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CHROMOSOME 19 INTERNATIONAL WORKSHOP

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**Proceedings and Final Report of the
Second International Workshop on Human Chromosome 19
January 25-26, 1992, Nijmegen, The Netherlands**

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The Second International Workshop on Human Chromosome 19 was hosted on January 25 and 26, 1992, by the Department of Human Genetics, University Hospital Nijmegen, The Netherlands, at the 'Meerdal Conference Center'. The workshop was supported by a grant from the European Community obtained through HUGO, the Dutch Research Organization (NWO) and the Muscular Dystrophy Association (MDA). Travel support for American participants was provided by the Department of Energy. The goals of this workshop were to produce genetic, physical and integrated maps of chromosome 19, to identify inconsistencies and gaps, and to discuss and exchange resources and techniques available for the completion of these maps. The second day of the meeting was largely devoted to region or disease specific efforts. In particular, the meeting served as a platform for assessing and discussing the recent progress made into the molecular elucidation of myotonic dystrophy.

The workshop participants were:

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Elizabeth Evans, Ph.D., HUGO Europe, London, U.K.
Jonathan Haines, M.D., Massachusetts General Hospital, Charlestown, MA
Jan M.N. Hoovers, Ph.D., Dept. of Human Genet., Academic Medical Ctr, Amsterdam
David Iles, Ph.D., Dept. of Cell Biology, University of Nijmegen (The Neth.)
Gert Jansen, Dept. of Cell Biology, University of Nijmegen (The Neth.)
Robert B. Jenkins, M.D., Ph.D., Dept. of Lab. Medicine, Mayo Clinic, Rochester,
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Keith Johnson, Ph.D., Charing Cross & Westminster Med. Sch., Univ. of London, London
Claudine Junien, Ph.D., INSERM U73, Chateau de Longchamp, Paris, France
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Marcy C. Speer, Ph.D., Dept. of Psychiatry, Columbia University, New York NY
Barbara J. Trask, Ph.D., Lawrence Livermore Natl. Laboratories, Livermore CA
James L. Weber, Ph.D., Marshfield Medical Research Foundation, Marshfield WI
Berend Wieringa, Ph.D., Dept. of Cell Biology, Univ. of Nijmegen, Nijmegen
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PROCEEDINGS OF THE 2ND INTERNATIONAL WORKSHOP ON HUMAN CHROMOSOME 19

1. RESOURCES AND TECHNIQUES

Somatic cell hybrids

In various laboratories, sets of somatic cell hybrids have been generated for the long arm of chromosome 19 as a part of efforts to map and characterize the region carrying the myotonic dystrophy gene. D. Shaw (Cardiff) reported on experiments performed by D. Brook et al (Genomics, in press) aiming at the construction of radiation reduced hybrids by selection for the retention of ERCC1 and for the loss of the poliovirus receptor gene (PVS). Their starting material was a hybrid containing approximately 30 megabases of human DNA including material from chromosome 19q and 17. One of the cell lines produced was shown to contain about 2 megabases of human DNA, including the ERCC1 and D19S51 markers which flank the DM locus. Several probes from this segment were isolated and a pulsed field map was presented.

M. Siciliano (Houston) presented a set of 22 somatic cell hybrids carrying different fragments of 19q. Probe screening allowed the arrangement of 41 genetic markers into 20 ordered subregions on the q-arm. The gene order determined was consistent with published physical and genetic maps. H.H. Ropers (Nijmegen) reported on the construction of a panel comprising of 119 hybrid clones with different fragments of chromosome 19, by making use of an irradiation-fusion rescue protocol. These clones were characterized with 10 different (CA)_n repeat markers that had been presented earlier by J. Weber at HGM11. Roughly 40% of these hybrids contained a single contiguous fragment, and the order of markers determined in this way was in keeping with their genetic order (see Figure 1). This hybrid panel will be available for collaborative mapping studies.

B. Wieringa and co-workers (Nijmegen) have used homologous recombination techniques to introduce selectable markers into the CKM gene of murine embryonic stem cells. By making use of various different vector systems and recipient cell lines, it was shown that efficient homologous targeting is only achieved if vector sequences and target ES cells have the same genetic background. This system should be very useful for tagging other genes on chromosome 19 and should greatly facilitate the generation of hybrids with region-specific sets of chromosome 19 fragments.

