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EDITORIAL

Pseudohemophilia of Erik von Willebrand caused by homozygous one nucleotide deletion in exon 18 of the VW-factor gene

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Abstract

The original description of a novel severe bleeding disorder as "Hereditary Pseudohemophilia" by Erik von Willebrand can currently be labelled as von Willebrand disease (VWD) type 3. VWD type 3 is autosomal recessive caused by homozygous or double heterozygous null mutations in the von Willebrand factor (VWF) gene and typically characterized by prolonged bleeding time and APTT, FⅧ: C levels below 2%, undetectable VWF: Ag, VWF: RCo and VWF: CB and absence of ristocetin induced platelet aggregation (RIPA). Autosomal recessive von Willebrand disease type 3 VWD with virtual complete VWF deficiency are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of mRNA). Reports on severe recessive VWD compound heterozygous for a null allele and a missense mutation and homozygous or double heterozygous for missense mutations are associated with very low but measurable FⅧ and VWF: Ag and should be reclassified as severe recessive type 1 VWD. Homozygous missense or compound missense/null mutations related to recessive severe type 1 VWD have been indentified in the VWF prosequence D1 and D2 domains, the D4, B1-3, C1-2 domains, and only a very few in the dimmerization site (D3 domain). The detection of even tiny amounts of VWF: Ag after desmopressin acetate (DDAVP) or in hidden sites like platelets allows the differentiation between patients with VWD type 3 and homozygous or double heterozygous recessive severe type 1. Carriers of a null allele related to VWD type 3 or a missense mutation related with severe recessive type 1 VWD may present with mild VWD with low penetrance of bleeding in particular when associated with blood group O. Heterozygous obligatory carriers (OC) of a null mutation or a missense mutation related to recessive VWD type 3 or severe type 1 both present with asymptomatic or mild VWD type 1 in particular when associated with blood group O. The response to DDAVP of OC of either a nonsense or a missense mutation appears to be abnormal and diagnostic with a 3-times higher response of FⅧ: C as compared to VWF: Ag. In contrast, the responses to DDAVP of F Ⅷ: C and VWF: Ag are equally good in individuals with low VWF levels related to blood group O and a normal VWF gene and protein (pseudo-VWD). These observations are completely in line with and extend the original observations of von Willebrand in a large family with VWD type 3 and asymptomatic or mild true type 1 VWD in OC.

Key words: Autosomal recessive von Willebrand disease type 3 and 1; Molecular etiology; Carrier of von

von Willebrand disease null or missense allele; Desmopressin acetate responses

Core tip: The novel lethal bleeding disorder described as "Hereditary Pseudohemophilia by von Willebrand (VW) in 1926 is caused by a homozygous nonsense mutation (one nucleotide deletion in exon 18) of the VW-factor gene consistent with autosomal recessive VW disease (VWD) type 3. Heterozygous carriers presented with VWD type 1 with variable penetrance of mild mucocutaneous bleeding manifestations. The present editorial reviews the clinical, laboratory and molecular features of severe recessive type 1 and 3 VWD and obligate heterozygous carriers of VWF nonsense and missense mutations.

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INTRODUCTION

In 1926 Erik von Willebrand first described a novel severe bleeding disorder which he named "Hereditary Pseudohemophilia" in at least 4 affected members of the original large family S, living on the Föglo Aland Island[1-5]. In this report we review the available clinical, laboratory and molecular features of the original family S, which can now be diagnosed as autosomal recessive VWD type 3 caused by a homozygous null mutation (one nucleotide deletion of exon 18) and of mild VWD type 1 with variable penetrance of bleeding manifestations in heterozygous carriers.

CLINICAL FEATURES

The pedigree of family S, originally described by Erik von Willebrand in 1926, has been updated and numbered by Blomback in 1999 (Figure 1)[4].

The proband Hjördis S, case 16, aged 5 years was admitted on April 29 1924 to the hospital Diakonianstalten in Helsinki, Finland. At the age of 1 year, her bleeding tendency was observed after falling and hurting her nose and bled unusually long. At 3 years of age, she fell and had a deep cut in the upper lip. She bled heavily for 3 d and became bloodless and almost unconscious. She had to be confined to bed for 10 wk before recovering. After this she had frequent bruising, and regular episodes of epistaxis and gingival bleeding. An ankle distortion was followed by a severe articular bleeding with intense pain for some weeks (hemarthrosis). When a bleeding time according to the Duke method was performed she continued to bleed for 2 h and this had to be stopped by compression. Erik von Willebrand never visited the Aland Islands himself. Eight years later at the age of 13, Hjördis bled to death during her fourth menstrual period.

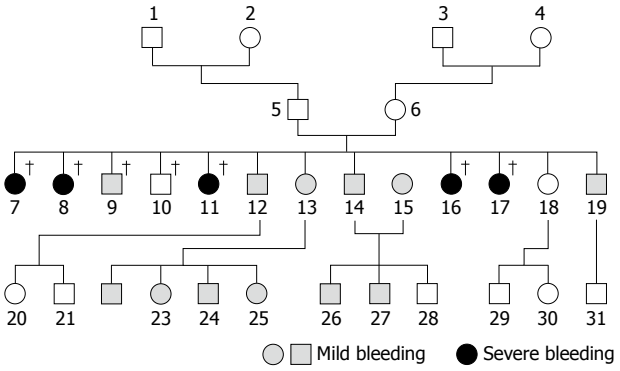

Figure 1 Pedigree of the original family S described by von Willebrand updated by Blombäck and Nilsson in the 1990s.

Her mother, Mrs Augusta, case 6, aged 44 years, had a history of frequent and persistent nose bleedings during her entire youth, but not lately. Menstruation (menarche) begun at age 16 years and had a duration of 6 d or more, always copious, especially lately. She had experienced normal deliveries without unusually severe bleeding. The bleeding time was normal. Augusta, the mother of Hjördis, (case 4, Figure 1), and four of 10 siblings in the family had an increased bleeding tendency.

Her father Mr Oskar S, case 5, aged 48 years, had rather sturdy nose bleedings, when he was young, and did not bruise more easily than other people. Neither of his parent (cases 1 and 2) had been bleeders. One of his sisters and several of her children (family E) had a moderate bleeding tendency.

Her oldest sister, Dagny S, case 7, had her first severe nose bleeding after a slight trauma at the age of 1 year. After this she had several nose bleedings and died from intestinal bleeding at the age of 2 years.

Her sister Anna S, case 8, began to have frequent nose bleeds from the age of 1 year. When she was 4 years old, she fell and two teeth penetrated her tongue. She had a bleeding that could not be stopped and died.

Her sister Dagny S, case 11, had thrush when she was a few weeks old. When her mother tried to loosen the membranes, there was a bleeding that almost would not stop. She bled much after insect bites and died at the age of 2 years from intestinal bleeding.

Her younger sister, case 17, aged 3 years, bruised easily since the age of 1 year and experienced heavy nose bleeds for the first time at age 1.5 years. Thereafter nose bleeds fairly often recurred. The bleeding often started spontaneously, and once went on for a whole week. The bleeding time was considerable prolonged. She had prolonged bleedings after insect bites. At the age of 5 she developed influenza, started to vomit blood and died within 20 h.

Her younger brother, case 19 (proven heterozygous for del 18 in 1990s), suffered in his youth from a slightly increased bleeding tendency, although he did not report

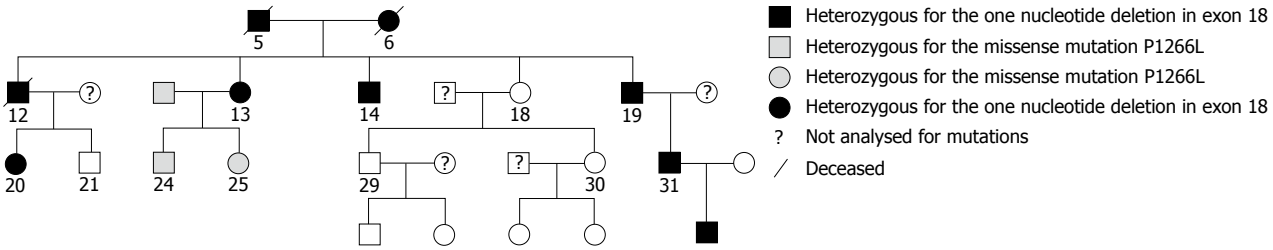

Figure 2 Results of molecular investigations in the original family S in the 1990s reveal a heterozygous nonsense mutation in seven affected members with mild bleeding diathesis[4].

Table 1 FⅧ: C and von Willebrand factor levels in family S together with finding of heterozygous non-sense mutation, deletion exon 18, consistent with mild von Willebrand disease type 1

Family S, Number	Mutation	FⅧ: C IU/dL	VWF: Ag IU/dL	VWF: RCo IU/dL	RCo/Ag Ratio
5		0.93			
6		0.51			
12	del 18	0.81	0.80	0.73	0.91
13	del 18	0.53	0.56	0.46	0.82
14	del 18	0.35	0.35	0.32	0.91
18	negative	0.96	0.86	0.65	0.76
19	del 18	1.10	0.67	0.81	1.20
22	unknown	0.50	0.38	0.25	0.66
23	unknown	0.73	0.39	0.39	1.00
24	P1266L	0.39	0.34	0.42	1.23
25	del 18/P1266L	0.69	0.20	0.30	1.5

VWF: von Willebrand factor; VWD: von Willebrand disease; FⅧ: Factor Ⅷ.

any problems in 1992.

