Retinal Hemangioblastoma in von Hippel-Lindau Disease: A Clinical and Molecular Study

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PURPOSE. To assess the natural history of retinal manifestations in von Hippel-Lindau (VHL) disease and to study the genotype-phenotype correlation.

METHODS. Data concerning 103 patients with VHL retinal manifestations and 108 patients without VHL retinal manifestations were extracted from the French VHL database. A retrospective study was performed by questionnaire. Patients were classified into three visual morbidity groups. Molecular analysis of the VHL gene was performed in 196 patients.

RESULTS. The mean age of ocular manifestations detection was 24.8 years. In half of the cases, the ocular manifestations revealed the disease. Half of the cases had bilateral involvement. Visual morbidity was significantly associated with the retinal hemangioblastoma count but not with other ocular or general characteristics. One third of the patients were classified in the worst visual morbidity group at the end of follow-up. Mutations were detected in 81% of patients with retinal hemangioblastomas and in 71% of patients without retinal involvement. Using a Poisson model and a marginal approach, the number of hemangioblastomas, age-adjusted, was 2.1 times greater for patients who had a substitution than in patients with a truncation (95% CI, 1.05–4.44; P = 0.05).

CONCLUSIONS. Visual loss remains one of the major complications of VHL disease, confirming the importance of early ophthalmologic screening. Visual morbidity was not related to the type of extraocular manifestation but appeared to be related to the type of germline mutation. However, only further genetic and clinical studies in a larger series of patients will clearly determine the genotype-phenotype relationship. (Invest Ophthalmol Vis Sci. 2002;43:3067–3074)

Von Hippel-Lindau (VHL) disease (Online Mendelian Inheritance in Man [OMIM] 193500) is a dominantly inherited familial cancer syndrome predisposing to various benign or malignant tumors: central nervous system (CNS) and ocular hemangioblastomas, renal cell carcinoma (RCC) and/or renal cysts, pancreatic tumors and cysts, pheochromocytoma, and endolymphatic sac tumors. The birth incidence is estimated to be 1 in 36,000 to 1 in 53,000. Hemangioblastoma is the most emblematic lesion of VHL disease because of its major clinical implications in the natural history of the disease. Benign tumor with a very singular vascular and cellular (stromal cell) differentiation occurs both in CNS and retina. The hemangioblastoma may be a single tumor, and sometimes it is the only manifestation of the disease, but the tumors are more frequently multiple. Ocular hemangioblastomas occur in 45% to 60% of patients with VHL. On fundus examination, the lesions have an easily recognizable globular reddish appearance with a dilated feeding artery and a tortuous draining vein. The size of the retinal hemangioblastoma may be variable, ranging from a pinpoint lesion or a discrete vascular tuft to a large globular lesion (Figs. 1, 2). In spite of its benign nature and classic slow-growing course, ocular hemangioblastoma may cause sight-threatening complications and remains a major cause of visual morbidity or sometimes blindness for patients with VHL disease. Early detection and treatment can change the visual prognosis.

Predisposition to VHL results from germline mutations in the VHL tumor-suppressor gene located on chromosome 3p25-p26, and development of tumors results from a somatic inactivation of the remaining wild-type VHL allele. Germline mutations in the VHL gene, spanning three exons and encoding a 213-amino-acid protein (pVHL), are now identified in most of the cases.

The main functional domains of the pVHL protein have been recently characterized. The major function of the pVHL protein is the negative regulation of hypoxia-inducible mRNAs such as the mRNA encoding VEGF by targeting hypoxia-inducible transcription factors (HIFs) for degradation by the proteasome. Indeed, the hallmark of VHL tumors, and moreover hemangioblastomas, is a high degree of vascularization due to overexpression of VEGF.

In spite of these important advances in the understanding of VHL disease, the overall extremely variable intra- and interfamilial expressivity of the disease remains unclear. A genotype-phenotype correlation has emerged only for the occurrence of pheochromocytoma, which distinguishes VHL type 1 (without pheochromocytomas) and VHL type 2 (with high risk for pheochromocytoma). Type 2 is subdivided in three subtypes, 2A (with low risk of renal cancer and pancreatic tumors); 2B (the full multisite subtype), and 2C (pheochromocytomas only, recently individualized by molecular genetics). Families with VHL type 1 typically have VHL deletions or truncations (95% CI, 1.05–4.44; P = 0.05).
protein truncating mutations, whereas families with type 2 have mainly missense mutations.

