are similar to those of other common respiratory diseases, making a specific diagnosis of SARS poses difficulties to medical professionals. Our enhanced real-time (ERT) polymerase-chain-reaction (PCR) method (first presented in June 2003 at a symposium on SARS) has been designed for the detection of SARS-CoV with high sensitivity and easy-to-interpret results. The power of the ERT technique has now been extensively explored with the development of ERT-based diagnostic tests for various infectious diseases, including avian influenza and foot-and-mouth disease.

Since the first report of ERT results for SARS, the ERT technique has been modified to increase its sensitivity for the detection of SARS-CoV by at least 10 times (Fig. 1). This improved sensitivity has been achieved by combining the reverse-transcriptase (RT) and PCR steps into a single step (described in Supplementary Appendix 1, available with the full text of this letter at www.nejm.org). In addition, the procedural change makes the diagnostic procedure more convenient. These salient features of one-step RT-PCR have thus far been overlooked by other researchers in this field. Because the single RT-PCR step and the subsequent real-time PCR step require only 35 cycles, the detection of SARS-CoV by the modified ERT technique yields results quickly and with higher sensitivity than regular real-time PCR assays reported to date.

As noted by the World Health Organization with respect to the shortcomings commonly seen in available diagnostic tests for SARS, it is important to unify a molecular test for SARS that can provide sensitive, reliable, and accurate results. Currently, many research groups claim that their methods are accurate, but the way in which they evaluate accuracy is not clearly described. The usefulness of an accurate test that lacks sensitivity has yet to be determined. Unless a unified molecular test for SARS with high sensitivity and reliability is available, we may face the risk of false negative test results, which would allow infected patients to slip into the community and avoid control measures set up to isolate carriers.

Over a year after the start of the 2003 SARS outbreak, many people are still struggling to recover from the physiological and psychological scars inflicted at that time. Identifying potential SARS-CoV carriers by a method with high sensitivity and reliability and as early as possible is crucial to avoid a repetition of the 2003 outbreak.

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Table 1. Congenital Anomalies Associated with First-Trimester Statin Exposure.*

<table>
<thead>
<tr>
<th>Case</th>
<th>Drug and Dose</th>
<th>Exposure</th>
<th>Pregnancy Outcome</th>
<th>Comments</th>
<th>Approximate Prevalence of Isolated Malformations</th>
<th>Estimated Total Exposed Infants</th>
<th>Cases Isolated (Malformation Only)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reported</td>
<td>Calculated</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cerivastatin, 0.25 mg/day</td>
<td>0–8</td>
<td>Holoprosencephaly</td>
<td>Therapeutic abortion after prenatal diagnosis</td>
<td>1/16,000</td>
<td>11</td>
<td>600</td>
</tr>
<tr>
<td>2</td>
<td>Lovastatin, 40 mg/day</td>
<td>0–7</td>
<td>Holoprosencephaly (defective septum separating lateral cerebral ventricles, with cerebral dysfunction), atrial septal defect, aortic hypoplasia, death at 1 mo of age</td>
<td>Corrective cardiac surgery not performed because of poor overall prognosis; no concomitant medications or illness</td>
<td>Holoprosencephaly, 1/16,000; aortic hypoplasia, 1/50,000; atrial septal defect, 1/370</td>
<td>84</td>
<td>6,050</td>
</tr>
<tr>
<td>3</td>
<td>Lovastatin, 40 mg/day</td>
<td>0–4.5</td>
<td>Aqueductal stenosis with hydrocephalus, concurrent limb deficiency (right banded, atretic thumb)</td>
<td>46,XX, no concomitant medications or illness</td>
<td>Aqueductal stenosis with hydrocephalus, 1–3/10,000; banded, atretic thumb, &lt;1/50,000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Lovastatin, 20 mg/day</td>
<td>First trimester</td>
<td>Cervicothoracic-to-lumbar neural-tube defect, myelole, duplication of spinal cord, cerebellar herniation with hydrocephalus; apparent agenesis of palate</td>
<td>46,XX, no concomitant medications or illness, duration of exposure reported inconsistently</td>
<td>Neural-tube defect, &lt;1/10,000; palate agenesis, 1/10,000</td>
<td>1</td>
<td>0 (complex neural-tube defect)</td>
</tr>
<tr>
<td>5</td>
<td>Atorvastatin, Until pregnancy recognized</td>
<td>Simvastatin, 10 mg/day</td>
<td>Spina bifida, right-arm abnormality</td>
<td>Type 1 diabetes</td>
<td>3/10,000 (approximate rate in diabetic pregnancy, year of case report [1999])</td>
<td>21</td>
<td>12,000</td>
</tr>
<tr>
<td>6</td>
<td>Simvastatin, 20 mg/day</td>
<td>0–6</td>
<td>Right leg: fibula and tibia 9% shorter than left side, agenesis of one tarsal bone; right foot 16% shorter than left (reported at 4 yr of age)</td>
<td>Concomitant medications: aspirin, codeine, acetaminophen, propoxyphene during 1st mo of gestation</td>
<td>“Unclassifiable” complex lower-limb deficiency, 1/100,000</td>
<td>393</td>
<td>7,075</td>
</tr>
<tr>
<td>7</td>
<td>Simvastatin, 10 mg/day</td>
<td>0–13</td>
<td>Left leg: femur 16% shorter than right side; foot: aplasia of metatarsals and phalanges 3, 4, and 5; additional VACTERL defects: left renal dysplasia, reversed laterality of aorta, disorganized lumbo-sacral vertebrae, single umbilical artery; additional findings: clitoral hypertrophy, vaginal and uterine agenesis</td>
<td>46,XX; concomitant medication: progestogen (10 days/mo), duration 0–13 wk</td>
<td>“Unclassifiable” complex lower limb deficiency, 1/100,000; four-component VACTERL, &lt;1/50,000</td>
<td>2</td>
<td>VACTERL associations with more than three features</td>
</tr>
</tbody>
</table>