LABORATORY FEATURES

The inheritance of the disease in Family S could be followed through four generations[1-5]. In the female bleeders, the bleeding diathesis is manifested in a mild form or in a severe form, whereas the males show only the mild form of bleeding diathesis (Figure 1). Among female affected members with severe bleeding five deaths in one generation had occurred, four in childhood and one shortly after menarche (Figure 1). The women are of two types, those with a single and those with double trait. The former (heterozygotes) may had a milder form of bleeding, the latter (homozygotes) a severe lethal form. There was no opportunity to study the so-called homozygote women because they died from fatal bleeding before the reproductive age and long before FⅧ and the von Willebrand factor were identified as causes of hemophilia and VWD more than 3 decades later in the late 1950s. This means that data on the level of F Ⅷ: C and VWF parameters are lacking in those deceased women with the double trait (Table 1). This may explain why Jurgens *et al*[6] have falsely interpreted the bleeding disorder as a constitutional thrombopathy mainly based on the very prolonged bleeding times. Jurgens *et al*[7] mentioned several years later a deficiency of an antihemophilic factor (FⅧ) in heterozygous affected family members with mild VWD, but the cases with severe bleeding could not studied anymore at that time.

Studies by Nilsson *et al*[5] showed decreased values for VWF: Ag and VWF: RCo and normal ratios for VWF: RCo/VWF: Ag in the affected heterozygous members of family S with mild VWD type 1 (Table 1) leaving the molecular etiology of the "homozygotes" elusive at that time.

MOLECULAR STUDIES

The DNA samples were screened for mutations with PCR, followed by direct sequencing in the "hot spot" regions in exons 18, 28, 32, 43 and 45 found in the Swedish-Finnish patients[8,9]. In the original family S one nucleotide deletion in exon 18 was identified in heterozygous carriers, which had been found to be a "hot spot" in the majority of VWD type 3 patients in Sweden[9,10]. This mutation interrupts the reading frame leading to an early translational stop (null allele). Five individuals (numbers 13, 14, 19, 20 and 25 in Figure 2 and Table 1) all having VWD type 1 were found to be heterozygous for the deletion. The deceased family member 12 must also have carried the same deletion, as his daughter is a carrier. In the third generation, at least four individuals (numbers 12, 13, 14, 19, Figure 2) carry the deletion. These results indicated that the deletion originated from the parents of family S (number 5 and 6, Figures 1 and 2) who are thought to be heterozygous. All five daughters, who died from uncontrolled bleedings, very likely would have been homozygous for the deletion in exon 18 consistent with pseudohemophilia, now called VWD type 3.

Table 2 Reports of autosomal recessive severe type 1 von Willebrand disease caused by homozygous missense or double heterozygous missense/null mutations in the D1 or D2 domain

Mutation	F/M, (yr)	BT (min)	Ⅷ: C U/dL	VWF: Ag U/dL	VWF: RCo U/dL	Domain/VWF	VWD type
D141Y/null[19]	F/63	> 30	0.03	< 1	< 1	D1	severe 1
C275S/null[19]	F/26	> 30	0.03	< 1	< 1	D1	severe 1
R273W/R273W[20]	Boy	15	0.20	0.06	0.06	D1	severe 1
R273W/R273W	Boy	15	0.33	0.09	0.04	D1	severe 1
R273W/R273W	Boy	> 20	0.09	< 0.01	< 0.01	D1	severe 1
W377C/W377C[12]	Child	> 20	0.02	0.03	0.03	D1	no data
C570S/C570S[21]	Boy	↑↑	0.12	0.05	0.05	D2	severe 1
Q77X/splice site Intron[24]		> 30	0.20-0.31	0.04-0.06	< 0.03 0.06	D1/D2	severe 1

VWF: von Willebrand factor; VWD: von Willebrand disease.

The three siblings, Harald, Sylvia and Runar, (cases 12, 13 and 14, proven heterozygous for del 18) had more less severe nose bleeds, especially in their youth but never experienced a pronounced bleeding tendency during life-long follow-up. These data demonstrate that the original family S, described by Erik von Willebrand as pseudohemophilia A, has to be diagnosed as autosomal recessive VWD type 3 caused by a homozygous null mutation (one nuclotide deletion of exon 18). In the family S in addition to the deletion in exon 18, two mutations at S1263 and P1266 in exon 28 were identified in two siblings (numbers 24 and 25, Table 1, Figure 2) with an unrelated and clinically normal father, who married into the family S. The transition G→A at S1263 is neutral, and the other C→T at P1266L results in an amino acid substitution of proline to leucin (P1266L). P1266L is a frequent mutation in Sweden and has been described as VWD type 1 Malmö with increased ristocetin induced platelet aggregation (RIPA) (mild 2B).

DIAGNOSIS AND MOLECULAR BIOLOGY OF RECESSIVE TYPE 3 VWD

The inheritance of a pronounced bleeding tendency in subsequent reports of families with VWD type 3 is autosomal recessive[11-15]. Patients with VWD type 3 are typically characterized by prolonged bleeding time (BT) and APTT, FⅧ: C levels below 2%, undetectable VWF: Ag, VWF: RCo and VWF: CB levels before and after desmopressin acetate (DDAVP) and absence of RIPA[15]. VWD type 3 patients with FⅧ: C levels above 2% and detectable levels of VWF: Ag and response of FⅧ: C to DDAVP should be reclassified as severe recessive type 1 VWD caused by double heterozygous for a nonsense/missense mutation or homozygous or double heterozygous missense mutation causing a severe secretion defect[12,13].

In 31 cases diagnosed as VWD type 3, (age 2 to 80, median 15 years) described by Schneppenheim *et al*[12] bleeding manifestations were recorded as easy bruising and prolonged epistaxis in 31 (100%), spontaneous joint bleeding in 23 (76%), muscle bleeding in 7 (22%) and gastrointestinal bleedings in 3 (10%). The bleeding manifestations and complications of childbirth have been nicely evaluated in 385 Iranian patients diagnosed as autosomal recessive type 3 VWD (SSC-ISTH classification) and compared to age matched severe hemophilia A[14]. Among patients with type 3 VWD the incidences of spontaneous hemarthrosis (37%) and muscle bleedings (52%) are lower most likely because FⅧ: C levels are higher (1%-9%) as compared to severe hemophilia A (≤ 1%).

Type 3 VWD with virtual complete VWF deficiency (severe VWD) and absence of FⅧ: C (pseudohemophilia) are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of mRNA) in the majority and rarely compound heterozygous for a null allele and a missense mutation or homozygous for a missense mutation[11-18]. The null alleles are located all over the VWF gene in nearly all exons 3-52[18]. The data base of the SSC of the ISTH reports 58 null alleles and 14 missense alleles involved in the etiology of type 3 VWD[18]. Missense mutations related to severe recessive VWD type 1 are mainly located in the D1-D2 domains (D47H, S85P, Y87S, D141Y, D141N, C275S, W377C, I427N, and in the D4, B1-3, C1-2, CK domains (P2063S, C2174G, C2362F, N2546Y, C2671Y, C2754W, and C2804Y), but not in the D3, A1 and A2 domains except one (C1071F/null)[18]. Consequently, some so-called type 3 VWD patients, who are compound heterozygous for a null allele and a missense mutation and may have detectable but very low VWF levels, are incorrectly diagnosed as VWD type 3 and should be reclassified as recessive severe type 1 VWD[19-27].

DIAGNOSIS AND MOLECULAR BIOLOGY OF RECESSIVE SEVERE TYPE 1 VWD

A considerable number of missense mutations related to autosomal recessive severe type 1 VWD have been identified in the VWF prosequence (D1 and D2 domains),) and the D4, B1-3, C1-2 and CK (dimerization) domains), but only a very few in the dimerization site (D3 domain (Tables 2 and 3)[19-26]. There are two reports on double heterozygous missense/null mutation D141Y/

Table 3 Laboratory features of recessive severe type 1 due to a double heterozygous missense mutation in the CK domain of the von Willebrand factor gene

Mutation	Age (yr)	Gender	BT	FⅧ: C	VWF: Ag	VWF: RCo	VWF: RCo/Ag	RIPA	VWD type
C2754W/C2754W[31]	13	F	> 20	0.12	< 0.05	< 0.05	-	nt	3
Father C2754W[31]	-	M	5	0.54	0.33	0.38	1.15	nt	Mild 1
Mother C2754W[31]	-	F	5	0.55	0.38	0.43	1.13	nt	Mild 1

VWF: von Willebrand factor; VWD: von Willebrand disease; M: Male; F: Female; FⅧ: Factor Ⅷ; RIPA: Ristocetin induced platelet aggregation.

null and C275S/null associated with VWD severe type 1 and not type 3 with documented hemarthos in one of them (Table 2)[19]. Expression studies the missense mutation D141Y and C275S showed a severe secretion defect of mainly dimers while higher molecular weight bands like tetramers and hexamers were barely detectable[26]. Homozygotes for the missense mutations W377C[12] and for R273W[20] in the propeptide D1 domain have been described to be associated with autosomal recessive severe type 1 (not type 3) VWD phenotype (Table 2). Homozygous missense mutation C570S in the D2 domain has been described as the cause of recessive severe type 1 VWD laboratory phenotype mimicking a type 2C (ⅡC) VWF multimers[21].

