Table 1. Hematological and DNA sequence data from the 3 patients.*

Variant	Mutation	Gender Age (years)	RBC 10^{12}/L	Hb g/dL	Ht %	MCV fl	MCH pg	Reticulocytes %	HbA_2 %
Hb Sens** (a243(CE1)Phe>Ile)	α2 CD 43 (TTC>ATC)	M 54	3.2	10.5	33.5	104	30	n.d	1.8
Hb Fez	α1 CD131 (TCT>TC-)	M 40	2.9	8.5	26.0	91	29.3	4.4	1.7
Hb Senlis	α1 CD134 (ACC>AC-)	F 62	2.5	8.8	27.0	105	34	3.9	1.9

** Molecular studies were performed as described in Moradkhani et al.[5] **splenectomized 18 years earlier.*

within the 3rd exon leads to FS with occurrence of a stop codon at position 156. In such cases where FS occurs proximal within the sequence, helix H is drastically altered by incorporation of several hydrophobic residues (six Leu, two Ile, four Trp), leading to dominant β thalassemia syndrome.[8] When the deletion is located at the very end of the 3rd exon, as in Hb Tak,[9] the mere outcome is a 10-residue-long C-terminal tail leading to moderate instability and high oxygen affinity.

Unlike most other α gene defects reported, the mutations described here have a dominant effect. Thus, the biological consequences of those mutations, whether missense or FS, are highly dependent upon their occurrence within the gene sequence.

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References

1. Hardison RC, Chui DHK, Giardine B, Riemer C, Patrinos GP, Anagnou N, et al. HbVar: a relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. Hum Mutat 2002;19:225-33.
2. Giardine B, van Baal S, Kaimakis P, Riemer C, Miller W, Samara M, et al. HbVar database of human hemoglobin variants and thalassemia mutations: 2007 update. Hum Mutat 2007;28:206.
3. Vasseur C, Domingues-Hamdi E, Brillet T, Marden MC, Baudin-Creuza V. The α-hemoglobin stabilizing protein and expression of unstable a-Hb variants. Clin Biochem 2009 May 29. [Epub ahead of print].
4. Wajcman H, Traeger-Synodinos J, Papassotiriou I, Giordano PC, Harteveld CL, Baudin-Creuza V, et al. Unstable and thalassemic α chain hemoglobin variants: a cause of Hb H disease and thalassemia intermedia. Hemoglobin 2008;32:327-49.
5. Moradkhani K, Mazurier E, Giordano PC, Wajcman H, Préhu C. An α 0-thalassemia-like mutation: Hb Suan-Dok [α109(G16)Leu_Arg] carried by a recombinant -α(3.7) gene. Hemoglobin 2008;32:419-24.
6. Feng L, Gell DA, Zhou S, Gu L, Kong Y, Li J, et al. Molecular mechanism of AHSP-mediated stabilization of α-hemoglobin. Cell 2004;119:629-40.
7. Seid-Akhavan M, Winter WP, Abramson RK, Rucknagel DL. Hemoglobin Wayne: a frameshift mutation detected in human hemoglobin α chains. Proc Natl Acad Sci USA 1976;73:882-6.
8. Préhu C, Pissard S, Al-Sheikh M, Le Niger C, Bachir D, Galactéros F, et al. Two French Caucasian families with dominant thalassemia-like phenotypes due to hyper unstable hemoglobin variants: Hb Sainte Seve [codon 118 (-T)] and codon 127 [CAG_TAG (Gln_Stop]). Hemoglobin 2005;29:229-33.
9. Flatz G, Kinderlerer JL, Kilmartin JV, Lehmann H. Haemoglobin Tak: a variant with additional residues at the end of the β-chains. Lancet 1971;1:732-3.

