Congenital mirror movements

Mutational analysis of *RAD51* and *DCC* in 26 cases

Aurélie Méneret, MD*
Christel Depienne, PhD*
Florence Riant, PharmD
Oriane Trouillard
Delphine Bouteiller
Massimo Cincotta, MD
Pierre Bitoun, MD
Julia Wickert, MD
Isabelle Lagroua, MS
Ana Westenberger, PhD
Alessandra Borgheresi, MD
Diane Doummar, MD
Marcello Romano, MD
Simone Rossi, MD, PhD
Luc Defebvre, MD
Linda De Meirleir, MD, PhD
Alberto J. Espay, MD, MSc, FAAN
Simona Fiori, MD
Stephan Klebe, MD
Chloé Quélin, MD
Sabine Rudnik-Schöneborn, MD
Ghislaine Plessis, MD
Russell C. Dale, PhD
Susan Sklower Brooks, MD
Karolina Dziezyc, MD
Pierre Pollak, MD
Jean-Louis Golmard, MD, PhD
Marie Vidailhet, MD
Alexis Brice, MD
Emmanuel Roze, MD, PhD

Correspondence to Dr. Roze: emmanuel.flamand-roze@psl.aphp.fr

Supplemental data at Neurology.org

ABSTRACT

Objective: We screened a large series of individuals with congenital mirror movements (CMM) for mutations in the 2 identified causative genes, *DCC* and *RAD51*.

Methods: We studied 6 familial and 20 simplex CMM cases. Each patient had a standardized neurologic assessment. Analysis of *DCC* and *RAD51* coding regions included Sanger sequencing and a quantitative method allowing detection of micro rearrangements. We then compared the frequency of rare variants predicted to be pathogenic by either the PolyPhen-2 or the SIFT algorithm in our population and in the 4,300 controls of European origin on the Exome Variant Server.

Results: We found 3 novel truncating mutations of *DCC* that segregate with CMM in 4 of the 6 families. Among the 20 simplex cases, we found one exonic deletion of *DCC*, one *DCC* mutation leading to a frameshift, 5 missense variants in *DCC*, and 2 missense variants in *RAD51*. All 7 missense variants were predicted to be pathogenic by one or both algorithms. Statistical analysis showed that the frequency of variants predicted to be deleterious was significantly different between patients and controls ($p < 0.001$ for both *RAD51* and *DCC*).

Conclusion: Mutations and variants in *DCC* and *RAD51* are strongly associated with CMM, but additional genes causing CMM remain to be discovered. ***Neurology*® 2014;82:1999–2002**

GLOSSARY

CMM = congenital mirror movements; **dbSNP** = Single Nucleotide Polymorphism Database; ***DCC*** = deleted in colorectal carcinoma; **EVS** = Exome Variant Server; **MM** = mirror movements; **OMIM** = Online Mendelian Inheritance in Man; ***RAD51*** = RAD51 recombinase.

Mirror movements (MM) are involuntary movements of one side of the body that mirror intentional movements on the opposite side. MM predominate in the upper limbs, mainly involving muscles controlling the fingers and hands.[1] Isolated congenital MM (CMM [OMIM #157600]) constitute a rare disorder characterized by MM that persist throughout adulthood. It has been described as a familial disorder with autosomal dominant inheritance, but simplex cases also exist. MM impair the ability to perform tasks requiring skilled bimanual coordination and are associated with pain in the upper limbs during sustained manual activities. MM result from various functional and structural abnormalities of the motor network, including altered decussation of the corticospinal tracts.[2] Recently, heterozygous mutations in *DCC* (deleted in colorectal carcinoma

*These authors contributed equally to this work.

