DIAN Dispatch From Hawaii: After Slow Start, Network Is Humming

12 July 2010. After more than a year of rumbling to life, the Dominantly Inherited Alzheimer's Network (DIAN) has kicked into gear and is now enrolling research participants at a healthy clip. Here's an update on how things are going, brought to you fresh from the lectern and hallway conversations in Honolulu. That's where DIAN scientists and some family representatives met on 9 July 2010 for an all-day meeting in advance of the International Conference on Alzheimer's Disease. DIAN leaders capped this internal meeting with an evening presentation of initial DIAN results to industry leaders, which formed part of their preparations for adding drug trials to the study as early as possible. "DIAN proved to be even more challenging to get underway than we anticipated," said its principal investigator **John Morris** of Washington University, St. Louis, Missouri. "For example, it took a long time to get regulatory and cyclotron issues ironed out across the 10 DIAN performance sites, and because of these issues we had to add staff to help with the tremendous administrative efforts. But in the past few months, all sites have become operational and our enrollment rate is exceeding expectations."

DIAN is a U.S./UK/Australian network of initially 10 research sites, whose investigators have banded together to offer the families with autosomal-dominant AD a two-pronged study tailor-made for them (DIAN-info.org). Primarily, the study aims to monitor the preclinical development of the disease biology 20 years earlier than a person's estimated age of symptom onset by way of comprehensively tracking, side-by-side, all major biomarker candidates research has produced to date. It is a demanding study for participants, who have said time and again that what they really are hoping for are treatment studies. Therefore, DIAN has started a process of essentially wooing drug company researchers, who are themselves looking for a way forward amid a three-way tug of sensing a new opportunity, wanting to do the right thing, but being loathe to shoulder additional risk for a candidate drug into which they have invested many millions.

So what's new? First, enrollment. Almost one-third into its funding period, DIAN has enrolled 90 participants as of 7 July 2010, currently adding about 15 per month for an expected 250 by next summer. Because only 32 people had joined by March 2010, the overall pace of enrollment is slower than originally anticipated. Why the sluggish start? For one, DIAN is highly complex. Not only does each site change its own local procedures to adhere to a centralized, uniform and intensive protocol, but also, IRB approval needs to be granted at each site. Every change and every important communication require coordination across three continents. "It's an honor to be part of DIAN," said **Paul Aisen**, who heads the ADCS in San Diego, a DIAN subcontractor. "But it involves lots of PIs in different time zones and a lot of data exchange, so the coordination effort is significant."

For another, technical problems outside DIAN's control have cropped up. Because the cyclotron (an expensive instrument that makes the radiochemical needed for amyloid imaging in DIAN) servicing the Boston and Providence sites is being replaced, no PET scans have been done there since last October, and the New York City site cyclotron went offline for repairs after an audit. This has slowed these sites down, and in response, DIAN participants may now undergo PET scanning at the Washington University site.

On the upside, however, enrollment has picked up speed since the spring. Moreover, the network is considering expansion from 10 sites to 11 by adding the University of Pittsburgh Medical School led by **William Klunk**. The Pittsburgh group has been studying approximately 20 carriers of autosomal-dominant AD mutations for up to seven years already through their Alzheimer's Disease Research Center. Discussions are underway to determine how at least some of them can join the more extensive DIAN protocol if they wish to do so. Newly recruited volunteers from Pittsburgh would also be offered the option to join DIAN. For example, a previously unknown family with autosomal-dominant AD approached Klunk following a local screening of the 2008 HBO special series on AD (for a segment with Klunk and Chet Mathis, see movie 8 in ARF Video Gallery). Sequencing confirmed a previously identified presenilin 1 mutation. This family, too, is interested in DIAN, Klunk said.

New families are joining at other sites as well. For example, **Ralph Martins**, who leads DIAN's Perth site in Western Australia, is expecting a one-month visit from a Libyan family this September. The father and four of his six children will spend the month to complete all of DIAN's baseline assessments, Martins said. One daughter is pregnant and one is a minor, otherwise they would all come, he said, adding, "They are highly motivated." The extended family has about 50 at-risk relatives. Martins has been working with this family for about 12 years, when the proactive husband of an affected mother of six contacted him. Because shipping blood from Libya is tricky, Martins requested it be dripped on filter paper and mailed as a letter. Martins's group found a previously known PS1 mutation with onset in the early forties when they sequenced the blood of the mother, her sisters, and all her children. To date, he has not disclosed the children's genotype. "I have worked with this family since the children were young but have never met them in person. It's very exciting to have them come," Martins said. Martins developed the early research on this Libyan family, as well as on other families in Western Australia that enabled Perth to become a DIAN site, with support from the Australian McCusker Alzheimer's Research Foundation, a private philanthropy.