Safety of cardiovascular magnetic resonance gadolinium chelates contrast agents in patients with hemoglobinopathies

Myocardial fibrosis/necrosis has been documented by histological and Cardiovascular Magnetic Resonance (CMR) studies in patients with hemoglobinopathies.[1] The delayed enhancement CMR technique with intravenous administration of gadolinium (Gd) chelates contrast agents is the only validated approach for detecting myocardial fibrosis non-invasively.[2]

Recently, serious concerns have been raised regarding the safety of Gd chelates. In particular, a link between gadolinium and nephrogenic systemic fibrosis (NSF) has emerged, due to the presence of gadolinium in skin samples of NSF patients.[3] However, NSF has also been diagnosed in patients who had not been exposed to gadolinium. NSF is a scleroderma-like disease mainly involving the skin and it is closely related to severe kidney failure (glomerular filtration rate (GFR) < 30 mL/min/1.73 m^2).[4] No cases have been reported in patients with GFR > 60 mL/min/1.73 m^2. Although cause and effect have not been proven for the NSF-gadolinium link, avoidance and care have been strongly recommended.[5] In addition, unconfirmed doubts have been raised about the use of Gd-chelate contrast agents in patients with hemoglobinopathies, characterized by heavy co-morbidity due to iron overload, which can also damage the kidneys and could be a co-factor, further enhancing the risk for NSF development.[6] To date, there have been no dedicated clinical studies on the safety of the Gd chelates in these

patients.

We administered Gd chelates contrast agents in 475 patients with hemoglobinopathies, enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network.[7] The patients older than 18 years were injected with gadobutrolo (Gadovist®; Bayer Schering Pharma; Berlin, Germany) and the patients younger than 18 years were injected with gadopentate dimeglumine (Magnevist®; Bayer Schering Pharma; Berlin, Germany), both at the standard dose of 0.2 mmol/kg. Kidney function was assessed by measuring the GFR, estimated by means of the Modification of Diet in Renal Disease formula[8] for patients older than 13 years and by Schwartz formula[9] for patients younger than 13 years. The values were normalized to body surface area. Seven patients (6 injected with gadobutrolo and one injected with gadopentate dimeglumine) had severe renal dysfunction. Since diabetes is a leading cause of kidney disease, its presence was also evaluated. The clinical characteristics of all patients are summarized in Table 1. All patients gave written informed consent. They were monitored at the reference thalassemia center every 45 days from the day of CMR up to December 2008 in order to detect the potential insurgence of adverse events (AEs), defined as unfavorable and unintended symptoms associated with the use of gadolinium. In particular, the possible development of first symptoms/signs for NSF, such as hardening of the skin and development of plaques affecting the buttocks, trunk and extremities were controlled. The mean time of the monitoring was 13±7 months; the monitoring time was more than 18 months in 133 patients (28%). We found myocardial fibrosis in 95 (20%) of the 475 thalassemia or sickle cell patients enrolled in the study, suggesting a clinical use of Gd chelates contrast agents in CMR in the hemoglobinopathies. Of the patients injected with gadopentate dimeglumine, none manifested AEs. Of the 426 patients injected with gadobutrolo, only 4 (0.94%) manifested AEs, all classifiable as mild. All patients were female with a mean age of 35±4.1 years and had thalassemia. Of 4 patients with AEs, 3 showed a moderate reduction (60-30 mL/min/1.73 m^2) of GFR. All AEs were resolved before the patients left the hospital and in only one case was it necessary to administer drugs. Table 2 shows the types of AEs observed and the characteristics of the patients who manifested them. The incidence of mild AEs resulted comparable with the data reported in literature for patients without hemoglobinopathies. Although 150 patients (32%) injected with gadobutrolo or gadopentate dimeglumine had moderate to severe kidney dysfunction, no cases of NFS have been detected. Although a potential risk factor for the development of NSF, such as iron overload, was significantly present in our population, it did not seem to play a rele-

Table 1. Patients' characteristics.