*Approximate prevalence of isolated malformations for each drug is based on published reports and the authors' experience. Numbers in parentheses indicate estimated prevalence for each malformation. The denominator is based on the total number of infants exposed to the medication, taking into account the duration of exposure and the estimated total number of infants exposed. The numerator is the number of infants reported to have the specific malformation. The expected number of cases is calculated based on the prevalence of malformations in the general population. The calculated number is based on the exposure data and the prevalence of malformations. The cases isolated (Malformation Only) column indicates the number of cases isolated for each malformation, with the number of cases isolated for each drug indicated in parentheses. The estimated total exposed infants column indicates the estimated total number of infants exposed to the medication, taking into account the duration of exposure and the estimated total number of infants exposed. The cases isolated (Malformation Only) column indicates the number of cases isolated for each malformation, with the number of cases isolated for each drug indicated in parentheses.
Table 1. (Continued.)

<table>
<thead>
<tr>
<th>Case</th>
<th>Drug and Dose†</th>
<th>Exposure</th>
<th>Pregnancy Outcome</th>
<th>Comments</th>
<th>Approximate Prevalence of Isolated Malformations</th>
<th>Estimated Total Exposed Infants‡</th>
<th>Cases Isolated (Malformation Only)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Lovastatin, 10 mg/day</td>
<td>6–11</td>
<td>Left arm: aplasia of radius and thumb, shortened ulna; additional VACTERL defects: left arthrogryposis, thoracic scoliosis, fusion of ribs on left, butterfly vertebrae in thoracic and lumbar region, esophageal stricture, anal atresia, renal dysplasia; additional findings: hemihypertrophy of entire left side, craniofacial anomalies (including asymmetric ears, ptosis of eyelids, high arched palate), torticollis</td>
<td>Concomitant medications: dextroamphetamine for wk 6–11 of gestation, 46,XX, negative for Fanconi’s anemia</td>
<td>Five-component VACTERL, &lt;1/500,000</td>
<td>84</td>
<td>6,050</td>
</tr>
<tr>
<td>9</td>
<td>Atorvastatin, 10 mg/day</td>
<td>0–9</td>
<td>Limb-reduction deficiency; transverse deficiency of otherwise normal radius and ulna superior to wrist structures, with aplasia of all distal structures</td>
<td>Transverse deficiency in distal third of forearm, absence of all metacarpal and phalangeal structures, no bony abnormalities above truncation, estimated 1/1,000,000</td>
<td>21</td>
<td>12,000</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Lovastatin, 40 mg/day</td>
<td>0–4.5</td>
<td>As described above (Case 3): banded, atretic thumb with aque ductal stenosis</td>
<td>As described above (Case 3)</td>
<td>Banded, atretic thumb, &lt;1/50,000; a queductal stenosis with hydrocephalus, 1–3/10,000</td>
<td>84</td>
<td>6,050</td>
</tr>
</tbody>
</table>

* The patient in Case 3 had both central nervous system and limb defects and is therefore listed twice. Eleven additional cases involving structural anomalies were as follows: simvastatin: cleft lip with intrauterine growth restriction, cleft lip (prospective report), polyactylly (prospective report), duodenal atresia (prospective report), hypospadias (prospective report), clubfoot, and “major abnormalities” not otherwise specified (therapeutic abortion); atorvastatin: cleft palate and esophageal atresia; lovastatin: microtia with absent auditory canal, and “severe deformity” not otherwise specified. Thirty-two other outcomes were as follows: intrauterine growth restriction (4, all simvastatin), intrauterine fetal death (3, simvastatin; 2, lovastatin), and healthy infants (including preterm births) (23). VACTERL denotes vertebral, anal, cardiac, tracheal, esophageal, renal, and limb defects.

