records kept by the Genetic Clinic, Polish-American Children's Hospital (PACH).

2. Records kept by the Orthopedic Clinic, PACH.
3. Register kept by the National Association of Children with OI.

A sample of 170 proband families were enrolled and 69 responded to our invitation. Table 1 shows the distribution of the families according to the source of data.

Table 1. Distribution of the families according to the source of proband registration

	Families consulted in PACH				Families not consulted in PACH
	Genetics	Orthopedics	Genetics and Orthopedics	Total	
Families from register of National Association of Children with OI	1	19	3	23	63
Families not included in register of National Association of Children with OI	18	19	47	84	–

Table 2. Distribution of 52 families according to the number of offspring and the number of affected children. Both parents healthy

Offspring number	Number of families		
	total	one member affected	two members affected
1	21	21	0
2	19	18	1
3	7	7	0
4	3	3	0
6	1	0	1
9	1	1	0
Total	52	50	2

Distribution of the families according to the number of offspring and the number of affected children is shown in the following tables. Table 2 contains data of 52 families with both parents healthy. Table 3 contains the data of 17 families with the father or mother affected by OI.

All the families which responded to the invitation and reported to the Department of Genetics PACH were subjected to the following:

1. Detailed history and pedigree analysis.
2. Physical and radiological examinations (unless done before).
3. Fibroblast culture and biochemical analysis (electrophoresis, measurement of melting temperature of protein) of α1 and α2 type I collagen chains was made in four cases in 1988-1991*.

Table 3. Distribution of 17 families according to the number of offspring and the number of affected children. One of the parents affected

Offspring number	Number of families		
	total	one member affected	two members affected
1	3	3	0
2	10	5	5
3	2	0	2
4	1	0	1

In the vast majority of the cases, the diagnosis and classification were based on the Sillence OI classification (SILLENCE et al. 1979, SILLENCE 1988) (Table 4).

Table 4. Sillence OI classification

Type	Clinical picture	Inheritance
I	Normal stature, slight or no deformity, blue scleras, hearing loss in 50% of families. Dentinogenesis imperfecta rare and may distinguish a subset.	AD
II	Lethal in perinatal period; minimal calvarial mineralization, beaded ribs, compressed femurs, marked long bone deformities, platyspondyly.	AD (new mutation) AR (rare)
III	Progressive bone deformities, usually with moderate deformity at birth. Scleral hue varied, often lightening with age. Dentinogenesis imperfecta common, hearing loss common. Stature very short.	AD AR
IV	Normal scleras, mild to moderate bone deformities and variable short stature. Dentinogenesis imperfecta common; hearing loss in some families.	AD

ADAD – autosomal dominant
AR – autosomal recessive

* The analysis was performed in the Division of Metabolism, Department of Pediatrics, University of Zürich.

Results

Table 5 shows the distribution of the families according to OI type and to the diagnostic criteria.

The definitive diagnosis of OI type was possible in 69.5% cases. Discrimination betwen type I and type IV was impossible in 11 cases, and between type II and type III in 10 cases, despite the biochemical collagen analysis performed in two patients.

Table 5. Distribution of the families according to OI type and to diagnostic criteria

OI type	Number of families (%)	Diagnostic criteria	
		clinical	clinical + biochemical
I	20 (29)	20	–
II	10 (14.5)	8	2
III	8 (11.5)	8	–
IV	10 (14.5)	10	–
II/III	10 (14.5)	8	2
I/IV	11 (16)	11	–

In fifty-two families enrolled in the study, both parents were unaffected. In this group 2 "multiplex" families (with two children affected) and 50 "simplex" families (with one child affected) were identified, among them 21 families with a single child.

The mother or the father were symptomatic in 17 families. One child was affected in 9 families, two children in 8 families.

In total, 50 "simplex" and 19 "multiplex" families were identified in our sample. Out of 19 "multiplex" families, in 10 OI type I was diagnosed, 6 families were affected by OI type I/IV and 3 by OI type IV. The distribution of OI types among the parents was similar (Table 6).

