Effectiveness of posthumous molecular diagnosis from a kept baby tooth

Helen Leonard, Mark R Davis, Gavin R Turbett, Nigel G Laing, Carol Bower, David Ravine

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Centre for Child Health Research, University of Western Australia, Telethon Institute for Child Health Research, Perth, Western Australia (H Leonard MbChB, C Bower PhD); **Neurogenetics Laboratory, Department of Anatomical Pathology, Royal Perth Hospital, Perth, Western Australia** (M R Davis PhD); **Forensic Biology Laboratory, Forensic Services, Western Australian Centre for Pathology and Medical Research, Nedlands, Western Australia** (G R Turbett PhD); **Centre for Medical Research, University of Western Australia, Western Australian Institute for Medical Research, Perth, Western Australia** (N G Laing PhD); **and Western Australian Institute for Medical Research, School of Medicine and Pharmacology, University of Western Australia, Western Australia** (D Ravine DM)

Correspondence to:
Dr Helen Leonard
hleonard@worra.ichr.uwa.edu.au

In April, 2004, we met the parents of a young girl who had died almost 13 years earlier. In September, 1991, their 7-year-old daughter had died of pneumonia after a 5-year history of undiagnosed neurological decline. She was the second live-born child of a couple with a healthy son, now in his mid-twenties. The girl was born at term after a pregnancy complicated only by slight early bleeding. Two previous pregnancies had resulted in stillbirths due to abruptio placentae. The child thrived during her first year and there were no parental concerns. At 16 months, however, they became uneasy because she was not walking independently. Parental anxiety was further heightened by loss of previously acquired words. At 17 months, she fell down steps, hitting her head on a cement floor but with no loss of consciousness. By her second birthday functional hand use, which had developed to the stage of self-feeding and pushing toy cars, was replaced by repetitive hand movements, including relentless finger chewing and sucking. Seizures, which responded only partially to anticonvulsant therapy, started after her third birthday. Between the ages of 2 years and 4 years she underwent intensive neurological investigations, which did not reveal a cause for her condition. Her deterioration continued and, in the absence of a diagnosis, her parents began to wonder whether they had somehow been responsible. As well as fears about the role of a vaccination a month before the onset of symptoms, the mother harboured concerns about a possible inherited disorder, and the father feelings of guilt about the circumstances of the child's fall.

During the child's lifetime no definite medical diagnosis was ever made, although Rett syndrome had been suggested by an occupational therapist visiting her school. An article about Rett syndrome in a women's magazine in 2002 rekindled the mother's interest in exploring this diagnosis. By then she had become concerned about the potential genetic risk for any future grandchildren. In 2004 contact with the Australian Rett Syndrome project,[1] as a result of an internet search, led to the family's participation and completion of a study questionnaire; this was our first contact with the family. Information provided was clinically consistent with Rett syndrome and the mother enquired about whether diagnostic confirmation could be provided by examination of DNA from a kept baby tooth. After discussion and explanation about the limitations of the test sensitivity (about 80%), we agreed to proceed. The tooth was cut open laterally (figure). The pulp was removed and digested with proteinase K and dithiothreitol, and the DNA was extracted with 5% Chelex 100.[2] Mutation screening by bidirectional sequencing of the *MECP2* coding regions revealed a nonsense mutation (c.502C>T; p.R168X), which is a well known recurrent mutation causative of Rett syndrome.[3] The result and its implications were explained to the family.

Figure: **Baby tooth; uncut and cut surfaces**

Beyond feelings of relief from finally understanding the cause of their daughter's condition and death, diagnostic confirmation has helped in other ways. The sense of guilt about the child's fall at 2 years of age has gone, as have the longstanding fears about her vaccination as a toddler. Explanations of the nature of X-linked inheritance (the *MECP2* gene is on the X chromosome) and that the rare male-associated *MECP2* phenotype involves neurological impairment, often of neonatal onset,[4] has allowed the couple to appreciate that their son's good health alone shows that he could not have inherited the mutation that caused his sister's illness. Posthumous molecular genetic diagnosis by use of DNA from commonly treasured baby teeth is now technically simple. Although common in forensic medicine, posthumous accessing of DNA from unconventional sources such as deciduous dental pulp has seldom been used in genetic counselling situations.[5] We proceeded with this investigation on the basis of a clear clinical rationale. As well as diagnostic confirmation, the result has also brought closure to many long-standing psychological stresses for a couple, one sign of which was the drive that eventually prompted the investigation 14 years after the death of their daughter.

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References

1 Colvin L, Fyfe S, Leonard S, et al. Describing the phenotype in Rett syndrome using a population database. *Arch Dis Child* 2003; **88:** 38–43.

2 Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991; **10:** 506–13.

3 Christodoulou J, Grimm A, Maher T, Bennetts B. RettBASE: The IRSA MECP2 variation database—a new mutation database in evolution. *Hum Mutat* 2003; **21:** 466–72.

4 Moog U, Smeets E, Van Roozendaal K, et al. Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females (MECP2). *Eur J Paediatr Neurol* 2003; **7:** 5–12.

5 Restagno G, Ferrone M, Doriguzzi C, Palmucci L, Mongini T, Carbonara A. Carrier detection of Duchenne muscular dystrophy through analysis of DNA from deciduous teeth of a dead affected child. *Prenat Diagn* 1995; **15:** 672–74.