† The number of reports that could be evaluated and the total number submitted are as follows: atorvastatin (Lipitor), 7 and 21, respectively; cerivastatin (Baycol, withdrawn from the market in 2001), 1 and 11; lovastatin (Mevacor), 15 and 28; and simvastatin (Zocor), 25 and 102. Information for other drugs (not shown) is as follows: pravastatin (Pravachol), 3 and 14; the 3 outcomes that could be evaluated were normal; estimated number of births, 5,000; and fluvastatin (Lescol), 1 and 2; the 1 outcome that could be evaluated was normal; estimated number of births, 1,500.

‡ The following method was used for the estimation of exposures. The smaller number (the reported value) equals the number of exposures reported to the FDA plus any additional exposures (according to information from the manufacturers) that did not require an FDA report (e.g., normal outcome). We then calculated a predicted number of births after statin exposure. The algorithm used incorporated sex- and age-specific prescribing data for each drug. Age-specific birth rates were used to yield an approximate total number of births potentially exposed to each agent through 2001. Assumptions include full compliance with the dispensed medication, discontinuation of the drug before conception in the estimated 50 percent of pregnancies that are planned, and comparability with the general population for age-specific birth rates. Limit their family size over time rather than remain at continual age-specific risk of pregnancy throughout the duration of their drug exposure.

§ The reported numbers reflect only those adverse birth outcomes included in the FDA data base, which is generally considered to underrepresent actual events. They are not true “observed” numbers, such as those that would be available if ascertainment were complete. Expected numbers are derived from predicted birth numbers and population background rates; they refer to a theoretical number of population events rather than an expected number of reports to the FDA.
bone shortening and aplasia or hypoplasia of the foot structures. The infant in one of these cases and a lovastatin-exposed infant also had rare forms of the VACTERL association (i.e., three or more of the following findings: vertebral, anal, cardiac, tracheal, esophageal, renal, and limb defects).

In all cases of adverse outcomes at birth, the associated statin was lipophilic. Cerivastatin, simvastatin, lovastatin, and atorvastatin all achieve embryoplacental concentrations similar to those of maternal plasma. In studies in animals, lipophilic statins have been shown to have adverse reproductive effects in the axial skeleton, viscera, or central nervous system. No malformations were reported among 14 infants exposed to pravastatin; this statin is hydrophilic, has low tissue penetration, and has not caused reproductive toxic effects in animals.

Holoprosencephaly and the VACTERL association have been linked to inhibition of cholesterol biosynthesis, down-regulation of the cholesterol-dependent sonic hedgehog morphogenetic pathway, or both. These malformations as well as neural-tube and cardiac defects are also associated with maternal diabetes; thus, diabetes might confound the association between statin use and these malformations. However, maternal diabetes was identified in only 7 of 178 case reports and 1 of 20 cases of malformation (spina bifida).

It is thought that only a small proportion of adverse events are reported to the FDA; however, reports are likely to be biased toward severe outcomes. The number of births after first-trimester exposures to statin are unknown. Table 1 presents both the number of reported exposures and the predicted number of exposures on the basis of prescription data and birth rates. There would be no expected cases of most of the malformations listed in the table, even allowing for the imprecision of estimating exposures; yet three rare anomalies are each observed twice in this small series.

Data from case series cannot be used to test hypotheses of teratogenicity. However, these findings support the need for controlled epidemiologic studies evaluating the potential teratogenic effects of individual drugs in this class.

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Gestational Exposure to Lovastatin Followed by
Cardiac Malformation Misclassified as
Holoprosencephaly

To the Editor: In 2004, we reported central nervous system and
limb anomalies that followed exposure to statin drugs in the first
trimester of pregnancy (April 8, 2004, issue).1 One case, in which
there had been exposure to lovastatin, was described as involv-
ing holoprosencephaly on the basis of three separate reports of a
“cerebral/brain ventricular septal defect,” with accompanying cardiac
malformations that had been submitted to the Food and Drug Ad-
ministration adverse-event database. We recently learned that the
manufacturer considered the structural anomalies to be solely car-
diac, and therefore we requested source documentation to clarify the
conflicting reports. A detailed clinical report that was located among
archival documents clearly described an atrial septal defect, a ven-
tricular septal defect, and aortic hypoplasia leading to cardiac failure,
with secondary central nervous system dysfunction. It was apparent
that a data-extraction error had occurred, incorrectly categorizing the
ventricular septal defect as an intracranial anomaly. Correcting this
misclassification reduces our reported number of lovastatin-exposed
fetuses with midline central nervous system anomalies from three to
two. We still believe that the preponderance of the evidence supports
the hypothesis that early gestational exposure to statin drugs may be
teratogenic and that prospective studies should be initiated.

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References

1. Edison RJ, Muenke M. Central nervous system and limb anom-
alias in case reports of first-trimester statin exposure. N Engl J Med