The purposes of this study were to analyze the natural history and the potential genotype-phenotype correlation of the ocular manifestations in a large series of French VHL patients.

PATIENTS AND METHODS

The patients were recruited through the French VHL database, which was created in 1992. During the period of the study (1996–1999), 610 patients with VHL were registered in the database. Detailed systemic follow-up data were available for 211 patients from 111 distinct pedigrees. The patients were divided into two groups: one group of 105 (48.8%) patients from 60 pedigrees with ocular involvement and a second group of 108 (51.2%) from 66 pedigrees with no ophthalmic manifestation reported on the last follow-up. Fifteen families were involved in both groups.

To be included, the patient had to meet the diagnostic criteria for VHL defined by the presence of two major manifestations, including at least one CNS or retinal hemangioblastoma, one major manifestation and a positive family history, or an isolated clinical feature with mutation in the VHL gene. Patients with solitary retinal hemangioblastomas and no mutation detected in the VHL gene were excluded from the study.

One hundred fifty-six patients had type 1 VHL, and 55 patients had type 2 (13 type 2A, 42 type 2B, no type 2C). In the group with ocular manifestations, 77 patients had type 1 VHL and 26 had type 2 (9 type 2A and 17 type 2B). In the group without ocular involvement, 79 patients had type 1 VHL and 29 had type 2 (4 type 2A, 25 type 2B).

General and Ophthalmological Data Assessment

A questionnaire (available on request) inquiring about the ocular and general status of the patients was sent to ophthalmologists treating patients with VHL. Extraocular features were also assessed with the VHL database.

General Data Evaluation

Personal and familial data were obtained and follow-up duration was evaluated. Detailed information concerning each extraocular manifestation if present were recorded and included: CNS hemangioblastomas, renal cell carcinoma, renal cysts, pheochromocytoma, pancreatic cysts, and/or neuroendocrine tumors, endolymphatic sac tumors.

Ocular Manifestations Assessment

The age of onset and the history of ocular manifestations were recorded. Vision at the first and last ophthalmic examination were noted. Detailed descriptions of the first and last ophthalmic manifestations were obtained.

Descriptive Data

The number and location (retinal or papillary) of the retinal hemangioblastomas were recorded for each eye at initial and final examination. Presence or absence of complications due to the retinal hemangioblastomas were noted. The following items were considered: macular exudation, extramacular exudation, retinal traction, retinal detachment treated successfully with surgery, retinal detachment with unsuccessful surgical treatment, optic disc or hemangioblastoma neovascularization, neovascular glaucoma, vitreous hemorrhage, phthisis, enucleation, and any other relevant ocular feature. Photographs and fluorescein angiograms were obtained if possible.

Impact on Visual Function

Visual acuity was measured with the Monoyer decimal charts. The results were converted to the Snellen 20/4 notation. The patients were classified according to visual morbidity in three categories: group 1 with normal vision (both eyes >20/40), group 2 with moderate vision loss defined by vision in at least one eye inferior to 20/40, and group 3 with severe visual loss defined by the vision in one eye inferior to 20/200.

DNA Mutation Analysis

This study was approved by the Ethics Committee at Le Kremlin-Bicêtre University Hospital (France) and was conducted adhered to the tenets of the Declaration of Helsinki. Blood samples were collected after written consent was received from each patient and processed for DNA isolation using standard methods. To detect the presence of a mutation, PCR and single-strand conformation polymorphism (SSCP) were used systematically. Briefly, we used six sets of primers, described elsewhere. If no abnormal migration pattern was detected, we performed direct sequencing. Finally, if both PCR-SSCP and direct sequencing produced normal findings, a Southern blot analysis was performed to detect large rearrangements.

