MOST Ancillary Study (AS14-06) D. Felson, D. Misra
"Fat and Osteoarthritis – SxOA study"

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1. Dataset description and Analyst Notes

Dataset: AS1406Sx_bioassay.sas7bdat
Observations: 1456 records (1456 participants, 2 assays, 2 selection batches)
Documentation:

- VariableGuide_ AS1406Sx_bioassay.pdf
- Distributions_ AS1406Sx_bioassay.pdf

AS1406Sx_bioassay dataset contains 1456 records (one record per participant) with 2 assays results (HMW Adiponectin and Total Adiponectin) performed at the University of Vermont Laboratory for Clinical Biochemistry Research on baseline serum between December 2015 (selection batch 1) and September 2016 (selection batch 2):

Note, for this study, the laboratory was blinded to clinical data (case/control selection) and demographic characteristics of the participants.

IMPORTANT ANALYST NOTES:

- Two adiponectin assay result variables (variable name HMWAdiponectin and TotalAdiponectin) were performed.

- When assay results were not obtained, special missing value were used:
  .L = below low detection level

  .H = above high detection level

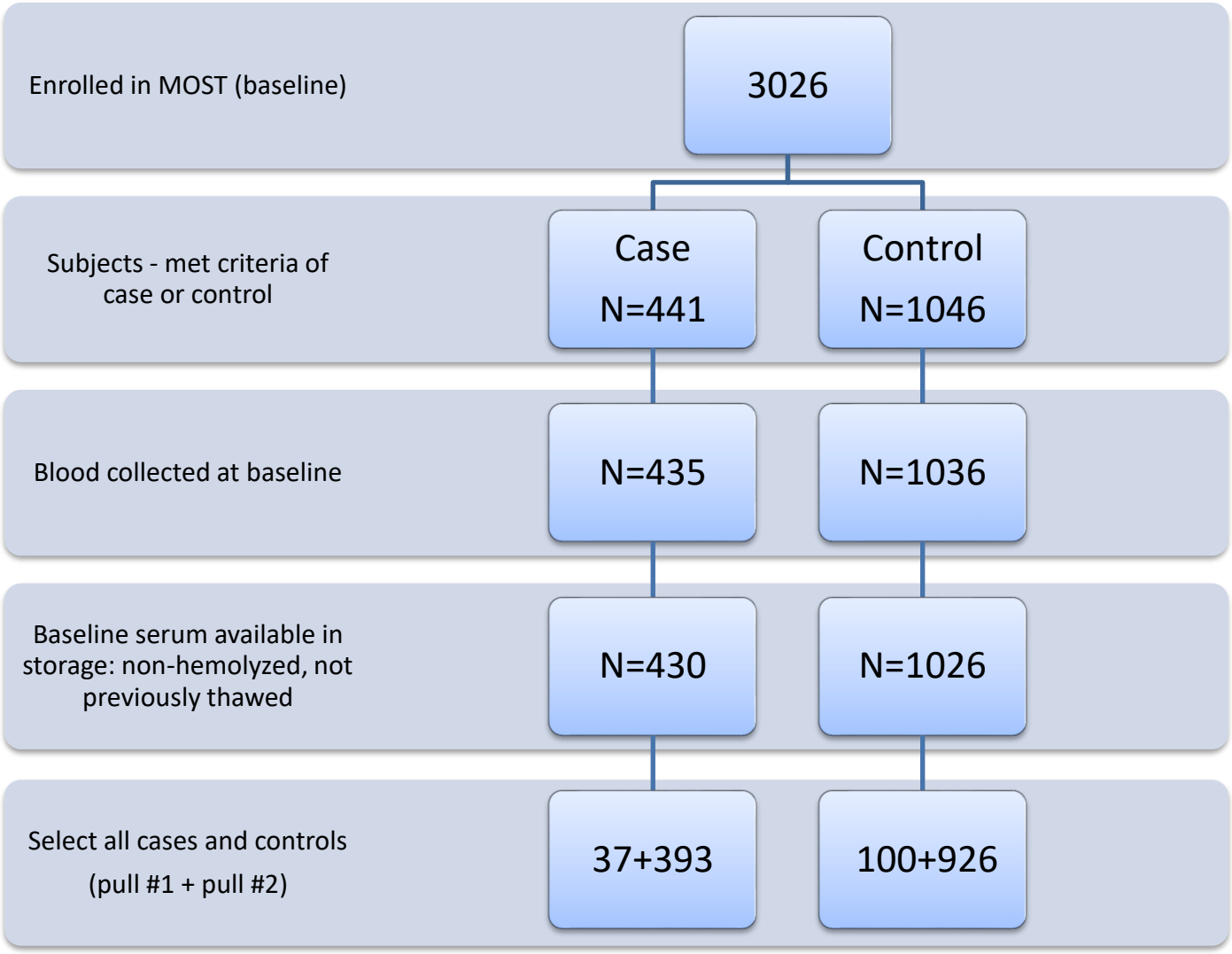
  Values .L and .H can be used in categorical analysis only. Alternatively, the analyst can assign the special value above detection or below detection if requested by investigator.
- If there was insufficient volume or some other reason assay could not be performed, and all assay values were missing, the record is not included in the analytical dataset.