The Libyan family is noteworthy not only for its size and willingness to travel the globe for a full month of scientific research participation, but also because it illustrates that families from across the globe can, in principle, join DIAN. The Libyan family speaks English fluently. This is a requirement at present, as results from cognitive tests need to be gathered in a standardized manner and entered into a common DIAN database. To help DIAN expand, both **John Ringman**'s and **Richard Mayeux**'s groups at the Los Angeles and New York sites, respectively, are working on translating the tests into Spanish to open the doors to known families from Mexico, Spain, the Dominican Republic, and other Spanish-speaking nations.

But enrollment remains hard work. **Stephen Salloway**, who heads the Butler Hospital DIAN site in Rhode Island, mentioned an extended family of 50 people, most of whom are reluctant to join, Salloway said. In discussion, **Alison Goate** of WashU noted how going to family reunions has enabled her to build rapport and to explain the purpose of research in a friendly setting outside the exam room. In discussion, **Denise Heinrichs**, a family representative, noted that while families are deeply scared, they do want help. "The key is to educate them. They don't want this to go on from generation to generation. If they know enough, they will want to do it for their children." DIAN family members, with help from WashU's **Randy Bateman**, **Wendy Sigurdson**, and the DIAN administration, have begun organizing a support

group that, once up and running, will operate independently from the scientists to facilitate open communication between otherwise isolated families who find themselves in a similar situation. Likewise, **Martin Rossor** and colleagues at the UK site have done the same. Efforts to allow these groups to connect are in planning. In particular, study participants within three years of their expected age of onset need added outreach, said **Natalie Ryan** of London's DIAN site at University College. Not only does anxiety intensify at that time, but people at this stage may already be finding it more daunting to organize the logistics of traveling long distances, taking off from work and arranging child care.

The support needs for this type of study are great, and yet, when asked what is the single biggest factor that would attract more family members to research participation, DIAN scientists and family representatives replied unanimously: a therapeutic trial. See Part 2 of this story.—Gabrielle Strobel.

Glimpse at Data, Push for Trials

13 July 2010. On 9 July 2010 in Honolulu, leaders of the Dominantly Inherited Alzheimer Network gathered to brief each other and—at the end of a long day cooped up in conference while everyone else was playing at Waikiki Beach—to present to some 30 pharmaceutical industry scientists the first cut of baseline data from this ongoing biomarker study. Results are preliminary and will evolve as more people enroll; however, they provided a tantalizing snapshot of how the science of DIAN is shaping up. **John Morris**, **Randy Bateman**, **Mark Mintun**, and **Anne Fagan** presented results on behalf of DIAN's coordinating site at Washington University School of Medicine in St. Louis, Missouri. CSF and imaging data behaved largely as expected, and plasma also yielded an intriguing signal. Read on for details.

The scientists cut off data on 73 participants on June 15 for analysis. As of this day, some two-thirds had completed the lumbar puncture, PIB-PET, and FDG-PET imaging, while nearly everyone had an MRI and the clinical assessments done. As the international sites ramp up enrollment, they vary in how many of their participants complete all assessments, and investigators are currently working on bringing this rate up near 100 percent at all sites. The WashU site achieves this goal, and Morris emphasized that it is possible for all others to do so as well. In Honolulu, a discussion of the reasons for gaps that exist to date led to two take-home messages. First, lumbar puncture—for which DIAN-wide acceptance lies currently at 65.8 percent—requires both education to counter misconceptions about its risks and adherence to best practices to minimize those risks. Second, where at all possible, scheduling all procedures into a single three- or four-day extended visit tends to work better than calling participants in repeatedly for individual procedures. Morris urged all investigators to take plenty of time to explain the purpose and importance of the CSF results to research volunteers. This is necessary, the scientists agreed, to enable analysis of DIAN's data at the statistical power they originally envisioned. **Martin Farlow** from DIAN's Indiana School of Medicine site urged all investigators to ensure that volunteers are well hydrated before the procedure, as that reduces the risk for headache, a rare side effect of lumbar puncture.