PCR-SSCP Analyses. PCR and SSCP analyses were performed according to the protocol of Gallou et al. PCR mixtures (25 μL) contained 20 ng of genomic DNA, 1× PCR buffer, 0.8 μM of each PCR primer, 200 μM of each dNTP, and 0.5 U Taq DNA polymerase (Invitrogen Life Technologies, Cergy Pontoise, France). The reaction mixtures underwent the following incubations in a thermocycler (Gene Amp 9600; Perkin-Elmer, Wellesley, MA): 94°C for 5 minutes; 30 cycles of 94°C for 15 seconds and 62°C to 70°C for 30 seconds (specific for each set of primers); and a final extension at 72°C for 5 minutes. SSCP analysis was performed as previously described. Four microliters of each PCR product containing 0.5 μCi [33P]-dATP was denatured for 10 minutes at 95°C in 4 μL gel-loading buffer and then chilled on ice. Samples were electrophoresed at 10 W for 10 to 16 hours at 20°C on mutation detection gel (Hydrolink Mutation Detection Enhanced gel; Bioprobe Systems SA, Montreuil, France).
**Sequence Analyses.** The PCR products were extracted with a purification kit (Wizard Prep; Promega, Madison, WI) purification kit. Cycle sequencing was performed on both strands either with a sequencing kit (Pharmacia France, St. Quentin Yvelines, France) or with a sequencing kit (Thermosequenase; Amersham, Succursale, France) on an automated sequencer (ALF; Pharmacia).

**Southern Blot Analysis and Hybridization**

DNA samples (6 µg) were digested by the restriction enzymes AseI and EcoRI and run using standard procedures. The membranes were hybridized with the g7 clone labeled with a random priming procedure for Southern blot analysis (Random Primed DNA labeling kit; Roche Molecular Biochemicals, Meylan, France). Washes were performed at 42°C for 10 minutes in 2× SSC and 0.1% SDS and two times for 5 minutes each at 65°C in 2× SSC and 0.1% SDS. Membranes were exposed to film for at least 12 hours with intensifying screens at -80°C.

**Statistical Methods**

The study was retrospective. For univariate analyses, differences between groups were analyzed with one-way analysis of variance for continuous variables. Comparisons between categorical covariates were performed by \( \chi^2 \) test or the Fisher test with contingency tables. All tests were two-tailed, and \( P \leq 0.05 \) was considered to indicate statistical significance.
Hemangioblastoma Count

The mean number of hemangioblastomas per gene carrier with ocular manifestation was 1.35 at initial evaluation (range, 1–20) and 2.97 (range, 1–20) at final evaluation. During the time of follow-up, the mean number of new hemangioblastomas per patient (of the group with ocular manifestation) per year was 0.36. The hemangioblastoma count increased with the duration of follow-up, but this increase varied according to age group. Indeed, the number of new hemangioblastomas per year was higher in the 10- to 20-year-old age group than in the 20- to 30-year-old age group, in which the number was higher than in the 30- to 50-year-old group. This estimation of the rate of new lesions appearing during 1 year in one gene carrier is probably imperfect, and the so-called velocity may vary considerably from one patient to the other.

Optic disc hemangioblastomas were diagnosed in 11% of the eyes at the first report (5% of the eyes had optic disc and retinal hemangioblastomas). At final report, 15% of the eyes had optic disc hemangioblastomas (11% of the eyes had optic disc and retinal hemangioblastomas). At initial report, 72% of the patients had retinal hemangioblastomas in one eye and 28% had them in both eyes. At final report, 51% of the patients had retinal hemangioblastomas in one eye only and 49% had bilateral involvement.

Evaluation of Ocular Complications

At initial report, macular exudation was present in 15% of the eyes, and extramacular exudation was present in 22%. At final report, macular exudation was present in 9% of the eyes and extramacular exudation in 12%.

Tractional retinal detachment was reported in 22% of the eyes at initial report. At final report, 11.5% of the eyes had a successfully treated retinal detachment, whereas 17% of the eyes had a retinal detachment for which surgery had been ineffective.