2. Reference.

Misra D, Felson DT, Silliman RA, Nevitt M, Lewis CE, Torner J, Neogi T. Knee osteoarthritis and frailty: findings from Multicenter Osteoarthritis Study and Osteoarthritis Initiative.
J Gerontol A Biol Sci Med Sci. 2015 Mar;70(3):337-42. doi: 10.1093/gerona/glu102. Epub 2014 Jul 25.
PMCID: 4351392 http://www.ncbi.nlm.nih.gov/pubmed/25063080

3. Selection plan

Enrolled in MOST: N=3026 participants / 6054 knees
Cases – participants with Sx OA incidence between baseline and 84m clinic visit pain assessment and knee x-ray
Controls – participants without Sx OA as of 84m clinic visit pain assessment and/or x-ray
Figure 1 – FLOW chart for Sx OA study selection.



Aliquots of serum from the baseline MOST examination will be sent to the University of Vermont laboratory where they will be thawed and analyzed for selected assays: Total Adiponectin and High Molecular Weight Adiponectin. The coefficient of variation for the Adiponectin assay is 9.8% to 12% and for High Molecular Weight Adiponectin is 6.7-8.6%.

Assays results were obtained in two batches:
Batch 1: 37 cases and 100 controls – results were done in February 2016 (Pilot study – results also included in the dataset AS1406pilot_bioassay);
Batch 2: 393 cases and 926 controls – results were done in June 2016

4. Appendix A - Assay Documentation Provided by Laboratory

Table 1. Adiponectin Assays from Batch 1:

Assay	Manufacturer	Method	Catalog#	Kit Lot	Volume	Sample Type	Range of Standard*	Low Detection	High Detection	Estimated Normal Range**
HMW Adiponectin	R&D Systems	Sandwich Elisa	DHWAD0	336275	15	Serum	2.9 - 250 ng/mL	390 ng/mL	25000 ng/mL	~ 1680 -10,906 ng/mL
Total Adiponectin	R&D Systems	Sandwich Elisa	SRP300	339922	15	Serum	3.9 - 250 ng/mL	390 ng/mL	40000 ng/mL	~ 1100 - 21,424

*The standard curve range is different from the detectable range (low and high) based on the dilution factor of the sample in the assay (in this case 1:100). Assay values can be detected from approximately 1500 pg/mL to 110,000 to 120,000 pg/mL at the 1:100 dilution. Most people just want to know the detectable range of the assay.

