Isolation of Sochi Virus From a Fatal Case of Hantavirus Disease With Fulminant Clinical Course

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Sochi virus, a novel genetic variant of Dobrava-Belgrade virus, was isolated in cell culture from a fulminant lethal case of hantavirus disease presenting with shock and combined kidney and lung failure. Sochi virus is transmitted to humans from host reservoir Apodemus ponticus and must be considered a life-threatening emerging agent.

Hantaviruses are pathogens that are transmitted from their reservoirs, small mammals, to humans. Hantavirus diseases present as 2 different clinical syndromes: hantavirus cardiopulmonary syndrome (HCPS) caused by viruses circulating in the Americas, and hemorrhagic fever with renal syndrome (HFRS) caused by viruses from Asia and Europe [1, 2]. The clinical severity of infections by the various hantaviruses is highly variable, with the American Sin Nombre and Andes viruses the most dangerous representatives exhibiting case-fatality rates of 30%–40% [3]. Both HFRS and HCPS present with acute thrombocytopenia and changes in vascular permeability. Although both syndromes may include renal and pulmonary symptoms, the disease caused by Old World hantaviruses (HFRS) predominantly shows renal impairment, whereas New World hantavirus disease (HCPS) is mainly associated with cardiopulmonary failure.

In Europe there are 2 main pathogenic hantaviruses, Puumala virus (PUUV) and Dobrava-Belgrade virus (DOBV). Of the latter virus, 3 genetic lineages (DOBV-Af, DOBV-Aa, DOBV-Ap) are hosted by different mice species of the Apodemus genus (A. flavicollis, A. agrarius, and A. ponticus, respectively) [4–6]. The first molecular, serological, and clinical investigations of HFRS patients in southern European Russia have demonstrated that the Sochi virus (representing the DOBV-Ap lineage) is endemic in this geographical area and is responsible for moderate to severe HFRS in humans [5, 7].

Fatal Sochi virus infections have not yet been described in detail, and no virus isolate from a patient exists. Here we report on a fulminant course of infection leading to shock, renal and pulmonary failure, and rapid death 4 days after the onset of fever. From this patient, the first Sochi virus isolate of human origin has been generated in cell culture and was named Sochi/hu. Sochi virus must be regarded as a highly pathogenic and life-threatening agent.

Methods
Hantavirus Serodiagnostics
Sera from HFRS patients were tested by immunofluorescence assay (IFA) for the presence of hantavirus antibody as described previously [8]. For screening of sera, we used IFA slides containing a mixture of Vero E6 cells infected with PUUV, DOBV, Hantaan, and Seoul viruses. For primary serotyping, we used monovalent antigens (Vero E6 cells infected by either DOBV-Ap, DOBV-Aa, or PUUV) for IFA. For fine serotyping, we performed focus reduction neutralization tests (FRNT) as described previously [9].

Virus Isolation and Identification in Cell Culture
Blood taken from the patient 4 days after the onset of fever was transported in liquid nitrogen to the laboratory. We homogenized 0.5 mL of whole blood with 0.5 mL Eagle’s minimal essential medium (EMEM) and added it to a Vero E6 cell suspension. After exposure for 1 hour at 37°C with agitation, the cells were twice washed in medium and then resuspended in 8 mL of EMEM with 10% newborn calf serum and seeded into a 50-mL vial. The infected cells were passaged every 10–13 days and checked for hantavirus antigen by IFA. Antigen positive cells were detected with 10% newborn calf serum and seeded into a 50-mL vial. The infected cells were passaged every 10–13 days and checked for hantavirus antigen by IFA. Antigen positive cells were detected by IFA. After 4 additional passages, the final isolate was prepared and serotyped by FRNT.

Rodent Trapping and Screening
Small mammals were trapped in the Sochi region, Lazarevsky district, in the summer of 2008. Lung tissues of mammals were
screened for the presence of hantavirus antigens by an antigen-capture enzyme-linked immunosorbent assay as described previously [10] and for hantavirus antibodies by IFA using slides with combined hantavirus antigen (see previous section).

**Genome Amplification and Characterization**

Hantaviral RNA was extracted by QIAamp Viral RNA Mini Kit (Qiagen) directly from cell culture supernatant according to standard procedures. Amplification and sequencing of the entire S and M segments were performed as described for the DOBV SK/Aa isolate [11]. The L-segment complementary DNA was obtained by long-distance polymerase chain reaction (PCR) in 2 overlapping parts, followed by cloning into Stratagene pSC-A PCR Cloning Vector (Agilent Technologies) and sequenced by genome walking. We performed the phylogenetic analysis based on complete S segment open reading frame sequences (1149 nt) using MEGA 5 software [12].