From INSERM, U 975, and CNRS 7225–CRICM (A.M., C.D., O.T., D.B., I.L., M.V., A.B., E.R.), Département de Neurologie (A.M., M.V., E.R.), Fédération de Génétique, Département de Génétique et de Cytogénétique (C.D., A.B.), Banque d'ADN et de cellules (I.L.), Department of Biostatistics (J.-L.G.), and Centre d'Investigation Clinique Pitié Neurosciences 1422 (E.R.), Hôpital Pitié-Salpêtrière, AP-HP, Paris; Université Pierre et Marie Curie-Paris-6 (A.M., C.D., M.V., A.B., E.R.), UMR_S 975, Paris; Laboratoire de Génétique (F.R.), Groupe hospitalier Lariboisière-Fernand Widal, AP-HP, Paris; INSERM UMR_S740 (F.R.), Université Paris 7 Denis Diderot, Paris, France; Unit of Neurology (M.C., A.B.), Florence Health Authority, Italy; Génétique Médicale (P.B.), CHU Paris Nord, Hôpital Jean Verdier, Bondy, France; Department of Human Genetics (J.W.), University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Institute of Neurogenetics (A.W.), University of Lübeck, Germany; Service de Neuropédiatrie (D.D.), Hôpital Trousseau, AP-HP, Paris, France; Unit of Neurology (M.R.), Villa Sofia-Cervello Hospital, Palermo; Department of Neuroscience (S.R.), Section of Neurology and Clinical Neurophysiology, Azienda Ospedaliera Universitaria of Siena, Italy; Neurologie et Pathologie du Mouvement (L.D.), Neurologie A, Hopital Salengro, Centre Hospitalier Universitaire, EA 1046, Lille, France; Pediatric Neurology and Metabolism (L.D.M.), Universitair Ziekenhuis Brussel, Belgium; James J. and Joan A. Gardner Family Center for Parkinson's disease and Movement Disorders (A.J.E.), University of Cincinnati Academic Health Center, Cincinnati, OH; IRCCS Fondazione Stella Maris (S.F.), Calambrone, Pisa, Italy; Department of Neurology (S.K.), University Hospital Würzburg, Germany; Service de Génétique Clinique (C.Q.), Hôpital Sud, Rennes, France; Institute for Human Genetics (S.R.-S.), Uniklinik RWTH Aachen, Germany; Service de Génétique (G.P.), CHU Clémenceau, Caen, France; The Children's Hospital at Westmead (R.C.D.), University of Sydney, Australia; Division of Medical Genetics (S.S.B.), Rutgers, Robert Wood Johnson Medical School, New Brunswick, NJ; 2nd Department of Neurology (K.D.), Institute of Psychiatry and Neurology, Warsaw, Poland; and Service de Neurologie (P.P.), Hôpitaux universitaires de Genève, Switzerland.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

[OMIM *120470]) and *RAD51* (RAD51 recombinase [OMIM *179617]) have been identified, respectively, in 3 and 2 families with autosomal dominant CMM.[3–5] *DCC* encodes the receptor for netrin 1 (NTN1 [OMIM *601614]), which promotes attraction and guidance of developing axons across the body's midline.[6] *RAD51* is mostly known for its role in DNA repair through homologous recombination,[7] but its recent implication in CMM has revealed its possible role in the development of the motor system.[2,4] So far, *DCC* and *RAD51* seem to account for most CMM families, but their implication has yet to be tested in simplex cases. In this study, we screened 6 familial and 20 simplex CMM cases for mutations in *DCC* and *RAD51*.

METHODS **Patients.** We studied 26 consecutive index cases with CMM including 6 families (total of 13 affected subjects) and 20 simplex cases. Each patient, as well as available family members, had a standardized neurologic assessment and DNA sampling. The severity of MM was scored with the Woods and Teuber scale.[8] Familial history, MM location, associated disorders, and reported functional disability were collected. A total of 658 unrelated healthy controls (348 Caucasians, 222 North Africans, 88 Turks) were also included to test for new variants.

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained from the patients (or the parents of minors) before genetic analyses. The study was approved by the ethics committee of the Pitié-Salpêtrière Hospital, Paris.

Genetic analyses. The coding and flanking intronic regions of *DCC* and *RAD51* were amplified as previously reported.[4,5] Forward and reverse sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA), and the products were analyzed on an ABI 3730 automated sequencer (PE Applied Biosystems). Quantitative multiplex PCR of short fluorescent fragments analysis was performed for all exons of *DCC* and *RAD51* and results were analyzed using GeneMapper analysis software version 4.0 (Applied Biosystems). To look for abnormal splicing, RNA was extracted from lymphocytes of patient 4 using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands), and *RAD51* cDNA was amplified and sequenced following reverse transcription with the Superscript III kit (Invitrogen).