YAC, P1 and cosmid libraries

As the first speaker of the Lawrence Livermore group, P. de Jong reported on the construction of chromosome 19-specific libraries from sorted chromosomes employing LAWRIST vectors. Host bacteria were employed that can retain palin-

dromic sequences and that do not degrade methylated human DNA. Chromosome 19-specific libraries have been put on gridded filters which are available on a collaborative basis.

As reported by A. Roses (Durham), a large insert P1 vector library is being constructed in his laboratory by Taylor and coworkers. Starting from a hybrid cell line containing only human chromosome 19 they have generated several hundred human specific clones with average insert sizes of 90-100 kb.

S. Brown and co-workers (London) have constructed a mouse YAC library which should be of use for the identification of clones that are homologous to genes and markers from chromosome 19. For these experiments, a yeast strain carrying a mutation in the RAD52 gene was employed which is required for recombination. Therefore, these YACs show reduced chimerism and increased stability. The library was made from a C57BL female mouse and encompasses 41,000 clones with an average insert size of 300 kb corresponding to 3.5 genome equivalents. Presently the group is screening for clones from the proximal segment of mouse chromosome 7 which is homologous to human chromosome 19. In collaboration with K. Johnson (London), YACs homologous to CKM and the pX75 (D19S112) marker, have been identified. PCR pools and filters are available for library screening, and collaboration with other groups is encouraged.

As reported by A. Carrano, the Livermore group has now fingerprinted over 10,000 chromosome 19 specific cosmid clones by restriction digestion and automated analysis, 15% of which contain alphoid sequences. 871 contigs have been identified with an average contig size of 8.2. The total length of the tiling path covered by these contigs is 46.6 megabases or 78% of the chromosome. Current attempts to fill the gaps between these contigs focus on YAC and cosmid walking, and on fluorescent in situ hybridisation (FISH). High density cosmid filter grids have been generated which are being sent to collaborators for probe and gene screening; moreover, the St. Louis YAC library has been pooled in several dimensions and is being used for collaborative studies. A database has been set up which stores all information thus far obtained, including contigs, mapping information, and the names of collaborators. The Lawrence Livermore data are accessible through Internet and readable on a Sun computer or any X-window system.

In situ hybridization

B. Trask (Livermore) has mapped 450 cosmids to chromosome bands by fluorescent in situ hybridization (FISH). These cosmids represent 165 contigs and over 70 genes. Cosmids have been detected that contain sequences present at or near most telomeres; in addition, (sub)telomeric sequences specific for 19p have been identified. Moreover, she has found alphoid sequences that hybridize specifically to the centromeres of chromosome 1, 2 and 19. All contigs with tiling path lengths of

above 7 have been regionally mapped to bands and sub-bands of chromosome 19. FISH mapping of multiple cosmids from single contigs revealed that 5 out of 100 hybridize to different chromosome regions. For 69 genes and/or DNA markers the regional map position could be determined or improved. The resolution of the FISH technique with metaphase chromosomes is in the order of 2 Mb employing two colour mapping. In this way, chromosome 19 can be divided up into 30 intervals. With interphase mapping, the resolution is optimal below 1 Mb, and levels off for greater distances, probably as a result of chromatin looping. Very small distances can be resolved by analysis of decondensed sperm chromosomes after fertilization of Chinese hamster egg cells. This technique is highly laborious, however, requiring statistical analysis and large numbers of observations. With metaphase and interphase FISH, the order of 62 markers has been unambiguously determined.

J. Hoovers (Amsterdam) reported on the isolation of 101 potential human zinc finger protein genes by using a oligonucleotide specific for the 'link' sequence between contiguous zinc fingers. Out of 69 zinc finger cosmids which were regionally localized to human prometaphase chromosomes by in situ hybridization, 37 mapped on chromosome 19. Of these, 14 mapped in band 19q13.4, one at or near the centromere, four in band 19p13.3, and the remainder mapped elsewhere on the short arm of chromosome 19. None of these putative zinc finger protein genes overlap, but several are arranged in tandem. Sequence comparisons allowed their classification in two groups, and there was clustering of group 1 sequences on the proximal short arm of chromosome 19. Previously, other groups have assigned zinc finger like sequences to chromosome 19, several of which may be identical to those identified by Hoovers et al. Whether or not these sequences are part of true genes remains to be determined.