The multimeric pattern of homozygous R273W and C570S clearly showed the absence of high molecular weight multimers and a pronounced monomeric band mimicking type 2C (ⅡC) subtype VWD[20]. Expression studies of recombinant R273W, W377C and C570S showed a severe secretion defect mainly consisting of dimers and failed to form intermediate and high molecular weigth multimers[19-21]. These findings demonstrate that mutations in the D1 and D2 VWFpp domain completely abolishes multimerization of VWF. Heterozygous asymptomatic carriers of such missense mutation are asymptomatic or diagnosed as mild type 1 VWD with borderline values of VWF parameters around 0.50 U/dL. Heterozygotes for a missense mutation in the D1 or D2 domain typically show a pronounced VWF dimer band.

Homozygous missense mutation C2364F in the B1-3 domain and double heterozygous C2364F/null has been reported to be associated with severe type 1 VWD featured by FⅧ: C levels of 12 to 32 U/L, very low but detectable VWF: Ag and undetectable VWF: RCo[22-26]. C2364F heterozygous carriers were asymptomatic, had normal or slightly prolonged BT, subnormal values for VWG: Ag and VWF: RCo with a normal VWF: RCo/Ag ratio, and a normal VWF multimeric pattern in a low 0.8% or 0.9% agarose resolution gel (asymptomatic "dominant" VWD type 1)[22]. However, analysis of VWF in plasma from cases with severe autosomal recessive VWD homozygous for a missense mutation C2362F or compound C2362F/null (exon 42 of the B1-3 domain) as well as heterozygous carrier of C2364F all showed a heightened proteolytic pattern with marked increase of 176 and 140 kDa degradation products mimicking type 2A (ⅡA) VWD[26]. Other causes of severe autosomal recessive type 1 VWD include homozygous C2364Y (B1-3 domain) or double heterozygous C2364Y/intron 13 splice site[23], homozygous C2671Y (exon 49) or double heterozygous missense mutation C2671Y/del (exon 49) of the VWF gene[27]. DDAVP in recessive VWD severe type 1 induces a poor for VWF: Ag and VWF: RCo but significant increase of FⅧ: C levels. In some cases of autosomal recessive severe type 1 VWD patients FⅧ: C, VWF: Ag and VWF: RCo reached values of > 0.50, 0.11 and 0.09 U/L respectively after DDAVP[22].

RECESSIVE SEVERE TYPE 1 VWD DUE TO MUTATIONS IN THE CK DOMAIN

The replacement of cysteine residues in the CK dimerization domain of the VWF gene causes two completely different laboratory phenotypes of VWD either severe VWD type 3 or VWD type 2D (ⅡD)[28-36]. Homozygous or double heterozygous loss of cysteines mutations C2739Y, C2754W, C2804 and C2806 results in severe autosomal recessive type 1 VWD with nearly complete absence of VWF[29,35]. Homozygous C2754W mutation is associated with VWD severe type 1 and a mild type 1 VWD in heterozygous carriers (Table 3). Expression studies of C2754W show intracellular production of mainly monomers and dimmers (indicating a dimerization defect) with no secretion of mutant VWF indicating that homozygous C2754W mutation indeed will lead to severe type 1 or 3 VWD[35].

Experimental and clinical data are in line with the concept that loss of a single disulfide band in the CK domain of VWF leads to a recessive quantitative VWF deficiency with very low VWF: Ag (VWD type 3) if an intrachain disulfide band is involved (C2739Y or C2754W), and to a dominant-negative qualitative defect of VWF with abnormal multimers if an interchain-disulfide bond is involved, which leads to the characteristic type dominant type VWD type 2D (ⅡD) multimeric pattern (Figure 3)[35,36].

RECESSIVE TYPE 1 VWD DUE TO A HOMOZYGOUS MISSENSE MUTATION IN THE D2 DOMAIN

The 1534C > A mutation in the consensus splicing site of intron 13 (D2 domain) induces exon 14 skipping with the introduction of a premature termination after codon 586, resulting in a truncated VWF[37]. Moreover, the 1534C > A mutation induces the activation of a cryptic

Patients	Blood group	[1]BT min	aPPT s	[2]RIPA	FⅧ U/dL	VWF: Ag U/dL	VWF: RCo U/dL	VWF: CB U/dL	Plat. VWF: Ag U/dL	Mutation
Ⅰ-1 (father)	O	7	34.3	89%	120	101.2	70	82.9	48.1	1543-3C > A/N
Ⅰ-2 (mother)	O	-	31.2	87.5%	123	64.6	44	56.4	56.4	1543-3C > A/N
Ⅱ-1 (proband)	O	20	37.8	5.4%	51	14.5	12.5	9.8	9	1543-3C > A/1543-3C > A
Ⅱ-2 (sister)	O	6	34.2	85.6%	146	76.5	77	86.7	56.8	1543-3C > A/N
Normal range		2-9	30-40	60%-84%	60-160	60-160	60-130	65-150	70-140	

[1]BT (bleeding time) was performed using Ivy method; [2]RIPA was performed with 1.2 mg/mL ristocetin; RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; aPPT: activated partial thromboplastin time.

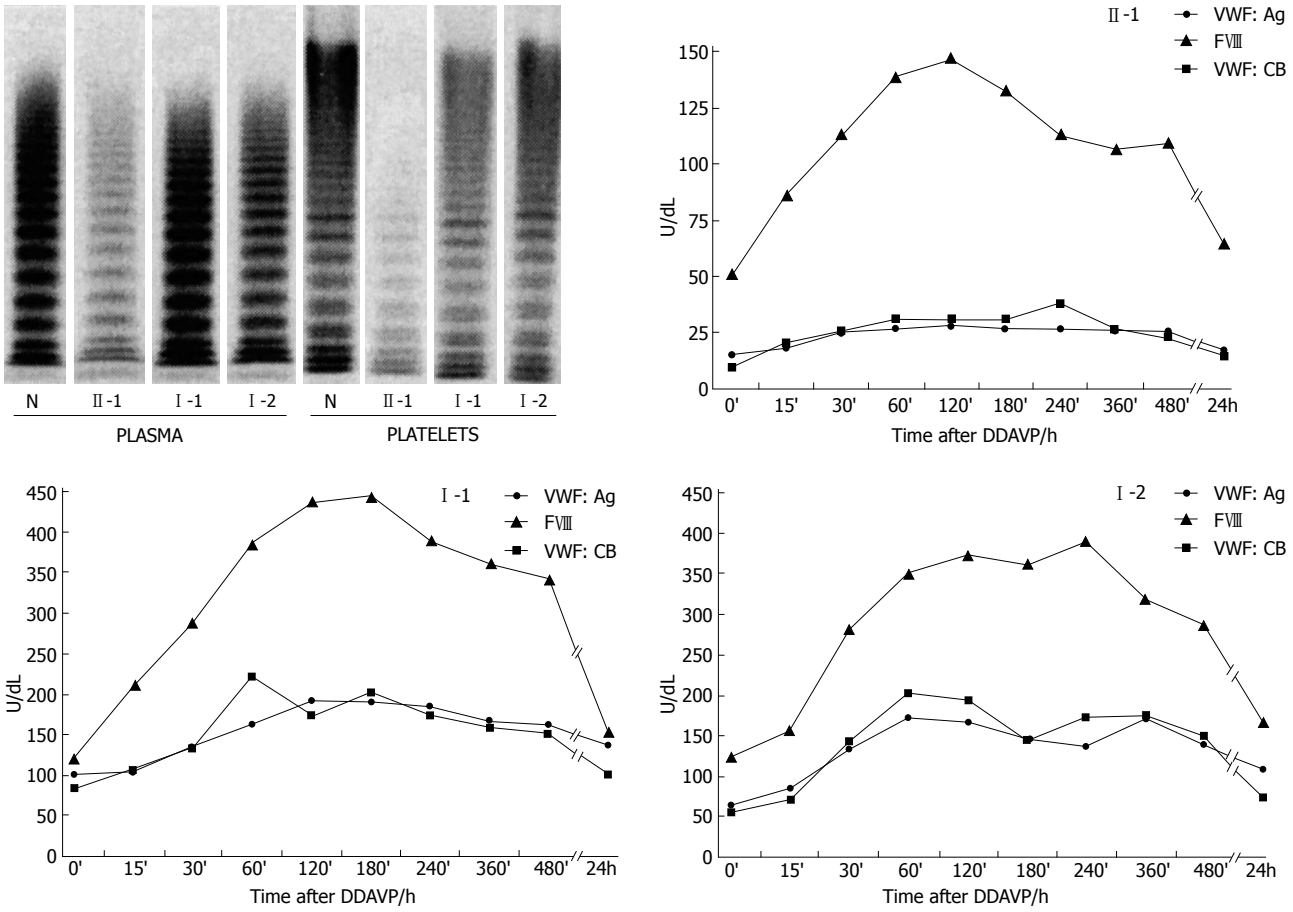

Figure 3 State of the Art characterization of a family with autosomal recessive von Willebrand disease type 1 by Casonato and co-workers using a complete set of laboratory assays related to von Willebrand disease diagnosis, von Willebrand factor multimeric analysis in plasma and platelets and the response of FⅧ, VWF: Ag and VWF: RCo to desmopressin acetate before and at several time points after desmopressin acetate according to standardized recommendations anno 2006. RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; VWD: von Willebrand disease; DDAVP: Desmopressin acetate.