One eye was reported as having new vessels (on a peripheral hemangioblastoma). No optic disc neovascularization was reported. Ten (5%) eyes had neovascular glaucoma and six (3%) had to be enucleated because of pain and/or phthisis bulbi.

Evaluation of Visual Impairment

Visual morbidity was evaluated by classifying the patients into three groups according to their visual acuity (Table 1). On initial examination 70% of the patients were classified into group 1, 9% into group 2, and 21% into group 3. On final examination 64% of the patients were classified into group 1, 6% into group 2, and 30% into group 3. No significant relation was found between final visual morbidity and the duration of follow-up, but this increase varied according to age group.

<table>
<thead>
<tr>
<th>Group 1 (Normal Vision)</th>
<th>Group 2 (At Least One Eye &lt;20/40)</th>
<th>Group 3 (Vision in One Eye &lt;20/200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial evaluation</td>
<td>70</td>
<td>9</td>
</tr>
<tr>
<td>Final evaluation</td>
<td>64</td>
<td>6</td>
</tr>
</tbody>
</table>

Average follow-up was 8 years. Data are the percentage of total patients classified in each group.
follow-up, the age at diagnosis, initial papillary involvement, and ocular involvement as a first sign of VHL disease. On the contrary, a significant association was found between visual morbidity and the count of hemangioblastomas ($P < 0.001$): Visual morbidity increased with the hemangioblastoma count. Patients with papillary hemangioblastoma did not differ in visual morbidity from those without papillary hemangioblastoma.

When the group of patients with retinal hemangioblastomas and the group of patients without ocular manifestations were compared, no significant association was found concerning the occurrence of retinal hemangioblastomas and central nervous system hemangioblastomas, renal cell carcinoma, pheochromocytoma, or pancreatic lesions (Table 2).

**Genotype Analysis**

In the group with ocular manifestations, 91 (88%) patients were screened for mutation in the VHL gene, and 74 mutations were detected (mutation detection rate: 81%). In the group without ocular manifestations, 105 (97%) patients were screened for mutations in the VHL gene, and 75 mutations were detected (mutation detection rate: 71%). Germline mutations in the two groups are detailed in Table 3, and their locations are represented in Figure 4.

Briefly, mutations in patients with retinal hemangioblastomas include missense mutations in 45 patients; nonsense mutations, frameshifts, or large deletions in 28 patients; and a splice site mutation in 1 patient. In the group of patients without retinal manifestations, there were missense mutations in 44 patients and nonsense mutations, frameshifts, or large deletions in 31 patients.

No significant differences were established comparing the location of the mutation (exon or main functional domains of pVHL). However, the type of mutation (leading to a truncated protein or to an amino acid substitution) between the two groups of patients showed a significant association with the number of hemangioblastomas. The count of hemangioblastomas was modeled with a Poisson model. The analysis, using a marginal model, took into account the familial structure and was age adjusted. The number of hemangioblastomas appeared 2.1-fold higher in patients who had a substitution when compared with patients with a truncated protein (95% CI, 1.05–4.44; $P < 0.05$).

In the group with ocular manifestations, no significant relation was found between any specific genotype and the velocity of new retinal lesions or the retinal hemangioblastoma count, and no significant association found with the visual morbidity, as defined earlier.

**DISCUSSION**

Retinal hemangioblastoma is one of the most frequent tumors occurring during the course of VHL disease and can be responsible for significant visual impairment. This study was designed to contribute to defining the natural history, the visual morbidity, and genotype–phenotype correlation in a French population with VHL disease, comparing patients with and without ocular manifestations. As opposed to other studies, this study took into account the duration of follow-up (on average, 8 years) for the two groups of patients.14–15, 48 However patients were managed in different centers with different observers, and therefore the variability of the care provided in the centers and by the ophthalmologists weakens the study.