Statistical analyses. We listed all sequence variants detected in *RAD51* and *DCC* in 4,300 controls of European origin on the Exome Variant Server (EVS).[9] We compared, for each gene, the frequency of rare (frequency $<2\%$) missense variants predicted to be pathogenic by either the PolyPhen-2 or the SIFT algorithm in our patients (excluding the ones with truncating mutations) and in the controls, using the Fisher exact test. Computations were performed using SAS version 9 statistical software (SAS Institute, Cary, NC).

RESULTS The characteristics and genetic results of the patients are summarized in table e-1 and figure e-1 on the *Neurology*® Web site at Neurology.org.

Two novel nonsense mutations (c.823C>T/p.Arg275X; c.377C>A/p.Ser126X) and 2 novel mutations leading to a frameshift (c.2871_2875dup/p.Pro960GlyfsX8; c.1366_1337insAGCC/p.Arg446GlnfsX27) were identified in *DCC* in 4 of the 6 families and one simplex case. They were present in all available affected family members and absent in 150 controls, confirming that they were responsible for MM in these families. A deletion of exons 4 and 5 of *DCC* was found in one simplex case (figure 1).

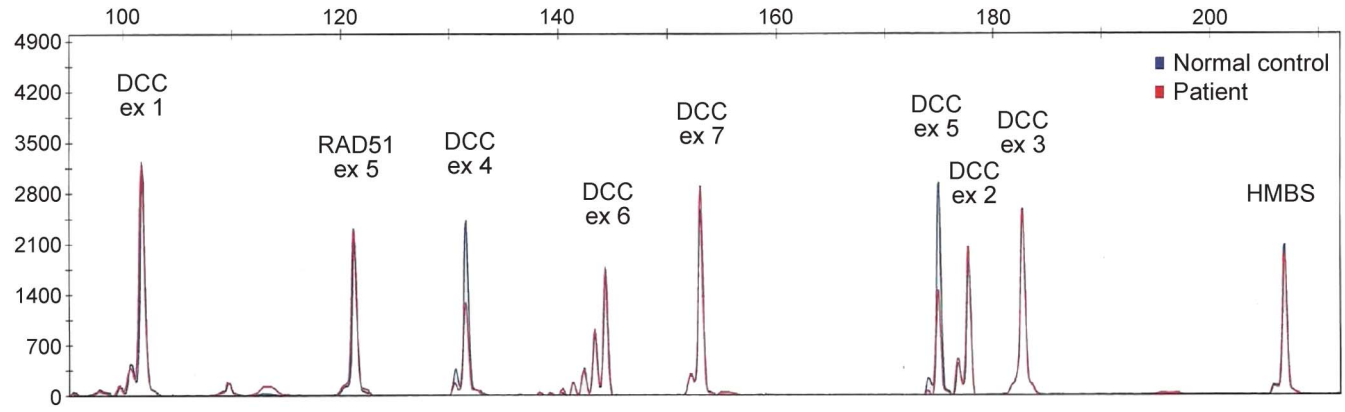
Five missense variants (c.527A>G/p.Asn176Ser, c.1409G>A/p.Gly470Asp, c.2407G>A/p.Gly803Asp, c.2000G>A/p.Arg667His, and c.2105A>G/p.Asn702Ser) were identified in *DCC* in 4 simplex cases (one individual had 2 variants) (figure 2A). All variants alter highly conserved amino acids and are predicted to be deleterious by both the PolyPhen-2 and SIFT algorithms (table e-2). Four of them (p.Asn176Ser, p.Gly470Asp, p.Arg667His, and p.Asn702Ser) are referenced in the Single Nucleotide Polymorphism Database (dbSNP). The p.Asn176Ser variant was transmitted by a healthy parent. The p.Arg667His variant was found at the heterozygous state in both healthy parents, who were first cousins, and a healthy brother. The variants p.Gly470Asp and p. Gly803Asp were present each on one allele in the same individual (*trans* configuration): the first one was transmitted by the patient's healthy mother, while the latter was absent from both parents, indicating its de novo occurrence. Two novel missense variants (c.140A>G/p.His47Arg and c.409A>T/p.Ile137Phe) were identified in *RAD51* in 2 simplex cases (figure 2B). These variants alter conserved amino acids and are predicted to be deleterious by at least one of the 2 algorithms (table e-2). Both variants were inherited from the patients' healthy mothers, and one of them (p.His47Arg) was also present in a healthy brother. A variant located next to a splice site (c.778-5A>G) was found in an additional simplex case. Although it was predicted to modify splicing, the study of the *RAD51* transcript in the patient's lymphoblasts revealed no abnormality, suggesting that it constitutes a rare benign variant.