splicing site, 62 nucleotides upstream from the normal site[37]. The spliceosome produces a normal transcript of normal VWF. Gallinaro described a family with autosomal recessive type 1 VWD caused by homozygous intron 13 splicing site mutation 1534-3C > A (Figure 3)[17]. The proband, a 34-year old man had a history of severe epistaxis requiring blood transfusion and recurrent mild epistaxis and gingivorrhagia not requiring medical attention. The proband had VWF values between 10 to 15 IU/dL with normal ratios of VWF: RCo/VWF: Ag (0.86), decreased VWF: CB/VWF: Ag ratio (0.6), and increased ratio of FⅧ/VWF: Ag (3.5) indicating a secretion defect. (dot) The plasma VWF multimeric pattern showed a homogenous decrease in all oligomers with a subtly loss of large VWF multimers and a more pronounced loss of platelet VWF multimers (VWD type 1 plasma low/platelet low). The response to DDAVP was restricted for VWF parameters but good for FⅧ: C thereby confirming a severe secretion defect. After DDAVP VWF: Ag increased from 15 to 28 IU/dL (1.9x) and FⅧ from 51 to 146 IU/dL (2.9x) followed by normal half life times for VWF and FⅧ (Figure 3). The father (Ⅰ-1) and sister (Ⅱ-2) heterozygous for the 1534-3C > A mutation never bled whereas the heterozygous mother suffered from mild menorrhagia, hematomas and bleeding after delivery. The plasma VWF values of affected family members were in the low normal range and relatively decreased as compared to FⅧ (Figure 3). Interestingly the response of FⅧ: C to DDAVP was rather good but severely restricted for all VWF parameters as compared the completely normal increase of FⅧ to values above 3.0 IU/dL consistent with carrier state of a missense mutation related to a secretion defect (Figure 3)[37].

Table 4 Laboratory phenotype and clinical symptoms in 69 patients with true von Willebrand factor deficiency type 1 heterozygous for the von Willebrand factor null allele (parents of type Ⅲ von Willebrand disease)

Author		Zhang *et al*[13]		Eikenboom *et al*[15]		
Number of patients		25	17	14		6
Blood Group		A	O	A		O
FⅧ: C (%)	Mean	81	74	93		81
	Range	37-121	11-128	69-138	58-93	
VWF: Ag	Mean	45	32	61		
	Range	13-94	12-70	37-98	40-66	52
VWF: RCo	Mean	-	-	56		53
	Range	-	-	30-92	39-68	
Mild bleedings[1]		13	11	1		1
		52%	65%	7%		17%

[1]Mild bleeding was defined by one or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruations without abnormal bleeding after both extraction or surgy, hemarthros or muscle bleeding. VWF: von Willebrand factor.

Table 5 Von Willebrand factor antigen (VWF: Ag) levels in heterozygous carriers for a null allele related to pseudohemophilia A-von Willebrand disease type 3 and for the mutation C2364F related to severe recessive type 1 von Willebrand disease

Carriers	Number of patients	VWF: Ag mean ± SD (IU/dL)	VWF: Ag range (IU/dL)
Null allele:			
Blood group O	15	43.2 ± 10.8	30-66
Blood group non-O	15	61.3 ± 23.6	25-98
C2364F:			
Blood group O	8	35.2 ± 16.2	25-55
Blood group non-O	15	61.5 ± 26.6	30-140

VWF: von Willebrand factor; VWD: von Willebrand disease.

DETECTION OF SYMPTOMATIC AND ASYMPTOMATIC OBLIGATORY CARRIERS OF RECESSIVE VWD TYPE 3 AND SEVERE TYPE 1

The only objective and correct way to characterise true type 1 VWF deficiency heterozygous for the VWF null allele or missense mutation is to analyse the bleeding manifestations and FⅧ: C/VWF parameters in obligate heterozygous parents of recessive type 3 or severe type 1 VWF patients. In the study of 27 patients with congenital type 1 VWF deficiency associated with one null allele analysed by Schneppenheim *et al*[12], 20 were asymptomatic and only 7 presented very mild bleeding, mainly bruising and epistaxis. All except one, had a normal BT. The mean values for FⅧ: C, VWF: Ag, and VWF: RCo were 0.76, 0.39 and 0.39 U/mL respectively with an increased FⅧ: C/VWF: Ag ratio of 1.9 and a normal VWF: RCo/Ag ratio of 1 consistent with true type 1 VWF deficiency. In the study of Zhang *et al*[13] including 25 patients heterozygous for the VWF null allele and blood group non-O, 12 had no history of bleeding and 13 presented with very mild bleeding (one or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruations with no abnormal bleeding after tooth extraction). The mean values for FⅧ: C and VWF: Ag were 0.81 and 0.45 respectively with an increased ratio for FⅧ: C/VWF: Ag of 1.8 (Table 4). In the same study of Zhang *et al*[13] out of 17 patients heterozygous for the VWF null allele but having blood group O, 8 had no bleeding history and 11 presented minor bleedings (65%, Table 4). The mean values for FⅧ: C and VWF: Ag were 0.74 and 0.32 respectively with an increased ratio for FⅧ: C/VWF: Ag of 2.3 (Table 2). In the study of Eikenboom the values VWF: Ag and VWF: RCo in carriers of a null allele ranged from 0.30 to 0.98 IU/mL (Table 4)[15]. In these studies[12,13,15], there is a wide range of values from 0.11 to 1.28 U/mL for FⅧ: C, from 12 to 0.94 for VWF: Ag, with ratios of FⅧ: C/VWF: Ag ranging from normal to increased above 2 indicating the difficulty to distinguish true congenital type 1 VWF deficiency from VWF deficiency related to blood group O.

Using the recently developed sensitive bleeding score assessment, Castaman *et al*[38] compared the severity of bleeding symptoms in 70 OC of recessive type 3 VWD, 42 OC of recessive type 1 VWD and in 215 normal controls. OC of VWD type 3 with a null mutation had clearly less severe bleeding than patients diagnosed as type 1 VWD. OC of type 1 VWD with a missense mutation were distinct from normal controls, presenting more epistaxis, cutaneous bleeding and usually did not significantly bleed after surgery, further pointing to the wide heterogeneity of VWD as a heterozygous congenital disorder of the VWF gene mutations (Table 5).

Obligatory carriers (OC) of a nonsense mutation related to VWD type 3 and OC of missense mutation related to severe recessive VWD type 1 in the population are asymptomatic or manifest mild bleeding, and have VWF levels at 50% of normal (true type 1 VWD according to the law of Mendel). Such OC of a null allele or missense mutation may become more symptomatic when associated with blood group O or another modifier of the VWF level. Castaman and Eikenboom demonstrated that ABO blood group significantly influences the VWF: Ag levels in OC of a null allele related to VWD type 3 or the missense mutation C2364F related to severe recessive VWD type 1 (Table 5)[39]. From a genotypic point of view, OC of a null allele in type 3 VWD are very similar to asymptomatic or mild type 1 VWD patients with a single missense allele.

Based on careful analysis of reports on recessive VWD we proposed in 2006 the Antwerp Classification of recessive VWD type 3, recessive severe type 1 VWD, and true type 1 VWD heterozygous for a null allele or missense mutation with variable penetrance of bleeding manifestations but symptomatic when associated with blood group O (Table 6)[40]. In subsequent studies the variable penetrance of bleeding manifestations mild VWD type 1 is clearly related to blood group O[41,42].

Table 6 The 2006 Antwerp Classification of recessive von Willebrand disease type 3, recessive severe on Willebrand disease type 1 and obligatory carriers of a null or missense allele with asymptomatic or mild on Willebrand disease type 1 and variable penetrance of bleeding tendency

Category VWD	BT	FVIII: C (%)	VWF (%) Ag	RCo	RIPA	Bleeding type	VWF gene mutation
Severe type 3 Recessive	↑↑↑	1-9	zero	zero	zero	Severe Hemophilia	Double Nonsense
Severe type 1 Recessive VWD	↑↑↑	9-40	1-10	0-6	zero	Moderate Severe	Double Missense
Blood group O (30-32) (Pseude-VWD)	N	35-150	35-150	35-150	N	Asymp Very mild	None
Carrier type 3 Minor influence (-10%) of bloodgroup O	N↑	30-140	15-90	15-90	N	Asymp Very mild	Single Non-sense (null allele)
Carrier type 1 (polymorphism)	N	N	N	N	N	Asymp	Single Missense
Mild type 1 Recessive or variable penetrance and multigenetic backgroud	N↑	20-80	20-50	20-50	N	Mild	Mis/Non-sense or Y1584C/ Bloodgroup O [19,50]
Dominant type 1 Secretion defect	N↑ ↑/↑↑	20-80 5-20	10-40 5-20	0-30 5-20	N	Mild Moderate	Single Missense
Dominant type 1 Vicenza	N/↑	< 15	< 15	< 15		Moderate	Single^{R120SH} Missense

VWF: von Willebrand factor; VWD: von Willebrand disease; RIPA: Ristocetin induced platelet aggregation.

Table 7 Response of FVIII: C and von Willebrand factor parameters to DDAVP (0.3 ug/kg) in an obligatory carriers of a null allele heterozygous for the nonsense splice site mutation IV7 + 1G > A in intron 7. (0874 + 1G > A) in intron 7

DDAVP	before	1	2	4	6	H post-DDAVP
	after					
FVIII: C	0.84	5	5.4	5.3	4.9	IU/mL
VWF: Ag	0.64	1.3	1.7	1.5	1.4	IU/mL
VWF: RCo	0.67	1.8	2	1.35	1.2	IU/mL
FVIII: C/VWF: Ag ratio	1.3	3.8	3.1	3.5	3.5	carrier of null allele
VWF: RCo/Ag ratio	1.05	1.38	1.17	0.9	0.86	mild type 1 VWD

VWF: von Willebrand factor; VWD: von Willebrand disease; DDAVP: Desmopressin acetate.