Probands' death was observed in 8 families. Type II was diagnosed in 6 cases, type II/III in 2 cases. Five children died within the first month of life.

Dermatoglyphics were examined in 104 patients with OI (55 females and 49 males). A higher number of patients in comparison to the number of patients in the clinical analysis is related to a longer period of data collection by the Anthropology Clinic, Department of Genetics and to access to additional

sources of information. Dermatoglyphics were analyzed without any correlation to OI types.

Table 6. Distribution of OI types among parents in "multiplex" families

	OI type		
	I	I/IV	IV
Mother	4	2	2
Father	4	4	1

The analysis of dermatoglyphics on finger tips was performed. An increased number of ulnar loops and a decreased number of whorls were observed both in males and females in comparison to the control group. "White lines" on prints were observed in 30.9% of the females and 28.6% of the males. Hypoplasia of dermal ridges was observed in 7.3% females and 8.1% males.

Genetic counselling was offered to families of children with OI. An algorithm of diagnostic management depending on family type ("simplex" or "multiplex") was developed.

Discussion

A large group of patients was enrolled in the study in the last few years. This is the largest population of patients with OI described in the Polish literature, which provides the opportunity to verify diagnostic criteria and to elaborate standards of management in different OI cases.

The patients were diagnosed according to Sillence classification (SILLENCE et al. 1979, SILLENCE 1988). The majority of patients could be classified on the basis of their medical history, physical and radiological examinations. In few cases it was difficult to classify the patient. Often, it was impossible to diagnose OI type during the first visit, and then the definitive diagnosis was established during a long clinical follow-up. Sillence classification is useful in the clinical practice, but in some cases it is not sufficient. The weak point of this classification is the oversimplified description of clinical manifestations of a very complex disease with an extreme molecular heterogeneity and variable inheritance pattern (SILLENCE et al. 1984, TRIPOURAS et al. 1985, STARMAN et al. 1989, COLE, DALGLEISH 1995). Additional diagnostic tests based on biochemical and molecular techniques are required in many cases.

Most of our patients presented symptoms fitting Sillence classification. However, deafness (reported as a common presentation of OI) was not observed in our population. Among 69 families, only two patients revealed a severe hearing impairment.

Variable OI symptoms expression was observed among different members of "multiplex" families. This phenomenon creates problems in genetic counselling, because a patient with mild symptoms carries the same risk of disease transmission as a fully symptomatic patient. Molecular diagnosis can be helpful in these cases.

Abnormalities in dermatoglyphics observed in the study population are of enough interest to continue work in this field. Dermatoglyphics examinations might be performed in "multiplex" families, as well as an analysis of their segregation among the affected and unaffected family members. A linkage analysis of molecular markers and dermatoglyphics patterns should be done in the next step. If such a linkage exists, then dermatoglyphics examination might be used as an initial screening test, which can be helpful, especially in cases with mild symptoms, and can help to establish indications for more accurate, but expensive molecular procedures.

Clinical and molecular OI heterogeneity warrant diagnostic efforts, which allow to establish an early and accurate phenotype and genotype diagnosis. An accurate diagnosis is of crucial importance for planning rehabilitation and potential orthopedic therapy, genetic counselling and appropriate obstetric management during the next pregnancy (LYNCH et al. 1991).

Conclusions

Pedigree analysis is not sufficient to determine the inheritance pattern in every case. Determination of inheritance is easy in a "multiplex" family; usually it is autosomal dominant. In "simplex" families, pedigree analysis is not informative. The disease can be caused by:

- *de novo* mutation,
- germinal mosaicism in one of the parents (COLE, DALDLEIH 1995),
- autosomal dominant inheritance with a milder expression of symptoms in one of the parents,
- autosomal recessive inheritance, when both parents are carriers.

The type of the patient should determine diagnostic measures.

1. "Simplex" family
   a) affected patient – diagnosis should be confirmed by molecular methods.

b) other members of the family – molecular diagnostics should be performed to exclude asymptomatic OI.