Expressed genes

M. Siciliano (Houston) described methods for optimizing the isolation of human genes present on a single chromosome or portions thereof in interspecific somatic cell hybrids. As previously published, the method employed in his laboratory involves the construction of a cDNA library from heterogeneous nuclear RNA transcribed in the hybrid cell by using consensus 5' splice sequences as primers for cDNA synthesis. Human clones are then isolated by screening the library with total human DNA. Using optimized primers and conditions for first strand synthesis, a 60-fold enrichment of exon sequences was achieved. Human inserts could be rapidly and efficiently isolated from primary clones by Alu-vector arm PCR. Human inserts averaged approximately 350 bp making sequencing practical for further analysis. 17 clones derived from a hybrid containing only a single human chromosome 19 are now being sequenced in collaboration with the Livermore group.

H. Epstein and his group (Houston) have employed a chromosome 19q-specific minisatellite sequence to isolate cosmid clones from a human placental library and

from a suitable somatic cell hybrid line containing the chromosome 19 segment carrying the DM gene. Clones were fixed onto nylon membranes and probed with cDNA lacking repetitive sequences which was prepared from various human tissues. Depending on the source of the cDNA, many clones yielded differential hybridization signals, and nearly all hybridized to at least one of the cDNA preparations. Seven positive cosmids localized to the ERCC1-D19S51 interval, which harbours the DM gene. As reported by A. Carrano, his group has sequenced 300 clones from normalized cDNA libraries. Clones mapping to chromosome 19 were employed for homology searches in databanks. Recently, the random approach has been abandoned and the group is presently concentrating on the analysis of chromosome 19 specific genes.

2. GENETIC MAPPING

J. Weber (Marshfield) and M. Speer (New York) have independently used the CEPH version 4 database to generate both comprehensive and framework maps of chromosome 19. Their maps were in general agreement with each other and with the consensus physical map. These maps will be included in the CEPH chromosome 19 consortium mapping report. Figure 1 includes a framework map of microsatellite repeat markers. The order given was favoured over all other orders by odds of 1000:1 or better. Estimates of inter-locus distances (in cM) are presented for both males and females separately as well as averaged over both sexes. The markers extend from distal 19p to 19q, but microsatellites have not yet been identified in the telomeric regions. Therefore, two RFLP markers, D19S20 (pter) and PRKGC (qter) have been chosen as the distal demarcations of these maps.

Several new short tandem repeat markers were reported at the meeting. D. Iles (Nijmegen) described a trinucleotide repeat (D19S190) and a dinucleotide repeat (D19S191) flanking the RYR1 locus (see below), and M. Speer and M. Pericak-Vance (Durham) reported a new dinucleotide repeat marker at the ATP1A3 locus. J. Weber reported five new tetranucleotide and 8 new dinucleotide polymorphisms. Four of these markers were developed by N. Dracopoli (Boston, MIT).

The order of the 'oldest' markers known to belong to the chromosome 19 linkage group, SE (= FUT2) and LU, is not yet firmly established. Haplotype analyses presented by S. Ball (Exeter) suggest the order: 19cen-D19S9-D19S47-LU-APOC2-FUT2-FUT1-ter, while previous analyses of H. Eiberg (Copenhagen) suggested that LU maps distal to FUT2. Retyping of the respective families with (the same set of) highly polymorphic markers may resolve this inconsistency.

Comparative mapping

Homologies between the long arm of chromosome 19 and the proximal segment of chromosome 7 of the mouse were discussed by S. Brown (London) and by

A. Roses, who presented the work of A. Saunders (Durham). A consensus map incorporating data from both groups is presented in Figure 1. As noted previously, the genetic map of the *Ryr-Ckmm* interval on mouse chromosome 7 is much smaller than the human RYR1-CKM distance. The gene order is inverted but internally preserved. *Pkcc* is the most proximal marker on the mouse map but a large group of genes including *Hrc*, *Snrp70* and *Rras* are not between *Pkcc* and *Ckmm* but much more distal on mouse chromosome 7. Other sequences from human 19 are distributed over at least 3 mouse chromosomes (8, 9 and 17). The *Jund-Mel-Junb* triplet appears to have an inverted order in mouse relative to man. Thus, at least two internal rearrangements of this chromosome and several translocations must have occurred since the evolutionary separation of mouse and man to explain these different gene orders.