**Results**

A 47-year-old woman living in the city of Sochi, southern European Russia, without previous serious disease, developed high fever (40°C), malaise, chills, general weakness, and abdominal pain. One day later (day 2), her clinical condition deteriorated significantly, and she additionally suffered from nausea, vomiting, increasing abdominal pain, and diarrhea. The patient was hospitalized under the suspicion of acute pancreatitis. At day 4, her body temperature became normalized; however, oliguria, hypotension, tachycardia, ventilation insufficiency, peripheral edema, and facial swelling appeared. Artificial ventilation was started in the morning of day 5. Urine production decreased continuously and the patient died from shock combined with acute renal failure and disseminated intravasal coagulation.

The most prominent biochemical results at day 4 showed significant elevations of serum creatinine, liver enzymes, and blood urea as well as thrombocytopenia and proteinuria. Postmortem investigation demonstrated disseminated intravasal coagulation with thrombosis, hemorrhagic infiltrations, and micronecrosis in the parenchyma of multiple organs, in particular, kidneys, liver, and adrenal glands. Furthermore, desquamative hemorrhagic gastroenteritis and pulmonary edema were found. In the blood taken before death, IFA antibodies against PUUV, DOBV-Aa, and DOBV-Ap showed titers of 1:128, 1:4096, and 1:4096, respectively. No sign of other acute infection was found. No specific risk of hantavirus infections was known from the patient’s medical history, besides regular walking with her dog in a suburban forest zone of the town of Sochi.

From a blood specimen of the patient, a virus isolate was established in Vero E6 cells and named Sochi/hu. In virus neutralization assays, both the Sochi/hu strain and the Ap1584/Sochi-01 (Sochi/Ap) strain, isolated from an A. ponticus animal, were found to be neutralized to an identical extent by sera of DOBV-Ap–infected patients (data not shown).

The complete sequences of the S, M, and L segments were obtained and compared with prominent representatives of European DOBV species as well as Hantaan and Seoul virus (Table 1). As expected, there is a strong similarity between Sochi/hu and Sochi/Ap isolated from an A. ponticus mouse from the city of Sochi [5], whereas the most similar of the other DOBV lineages is the AP/Af isolate from Greece.

In the S segment ORF-based phylogenetic tree, Sochi/hu clusters with the rodent-derived isolate from the city of Sochi, Sochi/Ap. The next most closely related are 2 newly analyzed rodent samples (nos. 43 and 79) from the vicinity of Sochi and sequences of human and rodent origin from a region in the northwest of Sochi (Krasnodar/hu and GK/Ap). There is a clear correlation between the geographical spacing of the sampling localities and the phylogenetic distance of the virus sequences. All these sequences form a well-defined genetic lineage of DOBV, named DOBV-Ap, which shares a common ancestor with A. flavicollis–associated DOBV lineage, DOBV-Af (Figure 1). Phylogenetic trees based on M- and L-segment sequences were also constructed. The placement of Sochi/hu as the closest relative of Sochi/Ap remained the same as in the

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<tr>
<th>Table 1. Sochi/hu Virus Complete Nucleotide and Amino Acid Sequence Lengths and Percent Identities Compared With Representatives of the Dobrava-Belgrade Virus Species and Related Viruses</th>
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<tbody>
<tr>
<td><strong>Sochi/hu</strong></td>
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<tr>
<td><strong>S segment</strong></td>
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<td>Length&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>M segment</td>
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<td><strong>Virus isolate&lt;sup&gt;b&lt;/sup&gt;</strong></td>
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<tr>
<td>Sochi/Ap</td>
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<td>Apodemus agrarius</td>
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<td>Lipetsk/Aa</td>
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<td>Saa/160V</td>
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<tr>
<td>HTNV&lt;sub&gt;76–118&lt;/sub&gt;</td>
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<td>SEOV&lt;sub&gt;80–39&lt;/sub&gt;</td>
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<sup>a</sup> Lengths of the complete genomic segments and the deduced proteins as determined in this study.

<sup>b</sup> Accession numbers for S, M, and L segments, respectively; Sochi/Ap, EU188449, EU188450, EU188451; Lipetsk/Aa, EU188452, EU188453, EU188454; SK/Aa, AY961615, AY961616, GU904039; Slo/Af, GU904029, GU904035; Apodemus agrarius, AJ104042, AP/Af, AJ410619, AJ410616, AJ410617; Saa/160V, AJ009773, AJ009774, AJ410618; HTNV<sub>76–118</sub>, M14626, M14627, NC_005222; SEOV<sub>80–39</sub>, AY273791, S47716, X56492. The S, M, and L segment sequences determined in this study.