2. "Multiplex" family

   a) affected patient – diagnosis is based on history, physical and radiological examinations, and pedigree analysis. Molecular diagnostics can be performed when it is planned in other clinically unaffected family members or when prenatal diagnostics is planned,

   b) asymptomatic family member – molecular diagnostics is recommended to exclude a mutation.

3. OI suspicion - molecular diagnostics is recommended to confirm the diagnosis, further investigations depend on thetype of the disease (sporadic or familial).

4. Prenatal diagnostics with molecular techniques is recommended in the case of pregnancy when the mother or father are carriers.

Acknowledgements. This work was supported by The State Committee for Scientific Research Project No. 4 PO5E 050 09.

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Appendix I

Table 7. General characteristics of the families

Probands number	Symbol of family	Number of affected children		Number of unaffected children						Remarks
		c = 1	c = 2	z = 1	z = 2	z = 3	z = 4	z = 5	z = 8	
1	2	3	4	5	6	7	8	9	10	11
1	B-0	√*		√						
2	B-1	√			√					
3	B-9	√		√						
4	B-10	√		√						
5	B-11		√							father +
6	B-12	√								
7	B-13	√				√				
8	C-4		√		√					mother +
9	D-4	√		√						
10	F-1	√		√						
11	G-1	√		√						
12	G-8	√								
13	H-1	√								
14	H-3	√		√						
15	I-2	√								
16	K-0	√*								
17	K-2	√		√						mother +
18	K-3	√		√						father +

1	2	3	4	5	6	7	8	9	10	11
19	K-17		√							mother +
20	K-24	√		√						
21	K-25	√								
22	K-26	√								
23	K-27	√								mother +
24	L-0	√*								
25	L-6	√								
26	L-7	√								
27	Ł-1		√							father +
28	M-0	√*		√						
29	M-1	√		√						
30	M-2		√							father +
31	M-3	√			√					
32	M-10		√	√						father +
33	M-11	√								father +
34	M-12		√	√						mother +
35	M-13	√*			√					
36	M-14	√			√					
37	N-3	√		√						
38	N-4	√								
39	N-5	√		√						
40	N-6	√			√					
41	O-1	√		√						
42	O-4	√								
43	P-10		√							
44	P-11	√			√					
45	P-12		√							father +
46	R-0	√*			√					
47	R-4	√		√						
48	R-5	√					√			mother +
49	S-0	√*								
50	S-1	√		√						
51	S-2	√								
52	S-10	√							√	
53	S-12	√								
54	S-15	√		√						
55	S-16		√				√			
56	T-2	√		√						mother +
57	T-3	√*		√						

1	2	3	4	5	6	7	8	9	10	11
58	T-4	√				√				
59	T-5	√				√				
60	U-1	√								
61	U-2	√			√					
62	W-1	√								father +
63	W-2	√								
64	W-3	√								
65	W-4	√								
66	W-6	√		√						father +
67	Z-1	√								
68	Z-2	√		√						
69	Z-3	√		√						mother +

+ – affected, * – expired.

Appendix II

Table 8. Families registered in the Department of Genetics, PACH

Patient's symbol	Relationship	OI type	DNA isolation	Fibroblast culture	Remarks
1	2	3	4	5	6
B-0/1	son (proband)	III	–	–	expired
B-0/2	daughter	–	–	–	
B-0/3	mother	–	–	–	
B-0/4	farher	–	–	–	
B-1/1	daughter (probant)	I	+	–	
B-1/2	son	–		–	
B-1/3	son	–	+	–	
B-1/4	mother	–	–	–	
B-1/5	father	–	+	–	
B-9/1	son (probant)	II	–	+	diagnosed in Zürich
B-9/2	son	–	–	–	
B-9/3	mother	–	–	–	
B-9/4	father	–	–	–	
B-10/1	daughter (proband)	III	+	–	
B-10/2	daughter	–	+	–	
B-10/3	mother	–	+	–	
B-10/4	father	–	+	–	
B-11/1	son (proband)	I/IV	+	–	
B-11/2	daughter	I/IV	+	–	
B-11/3	mother	–	+	–	
B-11/4	farher	I/IV	+	–	