**The normal range are values from assay run on a small representation of healthy donors.

Table 2. Inter- and Intra- CV Provided – by Assay for Batch 1:

Assay	Intra-Assay CVs	Inter-assay CV
HMW Adiponectin	5.29 - 8.95%	Not provided
Total Adiponectin	7.29 - 9.59%	Not provided

Table 3. Adiponectin Assays from Batch 2:

Assay	Manufacturer	Method	Catalog#	Kit Lot	Volume	Sample Type	Range of Standard	Low Detection	High Detection	Estimated Normal Range
HMW Adiponectin	R&D Systems	Sandwich Elisa	DHWAD0	336275	15	Serum	2.9 - 250 ng/mL	390 ng/mL	25000 ng/mL*	~ 1680 -10,906 ng/mL
Total Adiponectin	R&D Systems	Sandwich Elisa	SRP300	339922	15	Serum	3.9 - 250 ng/mL	390 ng/mL	40000 ng/mL	~ 1100 - 21,424

*Note: New data analysis software allows extrapolation within 20% of set detection ranges.

Table 4. Inter- and Intra- CV Provided – by Assay for Batch 2:

Assay	Intra-Assay CVs	Inter-assay CV
HMW Adiponectin	Not provided	*8.21 - 11.98%*
Total Adiponectin	Not provided	*5.05 - 6.56%*

5. **Appendix B - Quality Control Report for Serum Adiponectin Results by Selection Batch, Selection Case/Control Status and Sex/Gender**

The Coordinating Center performed QC procedures on the study results and generated this report. Note, results marked as special values (below or above detection level) are missing and therefore not included in this report.

Table 1. Assay by Selection Batch:

Selection batch	N Obs	Assay	N*	Mean	Std Dev	Minimum	Maximum
Batch1	**137**	**HMW Adiponectin**	131	7424.28	5016.46	512.88	22179.1
		Total Adiponectin	136	13219.58	8054.56	562.08	37306.69
Batch2	**1319**	**HMW Adiponectin**	1299	8327.09	5522.37	678.94	29947.92
		Total Adiponectin	1309	12938.56	7443.05	815.31	39690.12

*Note – difference in N obs (number of observations) and N is due to missing values not included in this preliminary analysis.

Table 2. Assays by Selection Batch and Selection Status:

Selection batch	incSxOA	N Obs	Variable	N	Mean	Std Dev	Minimum	Maximum
Batch1	**0**	**100**	HMW Adiponectin	96	7511.58	4835.83	742.1	22179.1
			Total Adiponectin	99	13479.45	7610.42	711.6	35838.08
	1	**37**	HMW Adiponectin	35	7184.81	5549.48	512.88	21143.13
			Total Adiponectin	37	12524.27	9216.05	562.08	37306.69
Batch2	**0**	**926**	HMW Adiponectin	912	8275.58	5611.84	709.3	29947.92
			Total Adiponectin	919	12850.3	7507.61	815.31	38600.87
	1	**393**	HMW Adiponectin	387	8448.47	5310.68	678.94	28097.18
			Total Adiponectin	390	13146.53	7293.94	1255.4	39690.12

Note: Each selection batch contains cases and controls and there is no significant difference in means values for either assay.

Table 3. Assays by Sex/Gender and Selection Status:

Sex/Gender	incSxOA	N Obs	Variable	N	Mean	Std Dev	Minimum	Maximum
Female	**0**	**593**	HMW Adiponectin	581	9814.48	5860.73	808.85	29947.92
			Total Adiponectin	587	15360.96	7634.34	2069.19	38600.87
	1	**274**	HMW Adiponectin	269	9774.58	5420.38	1403.05	28097.18
			Total Adiponectin	271	15249.43	7556.87	562.08	39690.12
Male	**0**	**433**	HMW Adiponectin	427	6009.91	4189.68	709.3	24903.17
			Total Adiponectin	431	9575.42	5902.53	711.6	34464.61
	1	**156**	HMW Adiponectin	153	5827.88	4129.26	512.88	21242.78
			Total Adiponectin	156	9345.83	5620.7	1255.4	29376.69

Note: Laboratory did not report sex/gender difference in standard range or normal range, but preliminary results show the **significant difference** in means by sex/gender for both assays regardless of selection (incident case or incident control).