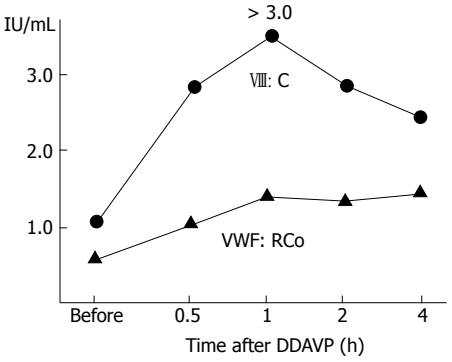

Figure 4 Good response of FVIII: C and restricted response of von Willebrand factor: RCo to desmopressin acetate (0.3 ug/kg) in a carrier of a null allele (Q2470X/normal). VWF: von Willebrand factor; DDAVP: Desmopressin acetate.

Similarly, the C1584 variant of mild VWD type 1 is associated with a slight decrease of VWF and FVIII: C levels, especially in combination with bloodgroup O[43,44].

We studied a consanguineous family with type 3 VWD. The propositus was a boy with VWD type 3, who presented with mucocutaneous bleeding and recurrent hemarthrosis of an ankle. Laboratory analysis found F VIII: C < 1% and an absence of VWF: Ag due to the homozygous nonsense splice site mutation IV7 + 1 G>A in intron 7 (0874 + 1G > A)[45]. Both parents were heterozygous for the nonsense mutation and completely asymptomatic with near normal to normal values for F VIII: C, VWF parameters and normal ratios for FVIII: C/VWF: Ag (Table 7)[45]. After DDAVP, the FVIII: C levels rose to much higher levels as compared to VWF: Ag, VWF: RCo and VWF: CB levels. FVIII: C/VWF: Ag ratios were 1.3 before DDAVP but more than 3 after DDAVP consistent with a carrier of a VWF null allele (Table 7). The VWF: RCo/Ag ratio was normal before and after DDAVP consistent with true congenital type 1 VWD disease (Table 7). This demonstrates that an increased ratio FVIII: C/VWF: Ag ratio after DDAVP is typically and diagnostic for true VWF deficiency type 1 heterozygous for a null allele. This important diagnostic clue t true congenital type 1 VWD has also been demonstrated by Lethagen *et al*[46] in a carrier of a null allele (Q2470X/normal, Figure 4).

From this analysis of the literature and personal experiences in VWD we conclude that heterozygous carriers of a null mutation related to VWD type 3 and a missense mutation related to recessive VWD severe type 1 both do present with asymptomatic or mild VWD type 1 in particular when associated with blood group O. The response to DDAVP of OC of either a nonsense or a missense mutation related to VWD type 3 or severe type 1 appears to be abnormal and diagnostic with a 3-times

higher response of FⅧ: C as compared to VWF: Ag. In contrast, the responses to DDAVP of FⅧ: C and VWF: Ag are equally good in individuals with low VWF levels related to blood group O and a normal VWF gene and protein (pseudo-VWD). These observations are completely in line with and extend the original observations of Erik von Willebrand in a large family with VWD type 3 and asymptomatic or mild true type 1 VWD in OC.

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BRIEF ARTICLE

Blood groups, hemoglobin phenotypes and clinical disorders of consanguineous Yansi population

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Abstract

AIM: To study frequency of blood groups, prevalence of sickle-cell anemia trait and glucose-6-phosphate dehydrogenase deficiency (G6PD), among consanguineous Yansi tribe.

METHODS: A total of 525 blood samples were collected, of which 256 among the Yansi population, and 269 for the unrelated control group in the Bandundu province of Democratic Republic of Congo. Blood group antigens were determined in the following systems: ABO, Rh, Kell, Duffy, Kidd and MNS. Blood grouping and extended phenotype tests were performed according to standard immunohematological procedures. Spot tests and tandem mass spectrometry were used respectively for the assessment of G6PD and sickle-cell anemia trait.

RESULTS: The frequency of ABO phenotypes conformed to the following order O>A>B>AB with notably 62.5%, 23.8%, 12.1% and 1.6% for the Yansi, and 54.6%, 27.5%, 14.1% and 3.7% for the unrelated control group, respectively (P = 0.19). As for the Rh phenotypes, the most frequent were ccD.ee, ccD.Ee, CcD.ee, corresponding to 71.5%, 12.1% and 12.1% for the Yansi, and 70.6%, 15.6% and 8.2%, for the unrelated control group (P = 0.27). The frequency of MN and Ss phenotypes were statistically different between groups (P = 0.0021 and P = 0.0006). G6PD was observed in 11.3% of subjects in the Yansi group, and in 12.4% of controls (P = 0.74). The sickle-cell anemia trait was present in 22.4% of Yansi subjects and 17.8% in the control group (P = 0.24). Miscarriages and deaths in young age were more common among Yansi people.

CONCLUSION: This study shows a significant difference in MNS blood group distribution between the Yansi tribe and a control population. The distribution of other blood groups and the prevalence of hemoglobinopathies did not differ in the Yansi tribe.

Key words: Blood group antigens; Blood group phenotype; Glucose-6-phosphate dehydrogenase deficiency; Sickle-cell anemia; Hemoglobin electrophoresis; Clinical disorders; Consanguinity; Yansi

Core tip: Assessment of blood group frequencies is important to evaluate the risk of alloimmunization after transfusion or pregnancy. Few documented studies have been published about the frequency of blood groups and extended phenotypes in the Congolese people in general and the consanguineous Yansi tribe in particular. This is also the case for the prevalence of glucose-6-phosphate dehydrogenase deficiency and sickle-cell trait. We show that the distribution of MNS blood groups is different in the Yansi tribe, compared to the general population. The Yansi also present with a higher frequency of medical disorders. This study may help in sensitizing the Congolese population about the medical risks associated with consanguineous unions and in building up a database of genetic diseases in the population.

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INTRODUCTION

The antigens of the ABO system were the first to be discovered among the blood group antigens and were known as the first human genetic markers[1]. Nowadays, there exist 308 antigens of red blood cells described in 30 systems, 12 collections, a 700 series, and a 901 series[2,3]. Apart from the ABO and Rh, there are many other systems of transfusion significance, such as Kell, Duffy, Kidd and MNS[4].

The blood groups antigens are most important in transfusion, pregnancy and transplantation because of their immunogenic capacity[5], and also in human genetics[6]. The frequency of blood groups antigens differs between populations[7].

A "genetic" population or a "mendelian" population is influenced by the way the procreating couples are constituted. It is characterized by a genetic pool and by cultural rules that govern the formation of those couples (rule of belonging to a community)[8]. The genes coding for the blood groups' markers are, like all genes, inherited from parents and their phenotypic expression is subjected to hereditary laws[9].

In the Yansi tribe from the Bandundu province in the Democratic Republic of Congo, the constitution of couples lies on two types of marriage: the ordinary marriage and the preferential or consanguineous marriage of "kitiul" type. Very few documented studies have been performed about the constitution of the preferential marriage among Yansi people. Mfukala and Hochegger mention nearly 60% of preferential marriages in which the grandfather marries his granddaughter to the son of his brother's daughter[10,11].

Consanguinity increases the probability that both spouses become heterozygote for the same recessive genes causing an increase of homozygosity for that gene in their descent[12,13]. Therefore, consanguinity increases the risk of developing genetic diseases such as hemoglobinopathies with the possibility of increasing the frequency of the deficient allele for glucose-6-phosphate dehydrogenase deficiency (G6PD)[14,15].

The G6PD deficiency is the most common enzymatic disorder in humans. It is a sex-linked genetic disease affecting more particularly African, Mediterranean, and Far Eastern people with an incidence of 25%[16].

The sickle-cell anemia is an example of recessive autosomic disease[17]. More than 90% of people with sickle-cell anemia are born in Africa where its prevalence varies in some regions between 10%-40%. In the Democratic Republic of Congo, prevalence of sickle-cell anemia trait varies between 15%-20%[18].

It is in this context that we have determined the frequency of antigens of ABO and Rh blood groups, and of the other blood systems such as Kell, Duffy, Kidd and MNS. We also evaluated the frequency of G6PD deficiency and sickle-cell anemia trait among the Yansi tribe practicing consanguineous marriage compared to an unrelated control group.

MATERIALS AND METHODS

A total of 525 blood samples were drawn, of which 256 among the consanguineous Yansi, and 269 among the control group. Subjects of all ages and of both sexes were selected in the scope of the study. The male/female ratio was not different with 44.9% and 52.4% of male subjects, 55.1% and 47.6% of female subjects in Yansi subjects and control group, respectively. The control subjects were younger than the Yansi subjects: the mean age was 40.86 ± 17.11 years (range: 3-79 years) in Yansi subjects and 28.87 ± 17.20 years (range: 0-79 years) in the control group. Our samples were collected in September 2010 and in June 2011 in the Bandundu province of Democratic Republic of Congo. The Yansi subjects were located in the district of Kwilu whereas control subjects where located in the city of Kikwit, both in the Bandundu province. Controls subjects did not belong to the Yansi tribe but lived in the same region of Congo. Information about subjects was collected through a questionnaire and blood samples were harvested for biological analyses.