1	2	3	4	5	6
B-12/1	son (probant)	IV	–	–	
B-12/2	mother	–	–	–	
B-12/3	father	–	–	–	
B-13/1	daughter (probant)	I	+	+	
B-13/2	daughter	–	–	–	
B-13/3	daughter	–	–	–	
B-13/4	son	–	–	–	
B-13/5	mother	–	+	–	
B-13/6	father	–	–	–	
C-4/1	daughter (probant)	IV	+	–	
C-4/2	daughter	IV	+	–	
C-4/3	son	–	–	–	
C-4/4	son	–	–	–	
C-4/5	mother	IV	+	–	
C-4/6	father	–	–	–	
D-4/1	daughter (probant)	I/IV	–	–	
D-4/2	daughter	–	–	–	
D-4/3	mother	–	–	–	
D-4/4	father	–	–	–	
F-1/1	son (proband)	III	+	+	
F-1/2	daughter	–	–	–	
F-1/3	mother	–	+	–	
F-1/4	farher	–	+	–	
G-1/1	daughter (probant)	IV	+	–	
G-1/2	son	–	–	–	
G-1/3	mother	–	–	–	
G-1/4	father	–	–	–	
G-8/1	daughter (probant)	III	+	–	
G-8/2	mother	–	+	–	
G-8/3	father	–	+	–	
H-1/1	son (probant)	II/III	–	–	
H-1/2	mother	–	–	–	
H-1/3	father	–	–	–	
H-3/1	son (probant)	III	+	–	
H-3/2	son	–	–	–	
H-3/3	mother	–	+	–	
H-3/4	father	–	+	–	
I-2/1	daughter (probant)	II/III	–	+	diagnosed in Zürich
I-2/2	mother	–	–	–	
I-2/3	father	–	–	–	
K-0/1	son (probant)	II	–	–	expired
K-0/2	mother	–	–	–	
K-0/3	father	–	–	–	
K-2/1	daughter (probant)	I	–	–	related to K-3
K-2/2	son	–	–	–	
K-2/3	mother	I	+	–	
K-2/4	father	–	–	–	

1	2	3	4	5	6
K-3/1	daughter (probant)	I	+	–	related to K-2
K-3/2	son	–	–	–	
K-3/3	mother	–	+	–	
K-3/4	father	I	–	–	expired
K-17/1	daughter (probant)	I/IV	+	–	
K-17/2	son	I/IV	+	–	
K-17/3	mother	I/IV	+	–	
K-17/4	father	–	+	–	
K-24/1	son (proband)	I	+	+	
K-24/2	daughter	–	+	–	
K-24/3	mother	–	+	–	
K-24/4	farher	–	+	–	
K-25/1	son (probant)	II/III	–	–	
K-25/2	mother	–	–	–	
K-25/3	father	–	–	–	
K-26/1	daughter (probant)	I	–	–	
K-26/2	mother	–	–	–	
K-26/3	father	–	–	–	
K-27/1	daughter (probant)	IV	+	–	
K-27/2	mother	IV	+	–	
K-27/3	father	–	+	–	
L-0/1	daughter (probant)	II	–	–	expired
L-0/2	mother	–	–	–	
L-0/3	father	–	–	–	
L-6/1	son (probant)	I	+	–	
L-6/2	mother	–	–	–	
L-6/3	father	–	+	–	
L-7/1	son (probant)	III	+	–	
L-7/2	mother	–	+	–	
L-7/3	father	–	+	–	
Ł-1/1	son (proband)	I/IV	+	–	related to T-2
Ł-1/2	daughter	I/IV	+	–	DNA was isolated
Ł-1/3	mother	–	+	–	from 3 relatives
Ł-1/4	farher	I/IV	+	–	
M-0/1	daughter (probant)	II	–	–	expired
M-0/2	son	–	–	–	
M-0/3	mother	–	–	–	
M-0/4	father	–	–	–	
M-1/1	son (probant)	II/III	–	–	
M-1/2	son	–	–	–	
M-1/3	mother	–	–	–	
M-1/4	father	–	–	–	
M-2/1	son (probant)	I	+	–	DNA was isolated
M-2/2	son	I	+	–	from 4 parental brothers
M-2/3	mother	–	+	–	
M-2/4	father	I	+	–	