Three of the variants identified in patients with CMM (p.Asn176Ser, p.Gly470Asp, and p.Asn702Ser in *DCC*) were found in controls, at a low frequency. The 5 remaining variants were not found in at least 150 ethnically matched controls (see details in table e-1).

Statistical analysis showed that the frequency of variants predicted to be deleterious was significantly different between patients with CMM and the EVS controls (2/20 vs 7/4,300, $p = 0.0007$ for *RAD51*; 5/20 vs 140/4,300, $p = 0.0004$ for *DCC*).

DISCUSSION We report 4 novel truncating mutations and one exonic deletion in *DCC* in 6 unrelated

Figure 1 **Deletion of exons 4 and 5 of *DCC* in a simplex case**

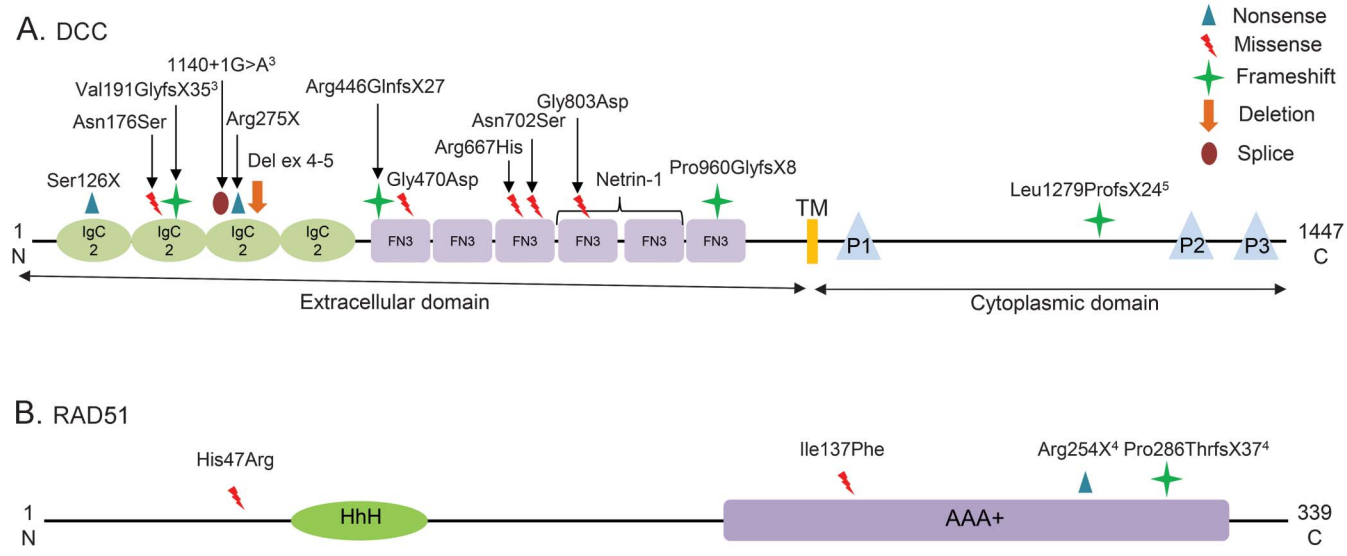


The quantitative multiplex PCR of short fluorescent fragments profiles of the deletion carrier (in red) and of a normal control (in blue) are superimposed. They are normalized using *HMBS* as a control amplicon. A 2-fold reduction of exons 4 and 5 is observed in the patient's profile. *DCC* = deleted in colorectal carcinoma; *HMBS* = hydroxymethylbilane synthase; *RAD51* = RAD51 recombinase.

CMM families. We also describe 5 *DCC* and 2 *RAD51* missense variants predicted to be damaging in 6 individuals. In total, 12 of the 26 probands (4/6 families and 8/20 simplex cases) had at least one variant in *DCC* or *RAD51* that certainly, probably, or possibly contributes to CMM.