For blood groups, samples were collected in EDTA tubes, whereas for G6PD and hemoglobin phenotypes, blood was collected on Whatman 903 filter paper. Blood grouping and extended phenotyping were achieved according to standard immunohematological procedures based on erythrocyte agglutination with specific antisera followed when necessary, by the addition of antiglobulin reagent[19]. The tube technique was used for all analyzes of blood grouping using monoclonal and polyclonal reagents in accordance with manufacturer's procedures. The phenotypes ABO and D were performed by the

double method of Beth-Vincent and Simonin. The ABO and D antigens were typed using monoclonal antisera of Pelikloon (Sanquin). Typing of C, c, E, e, Fya, Fyb, Jka, Jkb, M antigens was carried out with antisera from Immundiagnostika (Seraglu) while K, N, S, s antigens were typed using antisera from Seraclone (Biotest).

The detection of the sickle-cell anemia trait was carried out by tandem mass spectrometry[20,21] whereas assessment of the rate of G6PD deficiency was measured by the Beutler fluorescent spot test technique[22]. The principle of determination of G6PD deficiency is based on the visualisation of nicotinamide adenine dinucleotide phosphate produced in a blood spot sample. The reagent is prepared by mixing glucose-6P, β-nicotinamide adenine dinucleotide phosphate, saponine, Tris HCl and glutathione (all from Sigma). The reaction is observed under a ultraviolet lamp on Greiner Plate 655191.

Results are presented as frequencies and comparisons of variables between groups were done by a χ^2 test. Results were considered significant at the 5% level ($P < 0.05$). Calculations were done using SAS® version 9.3 (SAS® Institute; Cary, NC, United States).

RESULTS

We have studied the frequency of blood phenotypes among the consanguineous Yansi population that was compared with a population of unrelated subjects. Concerning the ABO phenotype, the distribution did not differ between the two groups ($P = 0.19$). These frequencies were determined as follows for the phenotypes O, A, B and AB, respectively: 62.5%, 23.8%, 12.1% and 1.6%; for the Yansi; 54.7%, 27.5%, 14.1% and 3.7% for the control group (Table 1). In a study based on a cohort of 2536 blood benevolent donors in the Democratic Republic of Congo, the frequency of ABO phenotypes was O>A >B >AB with 1427 (56.3%) for O, 558 (22.0%) for A, 464 (18.3%) for B and 87 (3.4%) for AB[23]. Overall the comparison of these data with our control group did not present significant difference ($P = 0.12$) but there was a significant difference ($P = 0.024$) between the Yansi and blood donors such that the blood group O frequency was higher in the Yansi (62.5%).

The frequency of Rh phenotypes did not significantly differ ($P = 0.27$) between the Yansi and the control group (Table 1), ccD.ee: 71.5% and 70.6%; ccD.Ee: 12.1% and 15.6%; ccD.EE: 0% and 1.1%; CcD.ee: 12.1% and 8.2%; CcD.Ee: 1.2% and 0.4%; CCD.ee: 0.4% and 0.4%; ccddee: 2.7% and 3.7%, respectively. Both populations were 90% Rh positive with a predominance of ccD.ee subgroup. Rh negative subjects accounted for 2.7% among the Yansi and 3.7% in the control group. Few documented studies have been published about the frequency of RHD antigen and RH phenotypes in the Congolese people. Two studies carried out on blood donors report frequencies of 98.9% and 99.5% for RHD[23,24]. As for the RH Cc and Ee phenotypes, the frequencies reported in blood donors was ccD.ee: 69.1%; ccD.Ee: 14.2%; ccD.EE: 1.2%; CcD.ee: 13.7%; CcD.Ee: 1.5%; CCD.ee: 0.2% et ccddee 0.1%[22]. These data are significantly different from our results in Yansi and unrelated subjects ($P < 0.0001$). This can be explained by the small number of samples. Larger studies are needed for the constitution of a reliable database of ABO blood groups and Rh phenotype.

Table 1 ABO, Rh and other phenotype frequencies in Yansi population and control subjects *n* (%)

	Yansi *n* = 256	Control *n* = 269	*P* value
ABO phenotype			0.19
A	61 (23.8)	74 (27.5)	
B	31 (12.1)	38 (14.1)	
O	160 (62.5)	147 (54.7)	
AB	4 (1.6)	10 (3.7)	
Rh phenotype			0.27
ccD.ee	183 (71.5)	190 (70.6)	
ccD.Ee	31 (12.1)	42 (15.6)	
ccD.EE	0	3 (1.1)	
CcD.ee	31 (12.1)	22 (8.2)	
CcD.Ee	3 (1.2)	1 (0.4)	
CCD.ee	1 (0.4)	1 (0.4)	
ccddee	7 (2.7)	10 (3.7)	
Kell phenotype			
K+k-	0	0	
K+k+	0	0	
K-k+	256 (100)	269 (100)	
Duffy phenotype			
Fy (a+b+)	0	0	
Fy (a-b+)	0	0	
Fy (a+b-)	0	0	
Fy (a-b-)	256 (100)	269 (269)	
Kidd phenotype			0.096
Jk (a+b+)	96 (37.5)	81 (30.1)	
Jk (a+b-)	141 (55.1)	173 (64.3)	
Jk (a-b+)	19 (7.4)	15 (5.6)	
MN phenotype			0.0021
MM	94 (36.7)	124 (46.1)	
MN	123 (48.1)	89 (33.1)	
NN	39 (15.2)	56 (20.8)	
Ss phenotype			0.0006
SS	10 (3.9)	35 (13.0)	
Ss	39 (15.2)	38 (14.1)	
ss	204 (79.7)	187 (69.5)	
S-s-	3 (1.2)	9 (3.4)	

The determination of extended phenotypes focused on the frequency of the most immunogenic systems, such as Kell, Duffy, Kidd and MNS (Table 1). In both populations, all subjects were Kell negative. In the Duffy system, the Fy (a-b-) phenotype was found in all subjects in both groups. The prevalence of Kell and Duffy antigens, which are strongly immunogenic, is low in the Democratic Republic of Congo[23]. This is also the case of Africans who reach 98% kk, 2% Kk and rare cases for KK[25].

The frequencies of MN and Ss phenotypes presented a significant difference ($P = 0.0021$ and $P = 0.0006$) between the two groups. These frequencies were respectively in Yansi and unrelated subjects 36.7% *vs* 46.1% for the MM phenotype; 48.1% *vs* 33.1% for MN; 15.2% *vs* 20.8% for NN; 3.9% *vs* 13% for SS; 15.2% *vs* 14.1% for Ss and 79.7% *vs* 69.5% for ss (Table 1). The frequencies

of the ss phenotype are close to data reported previously in Africans (68 %)[26]. In some studies carried out in Africa, the frequencies of the MNSs antigens reveal major differences between ethnicities. This was observed in Tanzania in three ethnic groups who have shown significant differences in the distribution of MNS phenotype[27], in Benin for the Ss phenotype[28], among Africans of Cape in South-Africa[29] and this is also the case for various ethnic groups including Somalia[30]. This system should be studied in a larger sample because this disparity in the distribution of antigens presents a high risk of alloimmunization during transfusions and pregnancy. Of note, the extended phenotype is not carried out in standard pre-transfusion workup in Africa.

DISCUSSION

We note the presence of S-s- phenotype in both populations with frequencies of 1.2% among the Yansi and 3.4% among the control group. The reported frequency of S-s- phenotype in Africans is around 1.5%[26]. There are individuals devoid of the U antigen who do not have both the S and s antigens. The U- phenotype is of low incidence in Africans and is absent in Caucasians[26]. Immunologic challenge of S-s- persons most often stimulates the production of anti-U, which is known to cause decreased survival of transfused antigen-positive red blood cells[26,31].

As for the Kidd system, there was no significant difference (Table 1) with 37.5% of Jk (a+b+) among the Yansi and 30.1% among the control group; 55.1% of Jk (a+b-) among the Yansi and 64.3% of Jk (a+b-) among the control group. Reported distribution in Africans is as follows: 34% for Jk (a+b+), 57% for Jk (a+b-) and 9% for Jk (a-b-)[32].

The distribution of AA and AS hemoglobin did not differ between groups ($P = 0.24$). Among the Yansi population, 78.1% and 21.9% of subjects exhibited AA and AS phenotype, respectively. Among the control group, 82.2% and 17.8% of subjects presented with AA and AS phenotype, respectively (Table 2). It is assumed that SS individuals were not found because of early mortality. In the Democratic Republic of the Congo, the sickle-cell anemia trait has a frequency ranging from 15% to 20%, which is also the case of Central Africa[18,33-35].

As for the G6PD phenotype, there was no significant difference between groups ($P = 0.74$). Among the Yansi, we noted 11.7% of subjects with G6PD deficiency, and among the control group, 12.3% were G6PD deficient (Table 2). Among the control group, the distribution according to sex showed a significant difference ($P = 0.023$): the proportion of males was much higher in the G6PD deficient group compared to the G6PD normal group (Table 3). The high frequency of G6PD deficiency in males is explained by the fact that the coding gene for G6PD is located on the X chromosome, and therefore, the deficiency is fully expressed in the boys affected in the hemizygote manner[36]. The distribution according to sex shows a non-significant difference ($P = 0.83$) in the Yansi: among G6PD deficient subjects, 46.7% were males and 53.3% were females. This may be due to selection of mutant alleles by consanguinity. Indeed, many variants of G6PD deficiency have been described with more than 140 known mutations, with variable enzymatic activity[36,37]. Two possibilities may arise: first, double heterozygous women carrying a completely silent allele and a partially silent allele may present with overall enzyme activity below the test cut-off. In this case they are categorized as deficient and their frequency will increase compared to controls. Second, men with a partially silent allele may be categorized as non deficient if the enzyme activity is above the test cut-off. In this case the frequency of G6PD deficient subjects will decrease compared to controls. On the other side, in normal G6PD subjects of the Yansi population, 44.7% were males and 55.3% were females (Table 3).