1	2	3	4	5	6
M-3/1	daughter (probant)	I/IV	–	–	
M-3/2	daughter	–	–	–	
M-3/3	daughter	–	–	–	
M-3/4	mother	–	–	–	
M-3/5	father	–	–	–	
M-10/1	daughter (probant)	I/IV	+	–	
M-10/2	daughter	I/IV	+	–	
M-10/3	son	–	+	–	
M-10/4	mother	–	+	–	
M-10/5	father	I/IV	–	–	
M-11/1	daughter	IV	+	–	
M-11/2	mother	–	+	–	
M-11/3	father	IV	+	–	
M-12/1	daughter (probant)	I	+	–	
M-12/2	daughter	I	+	–	
M-12/3	son	–	+	–	
M-12/4	mother	I	+	–	
M-12/5	father	–	+	–	
M-13/1	son (proband)	II	–	–	expired
M-13/2	daughter	–	–	–	
M-13/3	daughter	–	–	–	
M-13/4	mother	–	–	–	
M-13/5	farher	–	–	–	
M-14/1	son (proband)	I	–	–	
M-14/2	daughter	–	–	–	
M-14/3	daughter	–	–	–	
M-14/4	mother	–	–	–	
M-14/5	farher	–	–	–	
N-3/1	son (proband)	II	–	–	
N-3/2	daughter	–	–	–	
N-3/3	mother	–	–	–	
N-3/4	farher	–	–	–	
N-4/1	daughter (probant)	I	+	–	
N-4/2	mother	–	–	–	
N-4/3	father	–	–	–	
N-5/1	son (proband)	I/IV	+	–	
N-5/2	daughter	–	–	–	
N-5/3	mother	–	–	–	
N-5/4	farher	–	–	–	
N-6/1	son (proband)	III	+	–	
N-6/2	daughter	–	–	–	
N-6/3	daughter	–	–	–	
N-6/4	mother	–	–	–	
N-6/5	farher	–	–	–	
O-1/1	daughter (probant)	II/III	–	–	
O-1/2	son	–	–	–	
O-1/3	mother	–	–	–	
O-1/4	father	–	–	–	

1	2	3	4	5	6
O-4/1	son (probant)	IV	–	–	
O-4/2	mother	–	–	–	
O-4/3	father	–	–	–	
P-10/1	daughter (probant)	I	–	–	
P-10/2	daughter	I	–	–	
P-10/3	mother	–	–	–	
P-10/4	father	–	–	–	
P-11/1	daughter (probant)	III	+	–	
P-11/2	daughter	–	–	–	
P-11/3	son	–	–	–	
P-11/4	mother	–	–	–	
P-11/5	father	–	–	–	
P-12/1	son (proband)	I	+	–	children and father
P-12/2	daughter	I	+	–	additionally have defects
P-12/3	mother	–	+	–	of hair structure
P-12/4	farher	I	+	–	
R-0/1	daughter (probant)	II/III	–	+	
R-0/2	son	–	–	–	
R-0/3	son	–	–	–	diagnosed in Zürich, expired
R-0/4	mother	–	–	–	
R-0/5	father	–	–	–	
R-4/1	daughter (probant)	IV	–	–	
R-4/2	daughter	–	–	–	
R-4/3	mother	–	–	–	
R-4/4	father	–	–	–	
R-5/1	son (proband)	I	–	–	
R-5/2	daughter	–	–	–	
R-5/3	daughter	–	–	–	
R-5/4	son	–	–	–	
R-5/5	son	–	–	–	
R-5/6	mother	I	–	–	
R-5/7	farher	–	–	–	
S-0/1	son (probant)	II	–	–	expired
S-0/2	mother	–	–	–	
S-0/3	father	–	–	–	
S-1/1	daughter (probant)	I/IV	–	–	
S-1/2	son	–	–	–	
S-1/3	mother	–	–	–	
S-1/4	father	–	–	–	
S-2/1	daughter (probant)	I/IV	–	–	
S-2/2	mother	–	–	–	
S-2/3	father	–	–	–	
S-10/1	son (proband)	II	+	–	
S-10/2	daughter	–	–	–	
S-10/3	daughter	–	–	–	
S-10/4	daughter	–	–	–	
S-10/5	daughter	–	–	–	
S-10/6	son	–	–	–	