The impact of the ocular evaluation in VHL is considerable, because the first manifestation of VHL was ophthalmic in 50% of the patients. The ocular manifestations are known to occur early in the course of the disease, as confirmed by our series.3–7, 14–16, 48 Indeed, persons between 15 and 25 years of age are in a critical age group for the development of retinal hemangioblastomas. Patients with VHL in this age group warrant careful ophthalmic follow-up, keeping in mind the younger (6 years old) and older patients (60 years old) of this series diagnosed with retinal hemangioblastomas. The follow-up guidelines for the care of retinal hemangioblastomas in France advocate yearly ophthalmic examination from the age of 5 years. Between the age of 15 and 30 years, a funduscopy is recommended twice a year. After 30 years of age, a yearly examination is recommended. The rate of new hemangioblastomas in elderly patients may be higher than expected from the data available to date because of the increasing life expectancy due to considerable improvement in the clinical management of VHL.

Visual symptoms, usually related to long-standing or active lesions, revealed ocular involvement in 40% of the patients. This result in our series shows the necessity of improving the systematic early detection of lesions.

Eleven percent of the eyes at initial examination and 15% of the eyes at final examination exhibited papillary hemangioblastomas. Optic disc hemangioblastoma occurred in 8.3% of the eyes in a British series.15 In our series, no hemangioblastoma was present at the posterior pole other than at the optic disc. Macular exudation and retinal detachment are the major complications induced by retinal hemangioblastomas. During follow-up, a decrease of the number of patients with exudation was noticed. There are two explanations for this: efficient treatment for some patients and absent final evaluation of the exudation in patients with advanced retinal lesions. At final evaluation, 17% of the eyes had a retinal detachment for which surgery was ineffective, and 3% of the eyes had to be enucleated.

At final examination, 30% of the patients were classified in the severe visual loss group, compared with 21% at the beginning of follow-up. In this series, the risk of visual impairment increased with the hemangioblastoma count but not with the age at discovery or with the occurrence of visual symptoms. For these two last points, the results herein are not in accordance with those in a previous series.15

### Table 2. Incidence of Other VHL Manifestations in Patients of the Study

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Patients with Retinal Hemangioblastomas ($n = 103$)</th>
<th>Patients without Retinal Hemangioblastomas ($n = 108$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS hemangioblastoma</td>
<td>77 (75%)</td>
<td>83 (77%)</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>53 (51%)</td>
<td>46 (43%)</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>21 (20%)</td>
<td>23 (21%)</td>
</tr>
<tr>
<td>Pancreatic neuroendocrine tumor</td>
<td>13 (16%)</td>
<td>17 (16%)</td>
</tr>
</tbody>
</table>

Data are number of patients with each tumor type with percentage of total patients in each category in parentheses.

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**TABLE 2. Incidence of Other VHL Manifestations in Patients of the Study**

- CNS hemangioblastoma: 77 (75%) vs. 83 (77%)
- Renal cell carcinoma: 53 (51%) vs. 46 (43%)
- Pheochromocytoma: 21 (20%) vs. 23 (21%)
- Pancreatic neuroendocrine tumor: 13 (16%) vs. 17 (16%)

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14–15, 48
This study did not demonstrate a significant relation between retinal hemangioblastomas and other systemic VHL manifestation. Moreover, the incidence of retinal hemangioblastomas was not increased in the presence of CNS hemangioblastomas, which share the same histologic features. These results contradict those in a previous study in which patients with ocular hemangioblastoma had a significantly increased incidence of cerebellar hemangioblastoma and renal cell carcinoma. In this study, we could not show that the risk of development of a renal cell carcinoma or CNS hemangioblastoma was different between the two groups of patients with or without ocular involvement. However, our logistic approach did not take into account the date of onset of each extraocular lesion. The clinical follow-up of extraocular manifestations of the disease should be identical in the presence or absence of retinal hemangioblastoma.