We noticed during our survey that several subjects of the population Yansi died prematurely. That can be explained by the presence of congenital defects and/or malformative disorders associated to consanguinity[38,39], which has been reported in several populations[40,41]. Such occurrences generate a bias in the population Yansi as miscarriage and infant mortality are superior in Yansi than within control population (Table 4). However, our data do not provide a causal link between the occurrence of theses disorders and consanguinity. To get more reliable information on the consequences of consanguinity, biological parameters should be tested at birth, through newborn screening. This may help in sensitizing the Congolese population about the medical risks associated

Table 2 Distribution of glucose-6-phosphate dehydrogenase and hemoglobin phenotype in Yansi population and control subjects *n* (%)

	Yansi *n* = 256	Control *n* = 269	*P* value
G6PD			0.74
Normal	226 (88.3)	235 (87.4)	
Deficient	30 (11.7)	34 (12.3)	
Hemoglobin			0.24
AA	200 (78.1)	221 (82.2)	
AS	56 (21.9)	48 (17.8)	

G6PD: Glucose-6-phosphate dehydrogenase deficiency.

Table 3 Distribution of glucose-6-phosphate dehydrogenase deficiency in relation to gender in Yansi population and control subjects *n* (%)

Population	Male	Female	*P* value
Yansi (*n* = 256)			0.83
G6PD normal	101 (44.7)	125 (55.3)	
G6PD deficient	14 (46.7)	16 (53.3)	
Control (*n* = 269)			0.023
G6PD normal	117 (49.8)	118 (50.2)	
G6PD deficient	24 (70.6)	10 (29.4)	

G6PD: Glucose-6-phosphate dehydrogenase deficiency.

Table 4 Clinical disorders observed in the two populations *n* (%)

Clinical disorders	Yansi *n* = 256	Control *n* = 269	*P* value
	M = 115; W = 141	M = 141; W = 128	
Asthma	8 (3.1)	-	-
Rheumatism	20 (7.8)	-	-
Paralysis	2 (0.8)	-	-
Sterility	1 (1.4)	-	-
Sexual impotence	1 (0.4)	-	-
Epilepsy	13 (5.1)	-	-
Deafness	1 (0.4)	-	-
Miscarriage	46 (32.6)[1]	3 (2.3)[1]	< 0.0001
Stilbirth	4 (2.8)[1]	-	-
Death in young age	62 (24.2)	3 (2.3)	< 0.0001

[1]Percentage among women of childbearing age. M: Men; W: Women.

with consanguineous unions and in building up a database of genetic diseases in the population.

In conclusion, we present in this paper an overview of the blood group phenotypes, hemoglobin S and G6PD deficiency among populations for which few studies have been done to date. In view of our results we find that the different studied markers are not affected by consanguinity in general because the frequency of different variables of consanguineous subjects is not statistically different from that of the unrelated subjects. Nevertheless, the fact that level of MNS blood groups presented relevant difference in Yansi, and also the high frequency of clinical disorder show us that consanguinity brings genetic disorders in this population. Screening of a wider cohort could bring more clarification on the effects of consanguinity on the phenotypes under study in the present paper.

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COMMENTS

Background

The frequency of ABO and Rh blood groups, and of the other blood systems such as Kell, Duffy, Kidd and MNS differs between populations. Blood groups antigens are most important in transfusion, pregnancy and transplantation because of their immunogenic capacity. Distribution of blood groups is not known in Yansi tribe practicing consanguineous marriage. Consanguinity increases the risk of developing genetic diseases. Therefore this study also evaluated the frequency of glucose-6-phosphate dehydrogenase deficiency (G6PD) deficiency and sickle-cell anemia trait.

Research frontiers

The comparison between consanguineous Yansi and unrelated subject showed relevant difference in distribution of MNS blood groups. Authors also found high frequency of clinical disorders in Yansi population.

Innovations and breakthroughs

It is known that bloods group phenotypes, sickle-cell anemia trait and G6PD deficiency are significant on the medical and anthropological level but also that consanguinity brings genetics disorders in the population. Several studies have been conducted in various African countries but few studies have been done to date in Congolese population in general and in the Yansi tribe in particular. To our knowledge, this study is the first to report data on this specific population.

Applications

This study will contribute to build a database of genetic diseases in the population and it will help in sensitizing the population about the medical risks associated with consanguineous unions.

Peer review

The study aimed to determine the frequency of blood groups antigens in the ABO, Rh and other systems as well as the prevalence of sickle-cell anemia's trait and the G6PD among the consanguineous Yansi tribe in the Democratic Republic of the Congo. The study revealed a significant difference in MNS blood group distribution between the Yansi tribe and a control population. Thus new data has been provided that consanguinity brings genetic disorders in the population. The paper is interesting and timely.

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CASE REPORT

Gemcitabine cures metastatic hepatic carcinoma and bone metastasis

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Abstract

We report a case of a 59-year-old gentleman who had suffered from low back pain for several months. Abdominal sonogram showed multiple heteroechoic nodules in the bilateral liver and an enlarged pancreatic head. Abdominal computer tomography (CT) favored pancreas head tumor with liver and bone metastasis. Endoscopic retrograde cholangiopancreatography (ERCP) disclosed pancreatic duct invasion over the distal portion of the pancreatic duct with prestenotic dilatation. Liver biopsy showed undifferentiated carcinoma. As suggested by the pathologist, the nasopharyngeal area was checked by the ear, nose and throat doctor, was negative and nasopharyngeal carcinoma was excluded. Therefore, the patient was treated with Gemcitabine (1500 mg/wk), as the suggested treatment schedule, for 24 wk in opioid dependency program. Sequential abdominal CT during follow up showed the disappearance of liver metastasis and shrinkage of the pancreatic tumor. Repeated ERCP after treatment showed re-channelization of the pancreatic duct. During 11 years of follow up, 5 CT scans disclosed not only the disappearance of the hepatic tumor but also no cancer recurrence. Progressive shrinkage of pancreatic head was also noted. Therefore, we can say this malignant case was cured by monotherapy with gemcitabine.

Key words: Gemcitabine; Gemza; Cure; Metastatic hepatic carcinoma; Undifferentiated carcinoma; Bone metastasis

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INTRODUCTION

Gemcitabine is a nucleoside analog with a structural similarity to cytarabine. Initially, it has a low objective response rate of around 6%-11% in chemotherapy-naive patients with pancreas cancer who are given the single agent gemcitabine (800 mg/m^2 IV weekly for 3 of every 4 wk)[1].

CASE REPORT

In 1999, a 59-year-old gentleman was referred from another hospital due to an unknown malignancy with liver and bone metastasis. He suffered from lower back pain

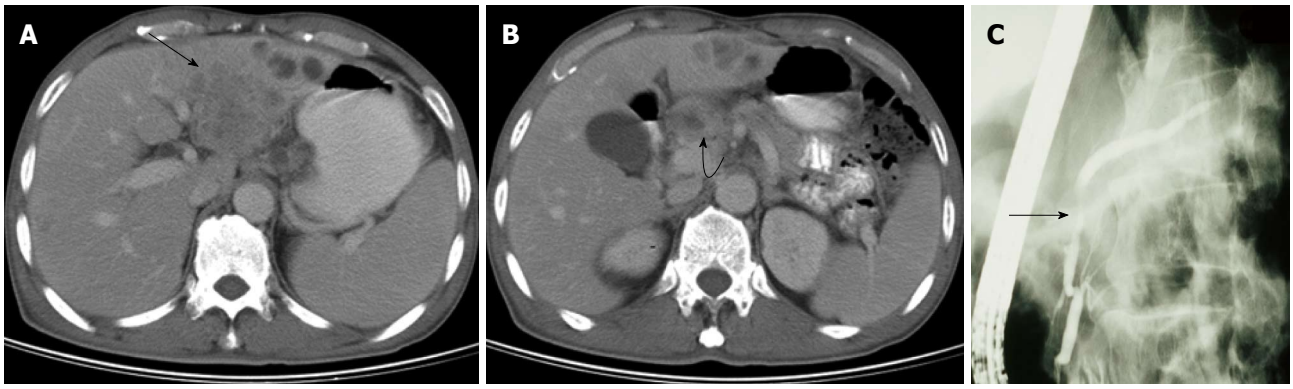

Figure 1 Diagnosis of pancreas adenocarcinoma. A: Multiple liver metastasis (arrow); B: Enlarged pancreas head (curved arrow); C: Focal stenosis over the distal portion of the pancreatic duct (arrow, apple-core like).