Pathogenicity of these variants is supported by the statistical analysis showing that the frequency of rare missense variants predicted to be damaging was significantly higher in affected individuals than in the EVS population. Strikingly, the 8 mutations reported so far in CMM families (2 in *RAD51* and 6 in *DCC*) were all truncating mutations,[3–5] whereas 7 of the 9 variants found in simplex patients were missense. Penetrance associated with truncating mutations in either gene was previously estimated to be 50% in CMM families,[3,4] and the most probable consequence of these mutations is haploinsufficiency resulting from the degradation of the mutated messenger RNA by nonsense-mediated RNA decay.[3,5] Missense variants possibly have different consequences at a molecular level since the mutated protein is theoretically expressed. Of note, some *DCC* variants are located within or in the vicinity of the netrin-binding domain, and might thereby alter axonal guidance. We hypothesize that missense variants could induce "apparently simplex" CMM by being associated with a lower penetrance than truncating mutations—which indicates

Figure 2 **Distribution of all the identified variants and mutations throughout the DCC and RAD51 proteins**



(A) DCC. (B) RAD51. AAA+ = ATPase domain; *DCC* = deleted in colorectal carcinoma; FN3 = fibronectin type III-like domain; HhH = helix-hairpin-helix domain; IgC2 = immunoglobulin-like type C2 domain; P1, P2, P3 = conserved domains of the cytoplasmic region; *RAD51* = RAD51 recombinase; TM = transmembrane domain.

that examination of a very large number of family members would have led us to detect more affected individuals. In keeping with this hypothesis, 4 of the 5 missense variants for which segregation data were available were inherited by an asymptomatic parent and also found in 2 siblings, whereas only one occurred de novo. Furthermore, 4 of the 7 missense variants were referenced in the dbSNP, and present at a low frequency in controls. Instead of representing monogenic mutations with reduced penetrance, missense variants may rather constitute susceptibility factors for CMM. Genetic or environmental factors might provide a second hit to induce the MM phenotype. Finally, we failed to identify mutations or rearrangements of *DCC* and *RAD51* in 2 familial and 12 simplex cases, implying that additional genes are involved in CMM and remain to be identified.

AUTHOR CONTRIBUTIONS

A.M., C.D., and E.R. drafted/revised the manuscript for content, including medical writing for content. C.D., A.B. and E.R. designed the study. A.M., C.D., O.T., D.B., M.C., J.W., F.R., I.L., A.W., A.B., D.D., M.R., S.R.S., L.D., L.D.M., J.H., A.E., S.F., S.K., C.Q., P.B., S.R.-S., G.P., R.D., S.S.B., K.D., P.P., and M.V. acquired and analyzed/interpreted data. J.-L.G. performed the statistical analysis. C.D., A.B., and E.R. supervised the study.

ACKNOWLEDGMENT

The authors thank the patients for participating in this study, the genotyping and sequencing platform of the ICM for technical assistance, the DNA and cell bank of CRICM for DNA extraction and cell culture, and Isabelle Dusart for proofreading the manuscript.

STUDY FUNDING

Supported by an unrestricted grant from Merz, Novartis, and Orkyn and also by INSERM, UPMC, IHU-A-ICM, and Djilali Mehri. Dr. Méneret received a grant "année-recherche" from AP-HP, and Dr. Roze received a grant "poste d'accueil" AP-HP CNRS. The Unit of Neurology of the Florence Health Authority was supported by Ente Cassa di Risparmio di Firenze.