3. PHYSICAL AND REGION-SPECIFIC MAPS

Because of the interest in myotonic dystrophy and more recently, malignant hyperthermia and Alzheimer's disease, regional mapping studies have focussed on the long arm of chromosome 19 while the short arm is much less well studied. H. Mohrenweiser (Livermore) gave an overview on the physical map of chromosome 19 referring particularly to the work done in Livermore. Much of the information presented during the meeting has been incorporated into the consensus physical and integrated maps given below (see Figure 1). In collaboration with others, the Livermore group now has cosmids for more than 90 genes and 20 other markers. A detailed map of CGM7-ATP1A3-PSG region of q13.1-q13.2 has been established by a combination of restriction enzyme mapping, directed cosmid walking, and fluorescence in situ hybridization to somatic interphase and sperm pronuclear chromatin (Brandriff et al, Genomics, in press; Thompson et al, Genomics, in press; Tynan et al, Nucleic Acids Res, in press). Twenty members of the CEA gene family have been localized to a 1.2-Mb region of the chromosome. The CEA subfamily is located centromeric to the PSG subfamily on the basis of hybrid analysis and two-color fluorescence in situ hybridization of metaphase chromosomes. The CGM7-CGM2-CEA-NCA-CGM1 cluster is separated from the BGP-CGM9-CGM6-CGM8 cluster by approximately 300 kbp. The ATP1A3 gene is located between the two clusters. The PSG subfamily consists of a series of 11 genes spanning approximately 500 kbp (Hammarstrom, personal communication) and located within ~30 kbp of CGM8. The anonymous marker D19S8 has been assigned to the PSG family (De Jong, personal communication).

RYR1, malignant hyperthermia and central core disease

M. Phillips from D. MacLennan's group (Toronto) presented an overview on the ryanodine receptor gene (RYR1) and its relation to malignant hyperthermia and the porcine stress syndrome. The human RYR1 cDNA encompasses 15,344 bp. Seventeen

overlapping phage clones spanning a total of 210 kb have been isolated as well as a cosmid clone containing the promotor region. Boundaries have been isolated for 81 of the 98 predicted exons. The porcine cDNA has been sequenced and 18 polymorphic sites have been detected. Only one of these had a changed the amino acid sequence. It co-segregated with the porcine stress syndrome gene in more than 400 meioses. For the detection of mutations in humans, flanking PCR primers have been generated for 10 exons. K. Johnson (London) reported on the activities of the European malignant hyperthermia group who developed diagnostic criteria for MH, including a standardized muscle contracture test. He confirmed previous studies indicating that MH is genetically heterogeneous (Deufel et al, see HGM11 report). S. Ball (Exeter) reported on linkage studies in 15 MH families, 8 of which show recombination with RYR1 or closely linked markers. (CA)_n repeat markers D19S75 and D19S47 flank the RYR1 locus but presymptomatic diagnosis is hampered by genetic heterogeneity. The gene frequency is not known but may be in the order of 1 in 10,000. The genetics of MHS may be more complex than previously thought, but there is no evidence that imprinting adds to this complexity. Using his new microsatellite markers D19S190 and D19S191 which flank the RYR1 locus, D. Iles (Nijmegen) has also observed recombination between MHS and the RYR1 locus.

C. Müller-Reible (Würzburg) reported on linkage studies in families with central core disease (CCO). In his series, no recombination and a lod score of 4.17 was obtained with the D19S18 marker which maps just distal to the RYR1 gene. Combining multipoint linkage data with those of previous studies (Haan et al, see HGM11 report) yielded the highest location scores for the same region. In CCO patients, no RYR1 mutations have been found so far.