Abbreviations: aa, amino acid; Aa, Apodemus agrarius; Af, Apodemus flavicollis; Ap, Apodemus ponticus; AP, isolate Ano-Poario; HTNV, Hantaan virus; hu, Sochi virus isolate of human origin; nt, nucleotide; Saa, Saaremaa virus; SEOV, Seoul virus; SK, isolate Slovak; Slo, isolate Slovenia.

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S-segment analysis (data not shown), and the overall tree topologies resembled the trees previously constructed for Sochi/Ap [5].

Discussion
We report on a fulminant course of HFRS and the cell culture isolation and genetic characterization of the causative virus, named Sochi virus. The virus belongs to the very recently described DOBV-Ap genetic lineage of DOBV carried by Apodemus ponticus as reservoir host. Whereas in a preliminary study the case-fatality rate of HFRS caused by DOBV-Ap infection was determined to be 6% [5], more extensive studies demonstrate a rate >10% [7; our unpublished data]. This lets us qualify Sochi virus as an emerging virus and new health threat.

Our patient is the first terminal case of DOBV-Ap infection with reported clinical course leading to death by shock combined with kidney and lung failure. Whereas for infections by most European hantaviruses the kidney impairment is a central element of the clinical course of HFRS, this patient’s pulmonary insufficiency resembles HCPS caused by American hantaviruses. Moreover, the patient developed coagulation disturbances typical for HFRS and HCPS, massive gastroenteritis, and adrenal inflammation. Based on the latter finding (adrenal inflammation), one might speculate that hormonal alterations observed...

Figure 1. Maximum-likelihood phylogenetic tree of Dobrava-Belgrade virus (DOBV) S-segment open reading frame (ORF). The tree shows the phylogenetic placement of the novel human isolate Sochi/hu (top of the tree, in bold) compared with Sochi/Ap virus, additional DOBV sequences from Apodemus ponticus (GK/Ap, 43/Ap, 79/Ap), and 1 additional human sample (Krasnodar/hu). The Sochi/hu S segment ORF has a length of 1287 nucleotides (nt); however, the data set compiled only 1149 nt because all positions containing gaps and missing data in the other sequences were eliminated. Different DOBV lineages are marked by boxes: dark gray, DOBV-Ap; light gray, DOBV-Af, DOBV-Aa, and Saaremaa virus. The Sochi/hu as well as the rodent-derived 43/Ap and 79/Ap S-segment ORF sequences were deposited in GenBank (accession nos. JF920148, JF920151, and JF920152, respectively). The tree was computed with the MEGA 5 software via the maximum likelihood method with general time reversible model with gamma distributed rates among sites and invariant sites (GTR+G+I) evolutionary model. The values at the tree branches are the bootstrap support values of the corresponding neighbor-joining tree (maximum composite likelihood method). The scale bar indicates an evolutionary distance of 0.1 substitutions per position in the sequence. Abbreviations: SANGV, Sangassou virus; HTNV, Hantaan virus; SEOV, Seoul virus; PHV, Prospect Hill virus; TULV, Tula virus; PUUV, Puumala virus; ANDV, Andes virus; SNV, Sin Nombre virus.
during hantavirus infection and mainly attributed to disturbances in the hypothalamic–pituitary–adrenal axis [13] could be also caused by direct impairment of adrenal glands due to virus infection.

Phylogenetic analysis indicated geographic clustering of the DOBV-Ap strains (Figure 1, and data not shown). This finding indicates long-term presence of the virus in the investigated area and its close association with local A. ponticus populations. Therefore, recent recognition of Sochi virus as an HFRS-causative agent [5] might be attributed to the increasing awareness of hantavirus infections by local physicians and improved diagnostics rather than recent appearance of the virus in the area.

Sochi virus, taken as a synonym for DOBV-Ap, is a highly dangerous pathogen that in this respect resembles the members of the DOBV-Af lineage from A. flavicollis (yellow-necked mice) causing HFRS with case-fatality rates of about 10% in the Balkan region [14]. One should note that other members of DOBV, such as DOBV-Aa from A. agrarius (striped field mouse), cause HFRS with case-fatality rates of ≤1% [5, 9, 11]. It would be important to understand which differences between the genetically highly related DOBV lineages are responsible for this dramatically different degree of virulence. The analysis of the Sochi/hu isolate may be used as an essential prerequisite for further studies on genetic factors of hantavirus pathogenicity. Early data based on in vitro genetic reassortment studies show that determinants on the S and L segments of the tripartite virus genome contribute to differences in DOBV phenotypes [15].

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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