	All patients	Patients injected with Gadovist®	Patients injected with Magnevist®
Total, n	475	426	49
Male/Female	233/242	201/225	32/17
Age (years)	31.95±10.47	34.11±8.69	13.17±3.37
N. (%) of patients with			
Thalassemia major	383 (80.6%)	337 (54.5%)	46 (93.9%)
Thalassemia intermedia	78(16.4%)	76 (12.3%)	2 (4.1%)
Thalasso-drepanocitosis	10 (2.1%)	10 (1.6%)	0 (0%)
Depranocitosis	4 (0.8%)	3 (0.5%)	1 (1.9%)
Hemoglobin (gr/dL)	9.59±0.84	9.53±1.19	9.53±6.4
Ferritin (ng/dL)	1515.6± 1525.01	1419.85± 1496.85	2322.77± 1536.36
ALT (u/dL)	46.04±38.87	45.86±38.99	47.61±38.1
GFR (mL/min/1.73 m^2)	74.59±25.53	73.42±25.67	84.77± 22.1
N. (%) of patients with GFR			
<30 mL/min/1.73 m^2	7 (1.5%)	6 (1.5%)	1 (2.1%)
30-60 mL/min/1.73 m^2	138 (29.1%)	133 (31.2%)	5 (10.2%)
60-90 mL/min/1.73 m^2	220 (46.3%)	200 (46.9%)	20 (40.8%)
>90 mL/min/1.73 m^2	110 (23.1%)	87 (20.4%)	23 (46.9%)
Type I Diabetes, n (%)	31	30	1
Type II Diabetes, n (%)	14	12	2
Months from the CMR	13.3±6.9	13.4±7.1	12.5±5.7

Table 2. Characteristics of the patients who manifested adverse events.

	Patient 1	Patient 2	Patient 3	Patient 4
Sex	Female	Female	Female	Female
Age (years)	40	30	35	35
Hemoglobinopathy	Thalassemia Major	Thalassemia Major	Thalassemia Intermedia	Thalassemia Major
Hemoglobin (gr/dL)	10.5	9.2	9.47	9.5
Ferritin (ng/dL)	1055	632	2250	822
ALT (u/dL)	45	22	46.6	59
Chelation therapy	Desferoxamine	Combination of deferoxamine and deferiprone	Deferasirox	Desferoxamine
GFR (mL/min/1.73 m^2)	45	55	54	71
Diabetes I	Yes	No	No	No
Diabetes II	No	No	No	No
Glucose intolerance	No	No	No	Yes
Months from the CMR	15	14	22	21
Gd-Chelate	Gadovist	Gadovist	Gadovist	Gadovist
EA	Nausea	Hives	Hives	Vomiting and headache
Treatment	None	None	Methylprednisolone	None

vant role.

In conclusion, in a large cohort of thalassemia and sickle cell patients the use of gadopentate dimeglumine and gadobutrolo in CMR seems to be safe and well tolerated, with a risk comparable to the general population. These data support the routine use of Gd chelates contrast agents in MR and especially in CMR to detect myocardial fibrosis/necrosis for diagnostic and clinical management of thalassemia or sickle cell patients. However, gadolinium should be used with caution in patients with hemoglobinopathies who have severe renal dysfunction.