DISCLOSURE

A. Méneret received a research grant from AP-HP, and received travel funding from ANAINF, JNLF, and the European Federation of the Neurological Societies. C. Depienne, F. Riant, O. Trouillard, D. Bouteiller, M. Cincotta, P. Bitoun, J. Wickert, I. Lagroua, A. Westenberger, A. Borgheresi, D. Doummar, M. Romano, S. Rossi, L. Defebvre, and L. De Meirleir report no disclosures relevant to the manuscript. A. Espay is supported by the K23 career development award (NIMH, 1K23MH092735); has received grant support from CleveMed/Great Lakes Neurotechnologies, and the Michael J. Fox Foundation; received personal compensation as a consultant/scientific advisory board member for Solvay (now AbbVie), Chelsea Therapeutics, TEVA, Impax, Merz, Pfizer, Solstice Neurosciences, Eli Lilly, and US WorldMeds; royalties from Lippincott Williams & Wilkins and Cambridge; and honoraria from Novartis, UCB, TEVA, the American Academy of Neurology, and the Movement Disorders Society. He serves as associate editor of *Movement Disorders* and *Frontiers in Movement Disorders* and on the editorial board of *The European Neurological Journal*. S. Fiori reports no disclosures relevant to the manuscript. S. Klebe is on an advisory board of UCB and receives royalties from Thieme edition. C. Quélin reports no disclosures relevant to the manuscript. S. Rudnik-Schöneborn receives funding from IZKF Biomat RWTH Aachen (project N5-4) and the DFG (project Ru 746 1-2). G. Plessis reports no disclosures relevant to the manuscript. R. Dale receives funding from University of Sydney, the NHMRC, and the Petre Foundation. S. Sklower Brooks, K. Dziezyc, P. Pollak, and J. Golmard report no disclosures relevant to the manuscript. P. Pollak received travel grants and compensation for speaking from Medtronic and served on the scientific advisory board for Aleva. M. Vidailhet received research grants from Metz, UCB, Novartis, INSERM (Cossec), and ANR, patients associations France Parkinson, AMADYS, and travel grants from Movement Disorders Society and Dystonia Coalition. A. Brice served on the Research Foundation Flanders, FWO-expertpanel Bio 1, as an editor for *The Lancet Neurology* (2003–2010), *Brain* (2007–), *Human Molecular Genetics* (2005–), *Neurodegenerative Diseases* (2003–), *Neurogenetics* (2001–), *Journal of Neurology* (1998–2003), *European Neurology* (1998–2009), *Neuromuscular Disorders* (1998–2000), *The Cerebellum* (2001–), *La Revue Neurologique* (1997–2005), and *Parkinsonism and Related Disorders* (2008–). He received grants from the French Agency for Research, the Programme Hospitalier de Recherche Clinique (academic research support), the Michael J. Fox Foundation, and the French Association Against Myopathies (nonprofit foundations). E. Roze is the recipient of a grant "poste d'acceuil" AP-HP/CNRS. He received research support from INSERM (COSSEC), AP-HP (DRC-PHRC), Fondation pour la Recherche sur le Cerveau (FRC), the Dystonia Coalition (pilot project), Ipsen, Merz-Pharma, Novartis, Teva, Lundbeck, and Orkyn; served on scientific advisory boards for Orkyn, Ipsen, and Merz-Pharma; received speech honorarium from Novartis and Orkyn, received travel funding from Teva, Novartis, the Dystonia Coalition, the Movement Disorders Society, and the World Federation of Neurology Association of Parkinsonism and Related Disorders. Go to Neurology.org for full disclosures.

Received November 17, 2013. Accepted in final form February 26, 2014.

REFERENCES

1. Bonnet C, Roubertie A, Doummar D, Bahi-Buisson N, Cochen de Cock V, Roze E. Developmental and benign movement disorders in childhood. Mov Disord 2010;25:1317–1334.
2. Gallea C, Popa T, Hubsch C, et al. RAD51 deficiency disrupts the corticospinal lateralization of motor control. Brain 2013;136:3333–3346.
3. Srour M, Riviere JB, Pham JM, et al. Mutations in DCC cause congenital mirror movements. Science 2010;328:592.
4. Depienne C, Bouteiller D, Meneret A, et al. RAD51 haploinsufficiency causes congenital mirror movements in humans. Am J Hum Genet 2012;90:301–307.
5. Depienne C, Cincotta M, Billot S, et al. A novel DCC mutation and genetic heterogeneity in congenital mirror movements. Neurology 2011;76:260–264.
6. Finger JH, Bronson RT, Harris B, Johnson K, Przyborski SA, Ackerman SL. The netrin 1 receptors Unc5h3 and Dcc are necessary at multiple choice points for the guidance of corticospinal tract axons. J Neurosci 2002;22:10346–10356.
7. Park JY, Yoo HW, Kim BR, Park R, Choi SY, Kim Y. Identification of a novel human Rad51 variant that promotes DNA strand exchange. Nucleic Acids Res 2008;36:3226–3234.
8. Woods BT, Teuber HL. Mirror movements after childhood hemiparesis. Neurology 1978;28:1152–1157.
9. NHLBI GO Exome Sequencing Project (ESP): Exome Variant Server [online]. Available at: http://evs.gs.washington.edu/EVS/. Accessed October 2013.