A genotype–phenotype correlation in VHL has emerged with confirmation of the clinical pheochromocytoma-based

### Table 3. Details of VHL Germline Mutations in Patients in the Study

<table>
<thead>
<tr>
<th>Mutational Event</th>
<th>Type</th>
<th>Localization</th>
<th>Codon</th>
<th>Wild-Type Codon</th>
<th>Wild-Type Amino Acid</th>
<th>Protein Consequence</th>
<th>Patients with Retinal Hb (n)</th>
<th>Patients without Retinal Hb (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>136G→T</td>
<td>Ts</td>
<td>Exon1</td>
<td>46</td>
<td>GAG</td>
<td>E</td>
<td>E46X</td>
<td>1</td>
<td>0</td>
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<tr>
<td>154G→A</td>
<td>Ts</td>
<td>Exon1</td>
<td>52</td>
<td>GAG</td>
<td>E</td>
<td>E52K</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>161insG</td>
<td>Fs</td>
<td>Exon1</td>
<td>54</td>
<td>ATG</td>
<td>M</td>
<td>Stop131/no initiation</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>179delG</td>
<td>Fs</td>
<td>Exon1</td>
<td>60</td>
<td>CGG</td>
<td>R</td>
<td>Stop66</td>
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<td>TCG</td>
<td>S</td>
<td>S60P</td>
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<td>197del24</td>
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<td>Exon1</td>
<td>66</td>
<td>GTC</td>
<td>V</td>
<td>Frame shift</td>
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<td>211insT</td>
<td>Ts</td>
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<td>CAG</td>
<td>Q</td>
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<tr>
<td>226–228delTTC</td>
<td>In</td>
<td>Exon1</td>
<td>76</td>
<td>TTC</td>
<td>F</td>
<td>In-frame deletion</td>
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<td>AAT</td>
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<td>N78S</td>
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<td>GGT</td>
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<td>Exon1</td>
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Nucleotides are numbered according to the international VHL data base. Hb, hemangioblastoma.
Retinal Hemangioblastoma in von Hippel-Lindau Disease

Acknowledgments

The authors thank Guy Allegre and Rachel Messaoudi for technical assistance.

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sight into natural history of pheochromocytoma. J Urol. 1999;162:
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APPENDIX

Participating Ophthalmologists and Their Locations

Jean-Pierre Agez (Enghien-Les-Bains); Claire Audouin-Barthelat
(Ambert); Franck Bacin ( Clermont Ferrand); Martine Banche-
reau (Le Mans); Dominique Baudet (Rennes); Franck Becquet
(Nantes); André Benko (Chezelles); Jean-Pierre Biestro
(Dieppe); Bernard Bievellez (Lyon); Mireille Bonnet (Lyon);
Mireille Bouchet (Mont De Marsan); Gilles Chaine (Boginy);
Isabelle Cocherneau-Massin (Paris); Dominique Commeau
(Cormelles En Parisis); George Constantinides (Lille); Philep
Cordier (Nevers); Gabriel Coscas (Créteil); Christian Creton
(Metz); Bernard Deroche (Angers); Pierre Devin (Marq-En
Baroeul); Curan Dominique (Le Mans); Annie Salvanet (Maisons-Alfort); Anny Segal (Reims);
Didier Grange (Lyon); Pascale Houtteville (Caen); Jean Ivanez
(Douai); Elisabeth Joubaud-Nenert (Gue-
rande); Marie Laval (Paris); Jean-Louis Dufier (Paris);
Claire Fagart (Le Mans); Jacques Flamant ( Strasbourg); Jean-Luc
Franco (Montluçon); Jean-Pierre Francois (Lille); Marie-Paule
Francis (Joueul); Chantal Gaianopoulo (Aulnay-Sous-Bois); Jean
Didier Grange (Lyon); Pascale Houtteville (Caen); Jean Ivanec
(Douai); Elisabeth Joubaud-Nenert (Guérande); Marie Laval
(Brive); Martine Logeais (Rennes); Raymond Louly (Cambrai);
Etienne Magniere (Montluçon); Jean Maugery (Saint-Etienne);
André Mathis ( Toulouse); Claire Monin (Paris); Dominique
Mouilleau (Le Mans); Michel Paques (Paris); Michel Pomes
(Maizon-Allfort); Antoine Raspiller (Nancy); Philippe Razemon
(Lille); Anne Robert ( Brest); Dominique Salame (Paray-Le-
Mion); Annie Salvanet (Maizon-Allfort); Anny Segal (Reims);
Gisèle Soubrane (Créteil); Jean-Pierre Turcat (Chambéry); Jean-
Pierre Vermelle (Lie-

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