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References

1. Meloni A, Pepe A, Positano V, Favilli B, Maggio A, Capra M, et al. Influence of myocardial fibrosis and blood oxygenation on heart T2* values in thalassemia patients. J Magn Reson Imaging 2009;29:832-7.
2. Wu E, Judd RM, Vargas JD, Klocke FJ, Bonow RO, Kim RJ. Visualisation of presence, location, and transmural extent of healed Q-wave and non-Q-wave myocardial infarction. Lancet 2001;357:21-8.
3. Deo A, Fogel M, Cowper SE. Nephrogenic systemic fibrosis: a population study examining the relationship of disease development to gadolinium exposure. Clin J Am Soc Nephrol 2007;2:264-7.
4. DeHoratius DM, Cowper SE. Nephrogenic systemic fibrosis: an emerging threat among renal patients. Semin Dial 2006;19:191-4.
5. Perazella MA, Rodby RA. Gadolinium use in patients with kidney disease: a cause for concern. Semin Dial 2007;20: 179-85.
6. Perazella MA. Gadolinium-contrast toxicity in patients with kidney disease: nephrotoxicity and nephrogenic systemic fibrosis. Curr Drug Saf 2008;3:67-75.
7. Meloni A, Ramazzotti A, Positano V, Salvatori C, Mangione M, Marcheschi P, et al. Evaluation of a web-based network for reproducible T2* MRI assessment of iron overload in thalassemia. Int J Med Inform 2009;78: 503-12.
8. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-70.
9. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976;58:259-63.

Platelet morphological changes in 2 patients with von Willebrand disease type 3 caused by large homozygous deletions of the von Willebrand factor gene

Platelet morphological defects have previously been described in von Willebrand disease type 2B (VWD2B), we now describe that they may also occur in patients with VWD3 lacking both platelet and plasma von Willebrand factor (VWF). Electron microscopy (EM) and immunofluorescence labeling (IF) were used to examine platelets from two VWD3 patients with a homozygous deletion involving *VWF* and *TMEM16B* genes. Platelet size heterogeneity was seen in both patients, with an unusual characteristic being the presence of a subpopulation of long thin platelets. The additional detection of circulating megakaryocytes and derived fragments suggests that the absence of VWF can affect megakaryocytopoiesis.

VWF is essential to platelet function mediating adhesion and shear-dependent thrombus formation on the vessel wall.[1] Yet relatively little is known about its role in megakaryocytopoiesis. Macrothrombocytopenia is found in about 30% of patients with von Willebrand disease type 2B (VWD2B) and the fall in platelet count can be severe.[2] In some families, circulating platelet agglutinates are present.[2-5] VWD2B results from mutations in exon 28 of the *VWF* gene that lead to amino acid substitutions in the VWF A1 domain. The result is a gain-of-function and VWF multimers that spontaneously bind to glycoprotein (GP)Ib on platelets. VWD type 3 (VWD3) is characterized by severely decreased or absent expression of VWF resulting from a variety of mutations or *VWF* gene deletions that are sometimes accompanied by alloantibody development.[1,6,7]

We have previously reported impaired megakaryocytopoiesis due to a precocious interaction between GPIbα with newly synthesized VWF in MKs of a family with VWD2B given by a R1308P mutation.[3] *In vitro* studies performed on MKs in culture have confirmed that proplatelet formation is inhibited by blockade of GPIb.[8] In continuing our investigations into the importance of VWF for platelet production, we have now examined platelet morphology for 2 patients with VWD3 caused by a previously characterized homozygous 253-kbp deletion involving *VWF* and *TMEM16B*.[7] Neither patient possessed detectable VWF:Ag in either their plasma or platelets and their bleeding scores[2] were high (P1, 24; P2, 25). Their platelet counts at the time of study were 241×10^9/L (P1) and 149×10^9/L (P2) (control range $150\text{-}300\times10^9$/L).

Electron microscopy (EM) was used to examine platelet morphology. Figure 1 (a-f) shows a wide range of platelet size heterogeneity in both patients. Illustrated are enlarged and sometimes rounded platelets with internal membrane complexes and a heterogeneous α-granule distribution (a,e). Enlarged α-granules were occasionally observed. An unexpected finding was the presence of very long thin structures (c, d) as these have not been reported in VWD2B.[2] The structure in (f) resembles more a MK fragment. In morphometric studies, a minimum of 100 platelet sections of platelets were analyzed for each subject and compared to the results obtained for 4 control donors. Platelet maximal and minimal diameters were measured using the Software Image J (NIH, Bethesda, MD, USA). Statistics were performed using Student's *t* test or Pearson's χ^2 test. Results showed that