1	2	3	4	5	6
S-10/7	son	–	–	–	
S-10/8	son	–	–	–	
S-10/9	son	–	–	–	
S-10/10	mother	–	–	–	
S-10/11	farher	–	–	–	
S-12/1	son (probant)	IV	–	–	
S-12/2	mother	–	–	–	
S-12/3	father	–	–	–	
S-15/1	daughter (probant)	I	–	+	
S-15/2	son	–	–	–	
S-15/3	mother	–	–	–	
S-15/4	father	–	–	–	
S-16/1	son (proband)	I	+	–	
S-16/2	son	I	+	–	
S-16/3	daughter	–	–	–	
S-16/4	daughter	–	–	–	
S-16/5	daughter	–	–	–	
S-16/6	daughter	–	–	–	
S-16/7	mother	–	+	–	
S-16/8	farher	–	+	–	
T-2/1	daughter (probant)	I/IV	+	–	related to Ł-1
T-2/2	son	–	–	–	
T-2/3	mother	I/IV	+	–	
T-2/4	father	–	–	–	
T-3/1	daughter (probant)	II	+	–	expired
T-3/2	son	–	–	–	
T-3/3	mother	–	–	–	
T-3/4	father	–	–	–	
T-4/1	son (proband)	IV	–	–	
T-4/2	daughter	–	–	–	
T-4/3	daughter	–	–	–	
T-4/4	son	–	–	–	
T-4/5	mother	–	–	–	
T-4/6	farher	–	–	–	
T-5/1	son (proband)	I	–	+	
T-5/2	daughter	–	–	–	
T-5/3	daughter	–	–	–	
T-5/4	daughter	–	–	–	
T-5/5	mother	–	–	–	
T-5/6	farher	–	–	–	
U-1/1	son (probant)	II	–	+	diagnosed in Zürich
U-1/2	mother	–	–	–	
U-1/3	father	–	–	–	
U-2/1	son (proband)	IV	+	–	
U-2/2	son	–	+	–	
U-2/3	son	–	+	–	
U-2/4	mother	–	+	–	
U-2/5	farher	–	+	–	

1	2	3	4	5	6
W-1/1	daughter (probant)	I/IV	–	–	
W-1/2	mother	–	–	–	
W-1/3	father	I/IV	–	–	
W-2/1	son (probant)	II/III	+	–	
W-2/2	mother	–	+	–	
W-2/3	father	–	+	–	
W-3/1	son (probant)	II/III	+	–	
W-3/2	mother	–	+	–	
W-3/3	father	–	+	–	
W-4/1	daughter (probant)	II/III	–	–	
W-4/2	mother	–	–	–	
W-4/3	father	–	–	–	
W-6/1	son (probant)	I	+	–	
W-6/2	son	–	–	–	
W-6/3	mother	–	+	–	
W-6/4	father	I	–	–	expired
Z-1/1	son (probant)	II/III	–	–	
Z-1/2	mother	–	–	–	
Z-1/3	father	–	–	–	
Z-2/1	daughter (probant)	I	+	–	
Z-2/2	son	–	–	–	
Z-2/3	mother	–	–	–	
Z-2/4	father	–	–	–	
Z-3/1	son (probant)	I	+	–	
Z-3/2	son	–	–	–	
Z-3/3	mother	I	+	–	
Z-3/4	father	–	–	–	