Who are the study participants? Of the 73, about a quarter were symptomatic, which this study defines as CDR 0.5 in one or more of its categories. Many of them are men in their late forties. The asymptomatic participants' ages range from the twenties to

the fifties, and two thirds of them are women—a preponderance frequently seen in AD research studies. As expected, half of the asymptomatic volunteers carry an eFAD mutation; curiously, a few of the symptomatic people appear not to. The scientists are investigating now whether these people are false positives—i.e., they have mild clinical symptoms that do not represent the actual start of a neurodegenerative disease—or whether a mistake in their genetic test will turn up and they eventually prove to have inherited the family's AD mutation, after all. A majority of cognitively normal participants to date are quite far off from their parents' age of onset. This reflects perhaps a new, young generation that is more proactive, said a family representative who asked that her name be withheld. However, the study needs more people who are closer to the expected age of symptom onset, both to observe the full dynamic range of biomarker changes and to start planning therapeutic trials, Bateman said.

What has DIAN learned about the volunteers? The numbers here get smaller as sample mailing and data analysis are actively ongoing, but in brief, the cerebrospinal fluid assessments trend along familiar lines. Among 27 volunteers, the mutation carriers had significantly lower CSF Aβ42 levels as measured by the Innotest ELISA. The same measurement done with an xMAP platform yielded a smaller signal going in the same direction, said Fagan. (For details on relative CSF test performance, see CSF quality control series). As expected, CSF Aβ40 levels were the same between carriers and non-carriers, but the Aβ42/40 ratio was markedly different, driven by the drop in Aβ42. CSF Aβ42 was low in people near their estimated age at onset, but tended to be as high as in non-carriers in those who were 15 years or more younger. "These are preliminary but highly informative data," said Fagan.

Also in the CSF, tau results shape up to reveal some trends. As measured with xMAP assays, carriers appear to have higher tau levels than do non-carriers; this is also true for tau phosphorylated at position 181. Starting some 15 to 20 years prior to age at onset, levels rise, and rise further as people become symptomatic.

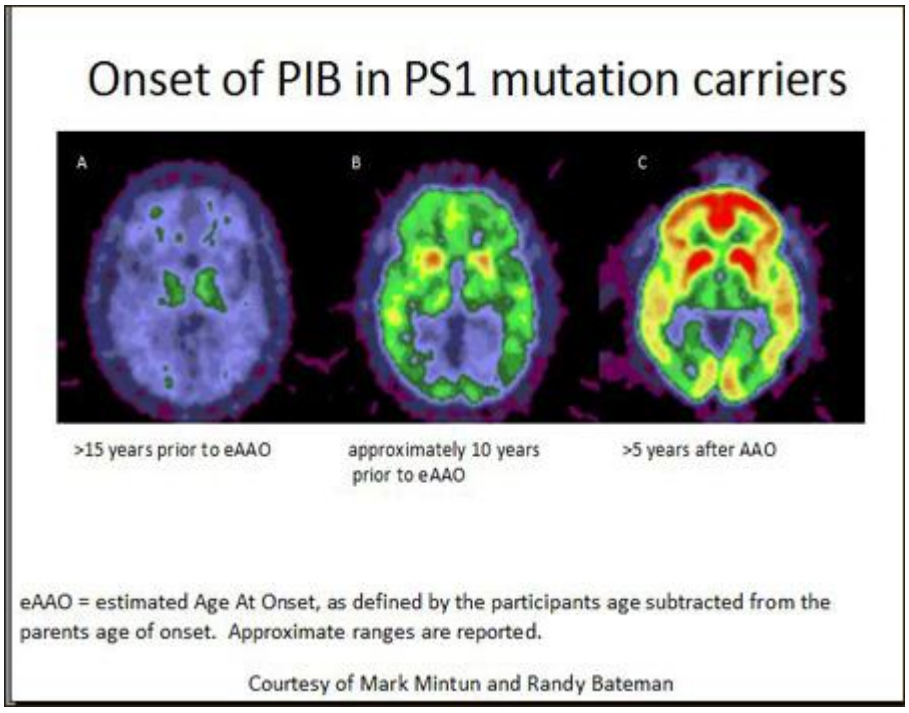
To some observers, perhaps the most exciting scientific finding came from plasma Aβ. Prior studies on this marker as a predictor of late-onset AD have been inconclusive in that no overall trend emerged from the literature, though small studies have indeed shown increases in plasma Aβ in FAD mutation carriers versus non-carriers. Among a first group of 26 DIAN participants whose plasma measurements are in, Aβ in the blood was higher in carriers, as was the Aβ42/40 ratio. "This, to me, is the biggest news tonight. It's an intriguing sign toward a simple and cheap predictive indicator," said one industry representative. "This result seems strong because it comes in the context of a comprehensive set of biomarker measurements."