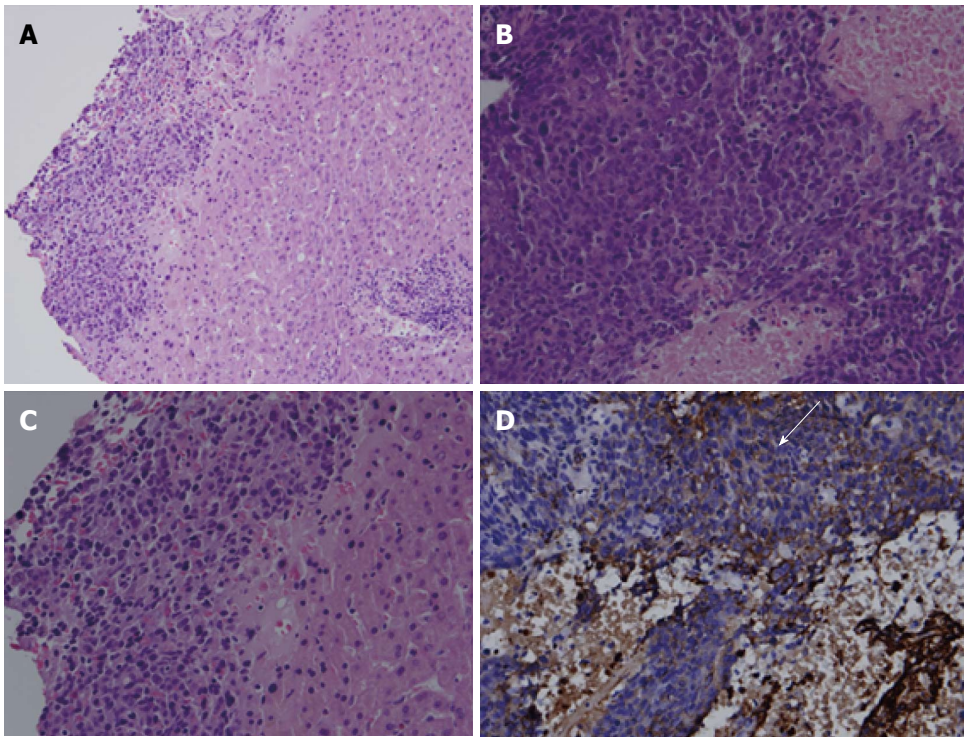

Figure 2 Liver aspiration. A: Liver tissue with tumor arranged in solid sheet on the left side of the picture (HE stain, x 200); B: High power view shows tumor cells with hyperchromatic nuclei and high nucleus-to-cytoplasm (N/C) ratio (HE stain, x 400). Two patches of necrosis are found in the lower and right upper part; C: Tumor cells in solid nest with hyperchromatic nuclei and high N/C ratio. The right side is normal liver tissue (HE stain, x 400); D: AE1/AE3 (IHC stain, x 400). Focal cytoplasmic positive reaction (white arrow).

and loss of body weight, about 10 kg within 3 mo. There was no familial history of malignancy. Physical examination showed pale conjunctiva, knocking pain over the lumbar spine and a mass lesion over the right sternoclavicular junction. The laboratory data showed only normocytic anemia and the hemoglobin was 10.4 mg/dL. The tumor markers, such as carcinoembryonic antigen and CA 19-9, were within normal limits. Abdominal sonogram showed many heterogeneous isoechoic tumors in bilateral lobes of the liver with enlargement of the pancreas head. Abdominal computed tomography (CT) showed suspicion of pancreas head cancer with liver and L3 metastasis (Figure 1A and B). The bone scan showed suspicion of metastasis over the right sternoclavicular area, L-spine and ischium. Liver biopsy was done. The pathologist reported undifferentiated carcinoma (Figure 2) because of the focally weakly positive result with cytokeratin AE1/AE3, a sole marker of the epithelial differential, and a nasopharyngeal check-up to rule out the possibility of nasopharyngeal carcinoma was suggested. The ear, nose and throat (ENT) doctor disclosed a smooth nasopharyngeal mucosa and biopsy showed no malignancy. Endoscopic retrograde cholangiopancreatography (ERCP) showed focal irregular stenosis, favoring tumor invasion over the pancreatic duct (Figure 1C). Clinically, pancreas cancer with liver and bone metastasis was highly likely.

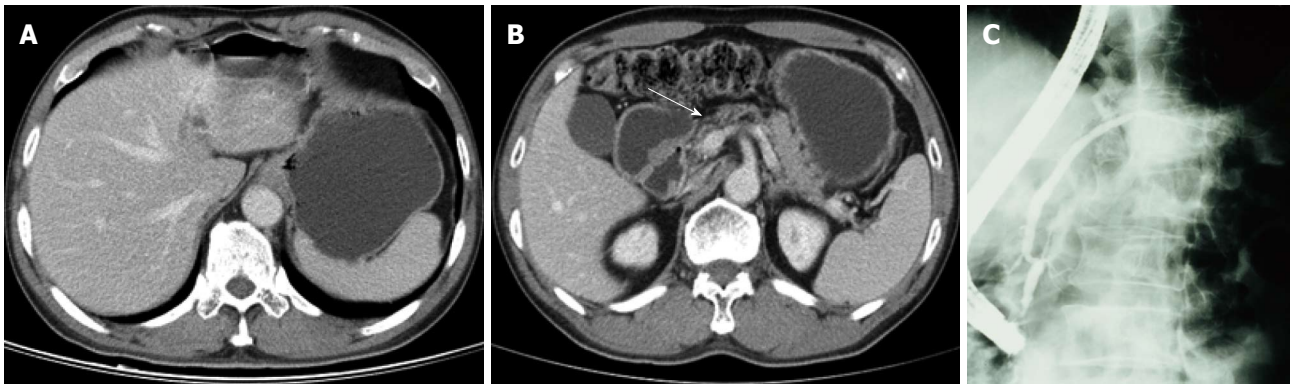

Figure 3 Tumor shrinkage post-chemotherapy. A: Liver nodules disappeared; B: The pancreas head was atrophied (white arrow); C: No stenosis of the pancreatic duct was noted after chemotherapy.

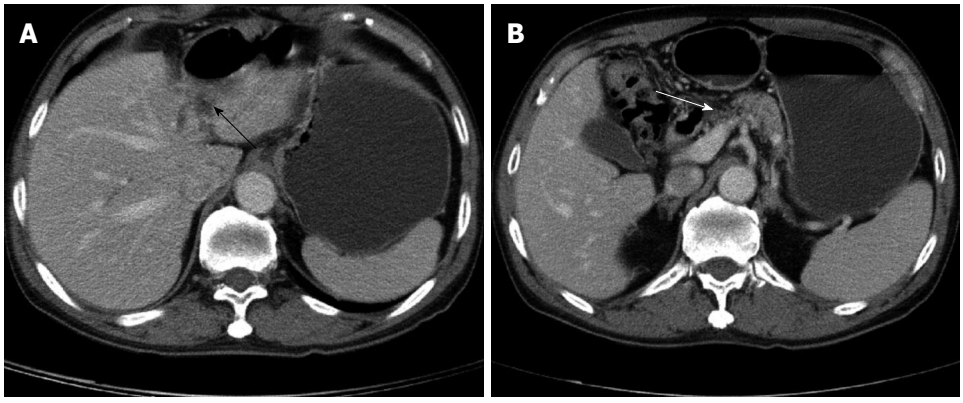

Figure 4 Shrinkage of left lobe of the liver (A, black arrow) and pancreatic head (B, white arrow).

The patient was treated with monotherapy of gemcitabine 1500 mg/wk in GI-OPD. The dose was 1000 mg/m^2 body surface area and medication was infused in 30 min. The patient received gemcitabine every week for the first 7 wk and then rested for 1 wk. Thereafter, he received treatment weekly for 3 wk and then rested for 1 wk. In total, the patient received 19 doses within 24 wk. Throughout, the patient tolerated the treatment well and blood transfusion was not necessary. Repeat CT scan showed disappearance of the liver mass (Figure 3A) and shrinkage of the pancreatic head (Figure 3B). Repeat ERCP showed re-channelization of the pancreatic duct (Figure 3C). The mass over the right sternoclavicular joint became softer and smaller gradually. Lumbago improved gradually during gemcitabine therapy and finally vanished after the treatment course. In the following 11 years, 5 CT scans showed progressive shrinkage of the left lobe of the liver (Figure 4A) and pancreatic head (Figure 4B). No cancer recurrence was noted. Also, repeat CA19-9 was normal. In 2010, 11 years after treatment, the patient had senile dementia but was still cancer free. Therefore, we can say this patient was cured by gemcitabine.

DISCUSSION

Undifferentiated carcinoma with multiple metastasis is common, about 20%-25%, in cancer of an unknown primary site[2]. In our case, NPC was excluded by biopsy on grossly normal mucosa of the nasopharyngeal area. In clinics, the mid or lower third portion of thoracic esophageal carcinoma with intra abdominal metastasis is also common, with an incidence of around 15.0%-17.4%[3,4]. However, the gastroscope was negative too. The positive reaction with immunohistochemistry stain of AE1/AE3 was specific for carcinoma but carcinoma with pancreatic duct metastasis is very rare. Image studies of CT and ERCP, together with liver biopsy, resulted in the diagnosis of pancreas adenocarcinoma with multiple metastases.

In 1997, the study of Burris *et al*[5] showed that the clinical benefit and survival of gemcitabine (1000 mg/m^2 weekly for 7 wk followed by 1 wk of rest, then weekly for three out of every 4 wk) was approved for first-line therapy of metastatic pancreatic cancer. However, there was no evidence to show that gemcitabine has the ability to cure advanced pancreas adenocarcinoma, even in cases with multiple organ metastases. But in our case, the liver mass disappeared and atrophy of left lobe of liver was noted in the following abdominal CT after treatment with gemcitabine. The repeated ERCP also showed the re-channelization of the irregular stenotic pancreatic duct. There was no cancer recurrence during the eleven years of follow-up. It was amazing that gemcitabine could cure

metastatic liver carcinoma.

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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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