Alzheimer disease

M. Pericak-Vance (Durham) summarized her published findings which gave rise to the contention that genes on chromosome 21 and on 19 are involved in the etiology of Familial Alzheimer Disease (FAD). After having excluded linkage with chromosome 21 markers in late onset families, multipoint lod scores of about 4 were obtained when only affected individuals from early and late onset families were examined with D19S13, ATP1A3 and BCL3 from proximal 19q. J.L. Haines (Boston) reported on the results of a chromosome 19/chromosome 21 linkage study from the combined analysis of both the Durham families and a series of families examined in Boston. This study on the expanded data set provided additional support for linkage to chromosome 19. In addition, they also found evidence for linkage to chromosome 21 as well as the existence of unlinked families (i.e. unlinked to either chromosome 19 or 21).

G. Schellenberg (Seattle) summarized the results of their linkage studies on chromosome 19. He reported support for linkage to chromosome 19 in his late onset families with a peak lod score of 2.08 at theta equal to 0.05 for the D19S13 and a

peak score of 2.52 at theta equal to 0.0 for KLK. Again, some of the families did not show linkage to chromosome 19 and chromosome 21 markers. In addition, Haines reported on the results of the chromosome 19 data in the late onset families of M. Mullan and coworkers (London). This group found significant linkage to chromosome 19, also using the Affected Pedigree Member method. In conclusion, the above studies present independent evidence for the involvement of genes on chromosome 19 in FAD.

Brain tumours

Starting from the observation that oligo-dendrogliomas are not infrequently characterized by chromosomal rearrangements of chromosome 9p, 19q, 10 and 13, R. Jenkins (Rochester) and coworkers have performed a systematic search for loss of heterozygosity employing markers from all human chromosome arms. In seven out of nine patients with oligodendrogliomas, loss of heterozygosity was found for the D19S8 marker. Similar observations were made with other long arm markers as well. These changes are also seen in part of the astrocytomas which may represent a subpopulation derived from oligodendrocytes. The frequency of these alterations points to changes on chromosome 19q being the primary genetic defect, and experiments are underway to test this hypothesis by linkage studies in familial cases.

Myotonic dystrophy

Since HGM11, remarkable progress has been made into the molecular elucidation of Myotonic Dystrophy (DM). All major groups working in this field were represented at the meeting which rendered the DM session particularly exciting. In his introduction, B. Wieringa (Nijmegen) gave a historic account of the findings that have led to the fine mapping of the DM locus between two closely flanking markers, D19S112 and D19S118, the detection of strong linkage disequilibrium between DM and D19S63 and the construction of a physical map of the region encompassing the DM locus. Moreover, he acknowledged the crucial role of the Muscular Dystrophy Association in stimulating international collaboration in this field.

K. Johnson (London) described the efforts of his group to find expressed sequences in the vicinity of the D19S51 marker and the mapping of YACs and cosmids by making use of a irradiation hybrid panel developed by D. Brook (Cardiff). YAC clones overlapping the D19S112-D19S118 interval which had been characterized previously by P. de Jong (Livermore) in collaboration with B. Wieringa (Nijmegen) and R. Korneluk (Ottawa) were subsequently employed to isolate cDNA from this region. When hybridized to genomic DNA from DM patients and controls, one cDNA yielded a consistently enlarged band in all patients. In British, Swedish and Finnish families, the expansion of this fragment was related to severity of the clinical phenotype.

D. Shaw (Cardiff) reported on the ordering of probes that were generated by his group and positioned on a 1.6 Mb macro-restriction map spanning the DM gene region. A phage contig encompassing a significant portion of this region was generated, and cDNAs were obtained by looking for evolutionary conservation, HTF islands, and by using exon trapping techniques (D. Brook in the laboratory of D. Housman, Boston). One of the cDNAs generated in this way displayed strong linkage disequilibrium supporting its location in the middle of the DM region. Prompted by the observation of Johnson (London), this cDNA was also used to screen genomic DNA of normal individuals and DM patients, and the same genomic changes were seen. Family analysis revealed increasing band sizes in successive generations and there was evidence for a smaller increment per generation if transmitted through males. Contraction of this DNA segment was never seen.