If this finding holds up, it would imply that drug trials could be offered more easily to eFAD families who are not part of DIAN, for example, families in Sweden and other countries. In this vein, researchers spoke about creating an international registry modeled on DIAN, into which families who are not participating in DIAN itself could enroll with a fraction of that study's data and in this way become eligible for therapeutic trials that grow out of DIAN.

Initial imaging results indicate that the hippocampus and cerebral cortex were increasingly atrophied as carriers approach age at onset and beyond, but in non-

carriers is the same across this age range. (These are all cross-sectional data at this point.)

Initial amyloid imaging with PIB-PET showed that starting 15 to 10 years prior to age at onset, carriers retain more of this amyloid marker than do non-carriers. This is particularly true in the precuneus, an early place of deposition in LOAD, and in the caudate part of the striatum (a brain region not traditionally associated with AD but one where fibrillar amyloid deposits early on in autosomal-dominant AD). The DIAN scientists presented one PIB slide (see below) from the Familial Adult Children Study, a precursor to DIAN. It shows, on the left, a cool blue PIB-negative brain of a mutation carrier approximately 15 years prior to the parent's age at onset; in the middle a green, mostly negative brain of someone 10 years prior to onset who had small red spots in the caudate; and on the right the flaming yellow-red amyloid- laden brain of a demented person five years after onset. **Bill Klunk** of the University of Pittsburgh Medical School noted in a brief review of six-year-old ongoing PIB studies with some 20 eFAD family members that to find APP or presenilin mutation carriers who are PIB-negative, one may have to image people in their twenties.



With regard to clinical observation, Bateman presented some early analyses of the concept of estimated age at onset (AAO). This is thought to be slippery because scientists generally estimate this age according to when the affected parent got ill. But in some families, siblings recall different ages for that, and typically people were diagnosed at a later stage one generation ago than they are today. Even so, plotting the age of each DIAN participant against that person's individual AAO (i.e., current age minus parents' age at onset) showed that, so far, all presymptomatic carriers are younger than this estimated age at onset, and most symptomatic carriers are older. Likewise, participants' MMSE starts dropping one to five years before their AAO, and their CDR moves up at that time from the perfect score of 0. This suggests that this estimated AAO measure reflects a symptomatic turning point and can be used for designing therapeutic trials, Bateman said. The MMSE is a crude tool to assess

cognitive impairment in middle-aged people; the more detailed psychometric tests that are part of the DIAN clinical battery have not been analyzed in this way yet.

"These results are very preliminary. The numbers will change after quality control and as the samples grow. But right now, things are trending in the right direction—perhaps we are beginning to see these signals," said Morris. With the exception of the caudate location for early amyloid deposition and the plasma Aβ result, all findings to date are similar to what is known about late-onset AD, reinforcing DIAN's hypothesis that the rare forms of AD model the common forms, Bateman said.

So what are pharma and biotech companies to do with this brand-new data, shared openly with the drug development community? A movement toward therapeutic trials is in place. On 23 March 2010, DIAN scientists and representatives of the NIA, the ADCS, the API, and a regulatory adviser met in Geneva, Switzerland, with representatives of 11 pharma and biotech companies that are developing AD drugs. The charge of that day was to discuss the underlying science, potential biomarker trial designs, and ways in which a drug-based prevention effort could be pulled off collectively. At the time, pharma representatives endorsed the concept. They professed their interest to act now, but said they needed to be consulted confidentially, one-by-one, to discuss potential drugs. Since March, the DIAN scientists and their colleagues from API have held 12 such meetings with individual pharma companies, with one more to come next week. They are following up with confidentiality agreements and are working on trial design. However, trial design can only go so far without knowing what the drug will be. "We are at an impasse where we cannot define the possible designs further without knowing which drug we'll use. We have provided the requisite scientific information," said Bateman.

As the next step, DIAN scientists will prepare a "nomination packet" and send it to the companies, which can then nominate a candidate drug, or drugs, for such trials. This need not yet be a binding commitment, said Bateman, but it does need to be a genuine effort on the part of pharma to advance the planning into a more concrete phase. Multiple treatments can be considered and evaluated initially in short biomarker studies that measure target engagement before any subsequent longer trials. These packets will arrive at the companies' doorsteps in August, putting the ball squarely in their court.—Gabrielle Strobel.