P. de Jong (Livermore) described the collaborative effort of the groups from Livermore, Nijmegen and Ottawa to clone the entire ERCC1-D19S51 segment. In close collaboration with the group of B. Korneluk (Ottawa), a cosmid contig of 350 kbp was generated extending telomerically from ERCC1. In collaboration with B. Wieringa's group (Nijmegen) a cosmid contig encompassing the D19S112-D19S51 segment was generated. A gap between these two contigs was closed by YAC and cosmid walking performed in the Livermore and Nijmegen laboratories. G. Jansen (Nijmegen) reported on strong linkage equilibrium between DM and D19S112 and on the construction of detailed restriction maps of the D19S112-D19S51 segment. Employing entire cosmid inserts as probes, several cDNAs were isolated from a mouse brain cDNA library. 4 distinct transcribed units were found just proximal to the D19S112 locus, and additional genes were found around the former cloning gap. One of these cDNAs, DMR-B15, hybridized to genomic DNA on both sides of the sequence that is expanded in DM-patients, which suggests that this gene is directly involved in DM. B. Korneluk (Ottawa) reported on Southern blot and PCR based analyses in DM families. In all cases, except for one family with uncertain diagnosis, the aforementioned variable DNA element was enlarged. In his series, the correlation between the length of this segment and clinical phenotype was less obvious than that reported by others. The length variability is due to a $(CTG)_n(CAG)_n$ repeat. In normal individuals the number of trinucleotides ranged from five to 28. In affected individuals several hundreds of trinucleotides were seen. Unsymptomatic carriers had a minimum of 60 repeat units (H. Smeets, Nijmegen) and there was no overlap with normal individuals. Combined PCR and Southern analysis allowed reliable diagnosis in 98% of the families studied.

As reported by H. Epstein (Houston), basically the same results were obtained independently by the Baylor group. Here, the expanding "multi-GCT repeat" sequence was found by systematic screening of cosmids (obtained from the Livermore-Nijmegen-Ottawa consortium) with a battery of oligonucleotides detecting di- and trinucleotide repeats. Preliminary data suggest that the repeat is part of a gene that is expressed in brain and skeletal muscle. In the close vicinity of, but not

overlapping this region, a sequence was identified with homology to a rat K⁺-channel gene.

A. Roses (Durham) reported that his group has isolated a cDNA encoding a K⁺-channel protein with six trans-membrane segments which is related to the *Drosophila* shaw gene. Although positioned just outside the region containing the expanding trinucleotide repeat, this gene may be a strong candidate for being involved in DM, in view of earlier biochemical and physiological findings demonstrating abnormal sodium and potassium transport.

4. GENOME DATABASE NEWS

R. Robbins (Baltimore) focussed on present and future possibilities to access GDB. In principle, all data and software in GDB are freely available to the scientific community. However, those who wish to run copies of GDB software must first obtain appropriate licenses from SYBASE, the commercial vendor whose products were used to build GDB.

Additional access routes to GDB have been developed. Those who wish to use the interface system developed by GDB can access the system directly in Baltimore on any of several official remote nodes around the world. A new, significantly improved version of GDB software will be released soon, which will support an X-windows interface into the database. Moreover, a dedicated SQL server has been established in Baltimore which allows direct access to the relational database. In addition, copies of the data in GDB are now available as ASCII files on an anonymous ftp server in Baltimore (mendel.welch.jhu.edu, or 128.120.59.42).

5. FUTURE CHROMOSOME 19 WORKSHOPS

E. Evans (London) discussed the past and the future of Human Gene Mapping meetings and in particular, a set of guidelines on the organization of single chromosome workshops which are about to be published by the Human Genome Organisation (HUGO). Single chromosome workshops will become the official platform for the presentation and validation of mapping data. These workshops should be planned, and future organizers should be nominated, two years in advance. In addition there will be annual chromosome coordination meetings (CCMs) which will resemble previous interim gene mapping workshops. The input of data into GDB has become the responsibility of Chromosome Editors; for chromosome 19, H.H. Ropers and H. Mohren-weiser have recently been appointed. The next chromosome 19 workshop, to be held in spring 1993, will be organized by A. Carrano and his group in the San Francisco Bay area. In 1994, the meeting will be organized jointly by K. Johnson and D. Shaw in England.

6. PUBLICATION

Ropers HH, Pericak-Vance MA, Siciliano MJ, Mohrenweiser HW. Report of the Second International Workshop on Human Chromosome 19 Mapping 1992. Cytogen Cell Genet 60:87-95, 1992.

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FIGURE 1.

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